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The Medically Important Aerobic Actinomycetes: Epidemiology and Microbiology

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INTRODUCTION

The term aerobic actinomycetes is an informal designation for bacteria that belong to the order *Actinomycetales*. Originally, microorganisms of this order were classified with the fungi because they possessed true aerial hyphae, which were considered to be a fungal characteristic. However, on the basis of their cell wall components, in particular, their cell envelope lipid and peptidoglycan compositions, these microorganisms are now recognized as true bacteria that are aerobic. Most actinomycetes are typically gram-positive, filamentous, partially acid-fast, branched bacteria that have many microbiologic characteristics in common with members of the genera *Mycobacterium* and *Corynebacterium*. Instead of formalizing subgroups of the order *Actinomycetales*, taxonomists have adopted a provisional, ad hoc ranking in which purely descriptive names for groups are applied. These transitional names for groups have no official standing in nomenclature. However, as knowledge is accumulated about the similarities among these bacteria, formal taxonomic families will likely be named. The major groups of the order *Actinomycetales*, actinoplanetes, maduromycetes, nocardioform actinomycetes, and streptomycetes, probably represent distinct groups, although additional strains need to be examined by molecular methods to confirm relationships based on chemical and morphologic indicators and to clarify apparent anomalies.

Although the aerobic actinomycetes are infrequently encountered in clinical practice, they are important potential causes of serious human and animal infections. The clinical manifestations and severity of disease and the prognosis in an infected host are extremely variable and may be determined by factors such as the route of infection and the presence or absence of a properly functioning immune system. The typical pathologic appearance of the disease is a granulomatous inflammatory reaction, which may progress to abscess formation. The diagnosis of these infections has been hindered by a combination of clinical and microbiologic difficulties, including their often nonspecific clinical presentation, a requirement for invasive diagnostic biopsy procedures, difficulty in isolation, and imperfect classification. Recent advances in identification and the application of recently described molecular methods for diagnosis and strain typing may enhance our understanding of the pathogenesis of these infections. Strategies to improve outcome for infected patients include a heightened awareness of clinicians and clinical microbiology personnel, which may enable the earliest possible diagnosis; standardization of antimicrobial susceptibility testing methods; and the evaluation of newer effective drug therapies for these patients.

In this article, we review current information on medically important aerobic actinomycetes and the sporadic and epidemic diseases caused by these bacteria in animals and humans. We also detail factors predisposing subjects to infection, the spectrum of clinical diseases, and antimicrobial therapy and discuss recent developments in identification, subtyping, and antimicrobial susceptibility testing.

NOCARDIA SPECIES

Historical Perspective: Introduction and Taxonomy

In 1888, a report by the French veterinarian Edmond Nocard of the disease bovine farcy on the island of Guadeloupe provided the first description of clinical disease caused by an aerobic actinomycete (417). Nocard characterized this often fatal illness of cattle as a granulomatous process with draining sinuses, abscess formation, and frequent pulmonary involvement (417). The etiologic agent isolated by Nocard from infected animals was characterized in 1889 by Trevisan and named *Nocardia farcinica* (591). Shortly thereafter, Eppinger reported the first human *Nocardia* sp. infection in a patient with pneumonia and brain abscess (159). In 1896, the microorganism was formally classified by Blanchard as *N. asteroides* (58). Four successive reviews of 180 cumulative cases reported in the medical literature established this microorganism as an unusual cause of human pulmonary infection (31, 250, 292, 310). However, it was not until the retrospective analysis by Presant et al. of 147 worldwide, culture-confirmed cases reported between 1945 and 1968 that standardized diagnostic and prognostic criteria were developed for human nocardial disease (457). In 1974, Palmer et al. published a review of human cases reported in the English literature from 1961 to 1971 and identified an additional 243 cases (434). These and other more recent reviews have suggested that nocardiosis is an increasingly recognized cause of opportunistic infection that may be associated with a variety of underlying disorders, receipt of chemotherapeutic agents, or both (41, 43, 124, 641).

The genus *Nocardia* comprises several species that are known to be unusual causes of a wide spectrum of clinical diseases in both humans and animals (43, 128). While the majority of nocardial infections have been attributed to *Nocardia asteroides*, other pathogenic *Nocardia* species that have been described include *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, and *Nocardia transvalensis*. In a recent taxonomic revision of the *N. asteroides* taxon, two new species—*N. farcinica* and *Nocardia nova*—were separated from it (330, 600, 621, 626).

A constant morphologic feature of *Nocardia* spp. is the tendency for both aerial and substrate hyphae to fragment into bacillary and coccoid elements (350). However, this characteristic is not pathognomonic for *Nocardia* spp. and, on the basis of morphology alone, other bacteria have been erroneously assigned to the genus *Nocardia*. Important biochemical tests that differentiate the three major pathogenic *Nocardia* species, *N. asteroides*, *N. brasiliensis*, and *N. otitidiscaviarum*, include the decomposition of casein, xanthine, tyrosine, and hypoxanthine. However, this identification method does not differentiate the *N. asteroides* complex from the nonpathogenic *Nocardia* species *Nocardia carnea*, *Nocardia amarae*, and *Nocardia brevicatena* or from species of the related genera *Mycobacterium*, *Rhodococcus*, *Gordona*, and *Tsukamurella*. In the past, the use of these few biochemical tests and morphology alone resulted in the genus *Nocardia* being characterized by extreme heterogeneity (196, 201, 203, 335, 512, 598). In particular, the consistency and composition of the growth medium can affect the growth and stability of both aerial and substrate hyphae. An inconsistent morphologic feature of the genus *Nocardia* includes well-developed conidia in *N. brevicatena* and less well-formed spores in some *N. asteroides* strains. Macroscopically visible aerial hyphae may be lacking, sparse, or very abundant, and colonies may have a superficially smooth appearance or, more commonly, a chalky appearance. Cell pigments may be off-white, gray, yellow, orange, pink, coral, red, tan, brown, or purple.

During the last two decades, major advances have been made in the development and application of new and reliable methods for classifying bacteria. In particular, techniques for the identification of cell wall amino acids and sugars, whole-cell sugars, fatty acids (including mycolic acids), menaquinones, and phospholipids and DNA relatedness studies have proven to be extremely useful for streamlining actinomycete taxonomy (120, 203, 649). As a consequence of these studies,

TABLE 1. Recent taxonomic changes in the genus *Nocardia*

New name	Previous name (*Nocardia*)	Reference
Amycolata autotrophica	*N. autotrophica*	339
Amycolata hydrocarbonoxydans	*N. hydrocarbonoxydans*	339
Amycolata saturnea	*N. saturnea*	339
Amycolatopsis orientalis	*N. orientalis*	339
Amycolatopsis orientalis subsp. *lurida*	*N. lurida*	339
Amycolatopsis mediterranei	*N. mediterranei*	339
Amycolatopsis rugosa	*N. rugosa*	339
Amycolatopsis sulphurea	*N. sulphurea*	339
Nocardia farcinica[a]	*N. asteroides* complex	330
Nocardia nova[a]	*N. asteroides* complex	600
Rhodococcus equi	*N. restricta*	200
Rhodococcus erythropolis	*N. calcarea*	200
Rhodococcus globerulus	*N. corynebacterioides* or *N. globerula*	208
Saccharothrix australiensis	*N. australiensis*	318
Saccharothrix aerocolonigenes	*N. aerocolonigenes*	317

[a] Formerly a subgroup of *N. asteroides*.

many taxa formerly assigned to the genus *Nocardia* have now been removed from this genus (Table 1).

Currently, the chemotaxonomic criteria used to assign bacteria to the genus *Nocardia* include the presence of the *meso* isomer of 2,6-diaminopimelic acid (DAP) and whole-cell sugars, arabinose and galactose (cell wall type IV) (331); intermediate-chain mycolic acids (46 to 60 carbons) (11, 88, 89, 201, 203, 335); a fatty acid profile showing major amounts of straight-chain, unsaturated, and tuberculostearic acids (336); and the presence of a menaquinone fraction containing a tetrahydrogenated menaquinone with eight isoprene units as the predominant isoprenoic quinone (119, 649, 650). Tests that have established the heterogeneity of *N. asteroides* include numerical taxonomy (48, 394, 431, 600), mycolic acid analysis by gas chromatography (651), DNA relatedness (651), and antimicrobial resistance patterns (625). These studies have indicated that many isolates recovered from clinical specimens and previously identified as *N. asteroides* are actually different species. One of these new taxa, *N. farcinica*, has been reestablished as a legitimate species by the Judicial Commission of the International Committee on Systematic Bacteriology (330). The clinical need for this separation from *N. asteroides* was once controversial. However, now this distinction has been supported by the specific antimicrobial resistance pattern of *N. farcinica* (626).

In *Bergey's Manual of Systematic Bacteriology*, *N. nova* was considered a species incertae sedis (330). However, in 1990, Yano et al. were clearly able to distinguish *N. nova* from *N. asteroides* and *N. farcinica* by DNA relatedness analysis, mycolic acid patterns, and numerical taxonomy (651). The taxonomic status of this species was further supported by a distinct antimicrobial resistance pattern (621).

Ecology

The aerobic actinomycetes are ubiquitous in the environment; they have been isolated worldwide from soil and organic matter (197). *Nocardia* spp. and related bacteria are considered saprophytic soil microorganisms, primarily responsible for the decomposition of organic plant material (197, 431, 449). In one Australian environmental survey, pathogenic species of the genus *Nocardia* were detected in house dust, beach sand, garden soil, and swimming pools (481). Also, there are reports of the isolation of *Nocardia* spp. from tap water in the Egyptian city of Ismailia (1) and from soil in Argentina (614, 615), Spain (611), and Sudan (7).

Also, although *N. asteroides* appears to be geographically widespread, most cases of *N. brasiliensis* infection in the United States have originated in the southeast or southwest, especially Texas, North Carolina, California, Oklahoma, and Florida (551).

Epidemiologic Aspects of Infection in Animals

There are several reports in the veterinary literature of *Nocardia* sp. infections in animals. However, clinical reports of animal and human disease do not always include a valid identification of the etiologic agent, a complete description of accompanying clinical findings, or histopathologic evidence to confirm invasive disease. *Nocardia* spp., in particular, *N. asteroides*, are the most common aerobic actinomycete species that are animal pathogens (45). They have been reported to cause infections in cattle (39, 145, 242, 323, 357, 368, 498, 535, 564), horses (55, 68, 122, 137), dogs (74, 504), and swine (296, 376). There are rare reports of *N. asteroides* causing infections in a variety of other wild and domestic animals, including birds (351, 436), cats (130), a fox (354), koalas (636), a mongoose (354), and cynomolgus and macaque monkeys (346, 496).

Since Nocard's original report, the aerobic actinomycetes have been found to be potential pathogens of domestic livestock (417). Bovine mastitis is an acute or chronic inflammation of the udder of milking cows that is caused by a variety of infectious agents and that results in decreased milk production ("dry cows"). Factors such as nonhygienic milking practices may contribute to the spread of mastitis within a herd. Thus, this disease has the potential to have a severe economic impact on the dairy industry in both developed countries and the developing world (39, 45, 242, 498). *Staphylococcus aureus* and *Streptococcus agalactiae* are the most frequently identified microorganisms causing sporadic mastitis in cattle; however, other potential etiologic microorganisms include *Nocardia* spp., other *Streptococcus* spp., coliforms, and yeasts.

N. asteroides is the *Nocardia* species most frequently associated with bovine mastitis, typically causing chronic mastitis that is often unresponsive to the usual therapy with broad-spectrum antimicrobial agents and corticosteroids. A characteristic of this infection is the tendency for the production of a granulomatous udder lesion. This characteristic, in itself, may present a barrier to the achievement of adequate therapeutic or bactericidal antimicrobial agent levels in tissue, causing specific antimicrobial therapy to be ineffective.

From 1985 through 1988, a large nocardial mastitis epizootic was reported across Canada (145, 357, 522, 564). Following the detection of an increased number of *Nocardia* sp. isolations in surveillance cultures of milk from Ontario dairy herds, a case-control study of Ontario dairy farms was conducted (564). Two significant risk factors identified in this study were the receipt of blanket dry cow therapy and a proprietary dry cow antibiotic product containing neomycin and hydrocortisone; however, no further details on the nature of these associations were obtained (564). Investigators hypothesized that intrinsic contamination of dry cow therapeutic agents, other, different unspecified milking and udder sanitation practices, and environmental contamination may have caused the epizootic (564). Other plausible explanations, unreported in this study (564), include the dissemination of an unusually virulent or antimicrobial agent-resistant *Nocardia* strain.

Equine nocardiosis has been infrequently documented. Rarely, horses may develop localized nocardial skin infections

that may form abscesses. These infections are thought to be acquired by contamination of superficial wounds. However, a more common occurrence is bronchopneumonia that may progress to disseminated disease. This condition is thought to result from inhalation of the microorganisms. Animals with disseminated disease may develop widespread noduloulcerative skin lesions, swollen lymph nodes, purulent nasal discharge, and other signs of respiratory tract involvement. At postmortem examination, pulmonary and pleural lesions that are often culture positive for *N. asteroides* may be present. An underlying immunosuppressive condition is the major predisposing factor identified in these infected horses. Two groups of horses appear to be particularly susceptible to developing *N. asteroides* infections: Arabian foals with combined immunodeficiency disease or, rarely, adult horses with hyperadrenocorticalism secondary to adrenocorticotropin-secreting pituitary tumors (55). There is also one report of a Spanish epizootic of nocardiosis among "serum" horses; 54 (22%) of 245 animals became infected, and 10 died (482). Fatal disseminated nocardiosis in association with combined immunodeficiency disease of Arabian foals was first described in 1974 (379) and has since been estimated to affect 10% of these animals (55). The circumstances favoring *N. asteroides* infections in horses appear to closely parallel those recognized for human infections. Of interest, Biberstein et al. found evidence that natural resistance may also occur in horses; in two immunologically normal animals that acquired infections following traumatic implantation, spontaneous recovery without receipt of specific antimicrobial therapy occurred (55).

N. otitidiscaviarum is a rare cause of animal infections, including invasive pulmonary and disseminated infections (101, 102).

Epidemiologic Aspects of Infection in Humans

The morbidity and mortality associated with nocardiosis have largely gone unrecognized because, as with invasive fungal infections, there is no comprehensive national reporting system. Therefore, information on these infections has been compiled largely by identification of case reports and data from a relatively few experienced investigators. To date, only one 1976 report, by Beaman and colleagues, has attempted to measure the incidence of nocardiosis in North America. The estimate obtained from this survey of U.S. infectious diseases physicians was between 500 and 1,000 new cases annually (43). Although no additional studies have been done, this often-quoted figure, projected to represent annual infections in both immunocompetent and immunocompromised patients in the mid-1970s, is very likely to be a gross underestimate of the annual incidence of the disease in the 1990s.

During the last decade, there has been a marked increase in the number of severely immunocompromised patients. Many of them are known to be extremely susceptible to the development of nocardiosis. This fact has been due mainly to recent technological advances, developed to prolong the life of severely ill hospitalized patients and, to a lesser extent, to the recent epidemic of AIDS. Since the numbers of severely immunocompromised patients show no tendency to stabilize or diminish, *Nocardia* sp. infections will likely continue to emerge as important opportunistic diseases.

In the United States and other countries, nocardiosis is usually recognized as a sporadic, community-acquired infection, although there have been a few reports of nosocomial outbreaks. Nocardiosis appears to have a slight predilection for males and usually affects adults in the third and fourth decades. *Nocardia* spp. can cause disease in the absence of any predisposing factor or may be opportunistic (124, 457). Cases may go undiagnosed because there is a delay in performing necessary diagnostic tests (invasive biopsies) for seriously ill patients or because the infection partially or successfully responds when prophylactic broad-spectrum antimicrobial therapy is prescribed. Failure by a clinical microbiology laboratory to isolate and correctly identify the pathogen in clinical specimens may adversely affect outcome for some infected patients.

Infections of the immunocompetent host. (i) Colonization. Since *Nocardia* spp. are ubiquitous in nature, the isolation of these microorganisms from a patient's sputum culture may not always indicate invasive infection and instead may reflect laboratory contamination or respiratory colonization (422, 597). *N. asteroides* may be a saprophyte on the skin and in the upper respiratory tract (341). Hosty et al. found that aerobic actinomycetes were isolated from 0.2% of respiratory tract specimens submitted for examination to a mycobacteriology laboratory (267). In a recent retrospective study of 102 isolates submitted to an Australian reference laboratory, 20% of *Nocardia* sp. isolates were found not to be associated with clinical disease (186). The majority of these nonsignificant isolates were cultured from the respiratory tract or a superficial lesion and were thought to indicate airway colonization or soil-derived contamination.

Nocardia spp. have also rarely been implicated as a cause of mild clinical infections in humans, including pharyngitis, bronchitis, and otitis media (341). Young et al. described seven patients (none on steroids) with fever or upper respiratory tract symptoms; all had sputum cultures positive for *Nocardia* spp., and all recovered without antimicrobial therapy (657). Underlying pulmonary disorders, in particular, those associated with bronchial obstruction or decreased bronchociliary clearance (e.g., malignancy, tuberculosis, cystic fibrosis, asthma, bronchitis, and allergic aspergillosis), may predispose patients to respiratory tract colonization with *Nocardia* spp. (488). However, invasive nocardial infections usually do not develop in these patients unless they are also given steroid therapy (488).

Criteria useful for judging the importance of a culture positive for *Nocardia* spp. from the respiratory tract and skin, in addition to signs of sepsis in the patient, include direct visualization of the microorganism on a Gram-stained smear, pure or predominant growth in the culture, and repeated isolation from serial clinical samples (488).

(ii) Cutaneous infection. In contrast to invasive pulmonary and disseminated nocardioses, which affect predominantly severely immunocompromised patients, cutaneous nocardiosis is usually a primary infection affecting apparently immunocompetent individuals. The same three major etiologic agents—*N. asteroides*, *N. brasiliensis*, and *N. otitidiscaviarum*—cause all three types of infection; however, *N. brasiliensis* predominates in the primary cutaneous infection (551). Cutaneous nocardiosis may be subdivided into four clinical types: mycetoma, lymphocutaneous infection, superficial skin infection (abscess or cellulitis), and secondary cutaneous involvement with disseminated disease (282). Primary cutaneous and subcutaneous actinomycete infections usually result from traumatic inoculation with these microorganisms, which may result from a variety of outdoor activities or occur at the time of surgery.

Mycetoma is a chronic, localized, slowly progressive and often painless subcutaneous disease. It usually involves the lower limbs and is characterized by tumefaction, subcutaneous nodules, destructive granulomata, deformity, and discharging sinus tracts with intercommunicating channels that exude pus, often with macroscopically visible granules of various sizes and colors (12, 52, 70, 104, 226, 293, 326, 527). Mycetoma is a

late-stage clinical manifestation of a subcutaneous infection produced by either bacteria (i.e., actinomycetes causing actinomycotic mycetoma, or actinomycetoma) or fungi (eumycetic mycetoma, or eumycetoma).

Microorganisms reported to cause actinomycetomas include *N. brasiliensis*, *Actinomadura madurae*, *Actinomadura pelletieri*, *Streptomyces somaliensis* and, less commonly, *N. asteroides*, *N. otitidiscaviarum*, *Nocardiopsis dassonvillei*, and *N. transvalensis*. In North America, South America, Mexico, and Australia, *N. brasiliensis* is the chief cause of actinomycetomas; however, in Africa *S. somaliensis* predominates (42). These infections most commonly affect patients in rural areas in Third World countries. Patients with these chronic infections may have a history of a specific minor localized traumatic injury (414, 557). The foot is the most common site of involvement (104, 110, 154, 620); however, the hand (12, 93, 189, 374, 414, 595), face (432), and neck (129) may also be affected. Walking barefooted may be the major mode of acquisition of these infections, since this practice potentially exposes the feet to repeated soil-contaminated puncture wounds.

Localized cutaneous *Nocardia* sp. infections may also present as either a chronically draining ulcerative lesion or a slowly expanding nodule and, less commonly, pustules, abscesses, cellulitis, or pyoderma. Frequently, there may be spread beyond the initial cutaneous infective focus to involve the regional lymphatics and, in up to one-third of cases, the disease may progress to form lymphatic abscesses (186). When regional lymph node involvement occurs, this form of the disease is referred to as the lymphocutaneous syndrome. Since this form of the disease bears a striking clinical resemblance to a superficial infection with the dimorphic fungus *Sporothrix schenckii*, it has also been termed the sporotrichoid form of cutaneous nocardiosis (47, 282, 505).

It is necessary to perform an appropriate laboratory diagnostic workup to exclude misdiagnosis of the disease as sporotrichosis (47, 595, 643). More localized lesions may also be misdiagnosed as staphylococcal skin infections. Since skin manifestations may be a complication of disseminated disease, the finding of a cutaneous lesion in a patient should not always be attributed to local inoculation.

(iii) Ocular infection. Rarely, ocular infections with *Nocardia* spp. have been reported to occur in both apparently immunocompetent and immunocompromised patients. Such an infection may occur secondary to a traumatic corneal injury, with exogenous inoculation with *Nocardia* spp., and result in keratitis and ultimately endophthalmitis (109, 116, 148, 246, 291). Less common exogenous mechanisms that have been reported to have resulted in ocular nocardial infections have included improper sterilization of extended-wear soft contact lenses (158) and postoperative *N. asteroides* infection in a patient following an ophthalmologic (scleral buckling) procedure (291).

Keratitis caused by *Nocardia* spp. may mimic noninfectious inflammatory eye conditions. Since infected patients may develop severe ocular complications if steroid therapy is inappropriately prescribed, it is essential that all patients who present with keratitis and in whom the diagnosis of *Nocardia* sp. infection is suspected should initially have corneal scraping specimens submitted for microbiologic examination to exclude that diagnosis (438).

Endogenous ocular nocardial infections may also occur in immunocompromised patients following bloodstream dissemination from a distant pulmonary or other infective focus. All patients who present with such an ocular infection should undergo a thorough evaluation to exclude underlying disseminated disease (164, 272, 295, 367, 460).

Infections of the compromised host. In severely immunocompromised patients, the most common clinical presentations caused by *Nocardia* spp. are invasive pulmonary infection and disseminated infection. However, each of these and other types of invasive disease may also occur in nonimmunocompromised patients (128, 487).

(i) Invasive pulmonary infection. Inhalation of infectious airborne spores or mycelia of *Nocardia* spp. is thought to be responsible for most cases of invasive pulmonary infection. However, respiratory tract colonization with these microorganisms may occur and may be an indirect source of the infecting microorganisms. Rarely, respiratory tract colonization with *Nocardia* spp. has been noted to occur in immunocompetent patients, in particular, patients with underlying chronic lung disease. However, there is evidence that the prevalence of respiratory tract colonization may be much higher in selected groups of severely immunocompromised patients. In a study of immunocompromised cardiac transplant patients, Simpson et al. found 44 to 51% of the patients to be colonized with *Nocardia* spp. (545).

N. asteroides is the most common cause of primary pulmonary nocardiosis: this species has been estimated to cause at least 80% of cases (43). Rarely, other species, in particular, *N. brasiliensis* (551), have also been implicated in causing pulmonary disease in both immunocompromised and nonimmunocompromised patients (101, 382).

Patients with impaired local pulmonary defenses may be predisposed to the development of clinical disease. In particular, pulmonary nocardiosis has been reported to occur in patients with chronic obstructive pulmonary diseases (chronic bronchitis and emphysema), asthma, and bronchiectasis (186, 407). Several reports have also described an association between pulmonary nocardiosis and alveolar proteinosis (13, 18, 87, 583).

In a review of 243 nocardiosis cases reported between 1961 and 1972, Palmer et al. found that 2% of patients with an underlying condition had alveolar proteinosis (434). However, it is unclear whether the reported association between nocardiosis and this disorder represents a true independent association. Since there is a strong association between alveolar proteinosis and hematologic malignancies, the coexistence of nocardiosis and alveolar proteinosis may merely reflect the independent correlation of each of these conditions with underlying hematologic malignancies (18).

Patients with systemic immunosuppression are also especially predisposed to the development of invasive pulmonary nocardiosis. Prior treatment with corticosteroids, cytotoxic agents, or both appears to be an important risk factor for the development of this infection. In particular, invasive pulmonary nocardiosis has been reported to occur in renal (16, 28, 33, 97, 249, 257, 328, 355, 424, 641) and cardiac (225, 238, 295, 305, 367, 544, 545) transplant recipients and patients with malignant neoplasms (17, 43, 53, 581), lymphoma (2, 38, 295, 423, 451, 494, 534, 582), sarcoidosis (407, 430, 661), collagen vascular diseases (especially systemic lupus erythematosus) (181, 222, 300, 645), dysgammaglobulinemia (434), chronic granulomatous disease (98, 163, 276, 278, 384, 587), chronic alcoholism (186, 610), diabetes mellitus (186, 434), trauma or surgery (434), and human immunodeficiency virus (HIV) infection (186, 302, 369, 442, 483). Intravenous drug abusers are another patient group at risk for the development of nocardiosis, which may result from intravenous administration of nonsterile substances (177, 178, 251, 442, 540, 612). Pregnant women may also rarely develop nocardiosis (76, 430). Thus, *Nocardia* spp. are uncommon pathogens in humans, involving mainly immunosuppressed or debilitated patients.

However, nocardial pneumonia may also occur in patients without any concurrent disease or therapy. The reported prevalence of such disease has ranged from 10 to 25% (43, 305, 657). Also, in another review of 455 cases of nocardiosis, 39% of infections were not associated with a preexisting illness or immunosuppressive therapy (128).

Like that in nonimmunocompromised infected patients, the disease in severely immunocompromised patients may be associated with nonspecific clinical findings. However, when compared with the more chronic course seen in immunocompetent patients, the course of the infection in severely immunocompromised patients is more often progressive, disseminated, and life threatening. The most frequent clinical presentation may be subacute or chronic, often necrotizing pneumonia, frequently associated with cavitation. Other presentations may include a slowly enlarging pulmonary nodule or pneumonia with associated empyema (124). Rarely, the disease may manifest itself as an acute fulminating infection, and diffuse, fulminant pneumonitis may occur in immunocompromised patients (229, 413), patients with pulmonary alveolar proteinosis (456), and nonimmunocompromised patients (524).

Infected patients are usually systemically ill, with symptoms of fever, night sweats, anorexia, weight loss, productive cough, and hemoptysis. Pleuritic chest pain may often precede or accompany the development of empyema. Following a pulmonary parenchymal infection, the usual pathologic process involves the formation of multiple necrotizing pulmonary abscesses (457, 492). Characteristically, nocardial abscesses are also poorly localized, and infection readily spreads to adjacent lung tissue, to the pleural surface, or to distant sites, predominantly the skin and the brain (457). The formation of tissue granulomata, especially in the lungs (407), is another common pathologic finding. Finally, indolent progressive fibrosis may result following treatment or successful containment without therapy (30).

The appearance on chest radiographic examinations of patients with pulmonary nocardiosis may be pleomorphic and nonspecific (30, 229, 305, 457, 492). The abnormalities commonly observed reflect the combination of necrotizing and granulomatous pathologies. They include localized infiltrates and irregular nodules (both of which may be associated with cavitation), pleural effusions, and hilar lymphadenopathy. Single or multiple nodules, masses, and abscesses, miliary lesions, diffuse alveolar and interstitial infiltrates, subpleural plaques, pleural empyema and, very rarely, calcification are less common radiographic findings in infected patients (30). In one case series, one or more areas of cavitation were found on chest radiographs for 42% of patients with pulmonary and disseminated nocardioses, although the authors did not suggest that this finding might be a characteristic pattern of lung involvement for the disease (186). The occurrence of cavitary pneumonia, particularly in a patient with compromised immunity, should alert the clinician to the possibility of nocardial infection (162). In particular, in immunocompromised patients, follow-up chest radiographic examinations may be important because there may often be a marked and rapid progression in radiographic findings.

Local complications of invasive pulmonary nocardial infections include pleural effusion, empyema, pericarditis, mediastinitis, superior vena cava obstruction and, rarely, the development of local chest wall and neck abscesses. Within the chest, primary pulmonary nocardiosis can invade local structures and cause mediastinitis and superior vena cava obstruction (452). In immunocompromised patients, involvement of the pericardium may occur and result in suppurative or chronic constrictive pericarditis or both (19, 287, 344, 454, 572). It has been proposed that the successful management of these patients requires an aggressive approach that combines appropriate antimicrobial therapy and surgical intervention (pericardiectomy) (287, 344, 454). Since the advent of antimicrobial therapy, the development of local chest wall sinus tracts or subcutaneous abscesses adjacent to a site of pleural involvement have become unusual complications (248). However, direct extension from a pulmonary infective focus may result in neck or mediastinal abscesses (185). *N. brasiliensis* was also reported to cause transtracheal neck abscesses after a transtracheal aspiration procedure for the diagnosis of pulmonary disease (140, 190).

Characteristically, invasive pulmonary nocardial infections have a propensity for hematogenous dissemination. In addition, metastatic infective foci may be present but unrecognized at the time of the patient's initial presentation with pulmonary nocardiosis. Progression of the infection in these sites may not become clinically evident until after the patient has begun specific antimicrobial therapy.

In severely immunocompromised patients, pulmonary nocardiosis may progress rapidly, and this diagnosis should be considered if these patients present with an acute necrotizing pulmonary infection. In addition, other bacteria known to cause cavitating pneumonia, including *Staphylococcus*, *Pseudomonas*, *Klebsiella*, and *Mycobacterium* spp., must also be excluded (168). However, pulmonary nocardial infections may also have a gradually progressive indolent course in these and other patients and may mimic pulmonary involvement with tuberculosis, fungal disease, sarcoidosis, and neoplasia. In patients with underlying hematologic malignancies or HIV infection, pulmonary infections caused by *Rhodococcus equi* must also be distinguished.

Cases of nocardiosis may go undiagnosed, either because they respond to empiric antimicrobial treatment given for many infections without a culture or Gram stain being performed or because *Nocardia* spp. may be difficult to identify in cultures of clinical specimens; they may be mistaken for nonpathogenic microorganisms (diphtheroids) and discarded.

(ii) Disseminated infection. Disseminated nocardiosis is often a late-presenting and potentially life-threatening infection. It is most frequently endogenous (i.e., secondary to bloodstream spread) from a primary pulmonary infection (128, 407, 434, 568, 641). However, very rarely, it may result from a primary nonpulmonary (cutaneous) infection (551). In patients with primary pulmonary nocardiosis, the development of a disseminated infection may result in brain and skin lesions and invariably has a significant adverse effect on the patient's prognosis. Disseminated nocardiosis has a poor prognosis, with a mortality rate of 7 to 44% (43, 434, 641). In severely immunocompromised patients, the mortality rate may be higher than 85% (457).

Disseminated infections in susceptible patients may be caused by any of the *Nocardia* spp. identified as causing invasive pulmonary and cutaneous infections. Patients with disseminated and potentially fatal *N. brasiliensis* (551), *N. otitidiscaviarum* (75, 101), *N. transvalensis* (29, 382, 384), *N. farcinica* (143, 389, 601, 606, 607, 626), and *N. nova* (518, 621) infections have been described.

As with pulmonary nocardiosis, the patients at highest risk for developing disseminated infections are severely immunocompromised patients (76). All of the previously listed underlying disorders and therapies that place patients at risk for developing invasive pulmonary nocardial infections may also predispose these patients to the development of disseminated infections. Recent reviews of invasive nocardial infections in high-risk, severely immunocompromised patients, including

renal transplant patients (249, 300, 328, 641), cardiac transplant recipients (305, 545), liver transplant recipients (173, 465), patients with carcinomatosis (53), and patients with HIV infection (274, 289), have been published.

Renal transplant patients are known to be predisposed to the development of nocardial infections. Immunosuppressive therapy and azotemia secondary to impaired graft function may be the major factors predisposing patients to these infections. Renal transplant recipients have been identified in many case series as being especially susceptible to the development of nocardial infections and have accounted for 2 to 13% of all invasive nocardiosis cases (43, 128). Invasive nocardial infections may account for approximately 4% of infections in patients following renal transplantation (641). Specific risk factors that have been identified for the development of nocardiosis in renal transplant patients include multiple early rejection episodes (more than two in the first 2 months), intensive immunosuppressive therapy (high-dose prednisolone and azathioprine), age (<10 and >40 years), type of graft (unrelated versus living related donor kidney), presence of granulocytopenia, and uremia resulting from impaired allograft function (6, 27, 170, 565). A recent development that may tend to reduce the number of nocardial infections that occur in this population is the substitution of cyclosporine for high-dose prednisolone and azathioprine in antirejection regimens.

Other organ transplant recipients, especially cardiac transplant patients, also have an increased risk for nocardial infections (545). In their case series of cardiac transplant recipients with nocardial infections, Simpson et al. identified patients with a history of high-dose prednisolone therapy, uremia, prolonged respiratory support, and frequent rejection episodes as having an increased risk for an adverse outcome (545). Of interest, the introduction of cyclosporine as an antirejection agent in both renal and cardiac transplant patients has been reported to have been accompanied by a marked decrease in the incidence of nocardial infections in these patients (258).

In cases of pulmonary nocardial infections, the frequency of disseminated disease has been reported to be approximately 28 to 50% (43, 434, 457). Georghiou and Blacklock found that 90% of patients with disseminated infections had radiographic evidence of pulmonary involvement at the time of diagnosis (186). However, despite the occurrence of a primary pulmonary infection in the majority of patients with disseminated nocardiosis, lung involvement may not always be clinically evident in these patients.

The brain is the most frequent nonpulmonary site involved in disseminated nocardiosis (167, 176, 270, 427, 580), and cerebral nocardiosis is an important cause of cerebral space-occupying lesions. However, the infection may also involve many other deep organs, including the kidneys (9, 307, 466, 497, 654), spleen (307, 654), liver (307, 460, 484, 497, 654) and, rarely, bone (10, 20, 26, 139, 232, 325, 327, 377, 446, 487, 525, 652), skin (71, 127, 282, 416, 658), and joints (71, 111, 123, 144, 299). In the brain and other organs, abscess formation is a particularly common pathologic manifestation of disseminated nocardiosis (457, 657).

Despite the relative rarity of *Nocardia* spp. as cerebral pathogens (i.e., they account for less than 2% of cerebral abscesses in the United States) (266), they demonstrate a marked tropism for the human central nervous system (128, 407, 434, 568, 641). Estimates of the prevalence of central nervous system involvement in nocardial infections range from 30 to 55% (43, 434).

Nocardial meningitis has been reported only rarely and was recently reviewed (80). Patients with nocardial meningitis typically present with subacute to chronic meningitis characterized by fever, stiff neck, and headache. Results of cerebrospinal fluid examination include neutrophilic pleocytosis, hypoglycorrhachia, and elevated protein levels. A common complication is an associated brain abscess that may be detected in 43% of cases (80). The case mortality rate in the recent review was 57%, and it was noted that the survivors were more often younger, had lower initial cerebrospinal fluid glucose levels, and were less likely to have a brain abscess (80). Also, a delay in making the diagnosis in these patients was a frequent finding, and often positive culture results or autopsy findings became available before nocardial infections were suspected for the patients (80).

The most common clinical manifestation of a central nervous system nocardial infection is a brain abscess. Patients may present with acute signs of sepsis and intracranial mass effects (128, 266). However, severely immunocompromised patients with a nocardial cerebral abscess may frequently be asymptomatic, and a prolonged latency (up to 3 years) may occur before this type of clinical presentation develops in patients infected following the commencement of immunosuppression (465, 545). Also, patients with a nocardial brain abscess may have a recent history of a respiratory tract infection or may have recently received a course of unsuccessful antimicrobial therapy prescribed for systemic sepsis or bacteremia (34). A cerebrospinal fluid culture may be positive for only up to 20% of these patients (434). There may be clinical evidence of a pulmonary nocardial infection in about one-third of these patients (80), and blood cultures may also be positive (25, 478). Computerized tomographic scanning is an extremely useful technique for making the diagnosis and may also be used to monitor a patient's response to treatment (115, 353, 634). However, a definitive diagnosis may only be established after brain biopsy clinical specimens from which nocardiae are cultured and/or show morphologically compatible microorganisms on histopathologic examination are obtained.

Byrne et al. reviewed 16 cases of cerebral nocardiosis and found that the important factors influencing patient survival included the early institution of antimicrobial therapy, surgical intervention, and the patient's level of immunocompetence (90). Surgical intervention may not be indicated for all cases, there are reports of successful treatment with antimicrobial agents alone (90, 418). In a retrospective analysis of patients with primary cerebral nocardiosis (i.e., patients presenting with a cerebral abscess but without obvious signs of a concurrent respiratory infection), Barnicoat et al. reported that the presence of underlying immunocompromise increased the rate of mortality from the disease in these patients from 40 to 95% (34). However, these authors also suggested that in immunocompromised patients, in the absence of other adverse prognostic factors, excess mortality may be substantially reduced if an aggressive surgical and antimicrobial therapeutic approach to management is undertaken (34).

Specific investigations for the detection of cerebral involvement are recommended for all patients with invasive pulmonary nocardiosis, since a brain abscess may be a common serious complication in these patients, and early lesions may be asymptomatic (166). The clinical presentation of disseminated nocardiosis as a skin or bone lesion may also need to be differentiated from disease caused by other unusual microorganisms, such as *Mycobacterium fortuitum* (562) and *Gordona bronchialis* in postcardiac surgery sternotomy infections (470).

Nocardiosis may also rarely be associated with invasive fungal infections; in particular, polymicrobial infections involving aspergillosis have been described for patients with a variety

of underlying severely immunocompromising conditions (97, 98, 305, 545).

(iii) Nosocomial transmission. Although nocardiosis is most often considered a late-presenting, community-acquired infection, nosocomial outbreaks of nocardiosis in immunocompromised patients have been reported (28, 124, 305, 434, 488, 545, 657). Houang et al. described an outbreak of nocardiosis affecting seven patients in a renal transplant unit in a United Kingdom hospital (268), and in a follow-up report from the same hospital unit, six further cases were diagnosed over a period of 4 years (249). In the United States, in an 11-year review of six children with nocardiosis complicating hematologic malignancies, Cox and Hughes noted a temporal clustering of three cases during a 2-month period (124). These authors also commented that, importantly, the spatial clustering of patients had occurred at a time when the putative index patients had microorganisms in their sputum or on their vocal cords, a fact which first suggested the possibility of nosocomial airborne transmission of these microorganisms (124).

Further evidence for possible nosocomial transmission of *Nocardia* spp. has been reported for severely immunocompromised heart transplant patients (305, 545). In addition, Sahathevan et al. recently described a cluster of patients with *N. asteroides* infections in a unit in which patients with acute and chronic liver disease and liver transplant recipients were cared for (495). In this outbreak, five patients were diagnosed with nocardiosis during a 2-month period. Two of these patients had underlying chronic liver disease, and three had undergone a liver transplant. Infection sites were the respiratory tract in three patients, a subcutaneous abscess in one patient who also had evidence of a cerebral abscess on computerized tomographic head scan, and blood (bacteremia) in one patient; two additional patients had delayed presentations—5 and 7 months after the first case (495). In this report, exposure to construction activity was identified as a possible risk factor for the infection and was present for all the cases; however, an extensive environmental investigation, which included environmental cultures, did not reveal any specific environmental source for these microorganisms (495). Three distinct biotypes were found for the isolates: three patient isolates fit each of two of the biotype patterns, and one patient isolate fit the third biotype pattern (495). Thus, available evidence did not support a single source for these infections.

In another report, Schaal suggested that nosocomial airborne transmission, possibly in the operating room environment, was responsible for a cluster of *N. farcinica* postoperative wound infections in patients undergoing cardiac and other vascular surgeries at a university hospital (511). An environmental investigation did not identify a definite source for these microorganisms; however, outbreak isolates had a characteristic antibiogram, and microorganisms demonstrating this same antibiogram were cultured from air samples from a storeroom in the operating suite (511). Also, Yew et al. reported that during a 3-year outbreak of *M. fortuitum* and *Mycobacterium chelonae* postoperative sternal wound infections, two additional patients developed *N. asteroides* infections 2 months and 7 months postoperatively; however, no additional epidemiologic information on these patients was available (656).

In one reported pseudooutbreak at a university hospital, 18 patient blood cultures were found to be false-positive for *N. asteroides* (439). An epidemiologic investigation revealed that the source of contamination was a malfunctioning automated radiometric blood culture machine, and the epidemic strain was confirmed by DNA fingerprinting. Control measures that were instituted and shown to be effective in halting the pseudooutbreak were changing the needle sterilizer and prolonging needle sterilization time on the machine (439).

An unusual cluster of nocardiosis cases was also reported among cancer patients who had received therapy in a clinic in Bahamas. An investigation by the U.S. Centers for Disease Control and Prevention (CDC) determined that the probable cause was contamination of parenterally administered cancer medications (17). There have also been other reports of cutaneous nocardiosis in patients following intravenous heroin abuse (182, 612).

The application of newer molecular typing techniques to the laboratory evaluation of epidemiologically important *Nocardia* isolates will assist in the differentiation of epidemic and endemic or colonizing isolates. In particular, in nosocomial nocardiosis outbreaks, the application of these methods may enable the identification of potential common sources of these microorganisms and aid in the formulation of effective infection control measures (439).

(iv) AIDS. Despite the characteristic severe degree of cellular immunodeficiency found in patients infected with HIV, recent reviews have emphasized that reports of opportunistic *Nocardia* sp. infections in HIV-infected patients have been relatively rare (274, 289). A review by Kramer and Uttamchandani identified 21 HIV-infected patients at the University of Miami who were diagnosed with pulmonary nocardiosis from 1983 to 1989 (302). However, in a retrospective study at St. Luke's/Roosevelt Hospital Center in New York, Kim et al. found that only 6 of 2,167 (0.28%) AIDS patients cared for at that institution from January 1980 through March 1989 were diagnosed with nocardiosis (289). Nocardiosis has been found as a complication of AIDS in only 0.19 to 0.3% of AIDS patients reported to the CDC (263); however, this value is probably an underestimation of the frequency of the infection, since the AIDS surveillance system rarely includes follow-up data on infections that these patients develop after the diagnosis of AIDS.

Factors postulated to be responsible for the relatively low reported incidence of nocardiosis compared with those of other opportunistic infections in patients with AIDS include (i) an undefined dysfunction of the host immune defense that is unrelated to cell-mediated immune mechanisms and that may not be present in all HIV-infected patients (274, 289), (ii) underreporting bias caused by the omission of nocardiosis from the list of the indicator diseases included in the CDC's definition of AIDS (274, 289), (iii) difficulty in making the clinical diagnosis of nocardiosis (289), (iv) difficulty in identifying *Nocardia* spp. (289), and (v) the frequent use in HIV-infected patients of prophylactic and therapeutic drug regimens that also have activity against *Nocardia* spp. (108, 289).

To date, the findings of postmortem case series on AIDS patients do not support the suggestion that underreporting and/or a failure to diagnose *Nocardia* sp. infections is an important factor responsible for the apparent low incidence of these infections in HIV-infected patients (415, 519). However, the ubiquitous occurrence of these microorganisms in the environment makes it certain that HIV-infected patients are exposed to them and provides evidence that specific noncellular immune mechanisms may exist and be active against these infections (274).

In 1985, Holtz et al. described four parenteral drug abusers with AIDS complicated by actinomycete infections; three patients had *N. asteroides* infections, and one patient had lymphadenitis caused by a streptomycete (263). In all three patients who had T-helper lymphocyte counts measured, these counts were $<100/mm^3$. In all four patients, other opportunistic infections were prominent, and two patients with *N. aster-*

oides infections had pericardial involvement. These investigators also emphasized that each infection episode in HIV-infected patients should prompt a search for a specific microbiologic diagnosis so as to avoid the administration of incorrect empiric antimicrobial therapy (263). Coexistent infections with mycobacteria were found for two patients in this study (263).

Kim et al. described six cases of nocardiosis in HIV-infected patients (289) and included in their report an additional eight cases identified from a review of the English literature (5, 263, 289, 358, 362, 377, 483). The three most common sites of involvement in these patients were the lungs, brain, and pericardium (289). The most commonly identified species was *N. asteroides* (289). Four patients had coexistent mycobacterial infections (*Mycobacterium tuberculosis* [three patients] and *Mycobacterium avium-Mycobacterium intracellulare* complex [one patient]) (289).

In a report by Javaly et al., 19 HIV-infected patients with nocardiosis were documented; 17 were reported in the literature (274). Again, *N. asteroides* was the most common species identified (274). Eighty-nine percent of the patients had an advanced degree of immunodeficiency, and for 42% of the patients the nocardial infection represented their first serious opportunistic infection (274). Disseminated infection—defined as (i) the isolation of *Nocardia* spp. from a noncutaneous extrapulmonary site or (ii) the presence at the time of diagnosis of the microorganisms in the brain or at least two separate noncontiguous sites—occurred in 42 or 84% of the patients, respectively (274). An invasive procedure for obtaining clinical specimens for culturing or histopathologic examination was performed for 89% of the patients to establish the diagnosis. The early aggressive use of such a procedure for patients in whom this diagnosis is suspected has been emphasized by these and other authors (274, 289).

Reports of infections with other *Nocardia* spp. have been uncommon for HIV-infected patients and have been limited to *N. brasiliensis* infections (69, 289, 542, 543).

Of interest, there have been reports of patients with simultaneous infections with *N. asteroides* and *M. tuberculosis* (244, 341, 630). A nontuberculous mycobacterial infection has been described for up to 6% of cases of nocardiosis (183). Also, a statistically significant association has been noted between primary pulmonary nocardial disease and the subsequent development of nontuberculous pulmonary mycobacteriosis caused by *Mycobacterium kansasii* and the *M. avium-M. intracellulare-Mycobacterium scrofulaceum* complex in cardiac transplant recipients (544). Hypotheses to explain this apparent association in cardiac transplant patients have included simultaneous infection with these microorganisms, resulting in variable clinical expression; a mutational or recombinational mechanism; and induction of antigen-specific immune tolerance that is secondary to nocardial infection and that subsequently facilitates the development of mycobacterial infection (544). Although coinfections with *Mycobacterium* spp. have been identified for some of the HIV-infected patients reported in the above-mentioned studies, determination of whether these coinfections represent a true association that may indicate some interrelationship between these microbial agents, analogous to that for infected cardiac transplant recipients, will await future studies.

Despite no clearly defined optimal therapy, several reports have suggested that HIV-infected patients diagnosed as having nocardiosis may be treated successfully with a variety of antimicrobial regimens (some without sulfonamides), sometimes in combination with surgery (usually a drainage procedure) (5, 270, 280, 289, 290, 483, 542, 584). Frequently, in these patients, the cessation of specific antinocardial drug therapy may be followed by a recurrence of the infection, so that lifelong maintenance antimicrobial therapy is generally recommended. Importantly, trimethoprim-sulfamethoxazole, which is the most commonly used initial drug therapy for non-HIV-infected patients, has been found to be responsible in 50% of HIV-infected patients for adverse reactions (severe hypersensitivity reactions and prolonged myelosuppression) that are frequently of sufficient severity to cause its discontinuation (214).

Alertness on the part of clinicians to the diagnosis of nocardial infections in patients with AIDS and aggressive diagnostic approaches are important, since early diagnosis and timely initiation of drug therapy may lead to an improved outcome. It is also possible that, together with improved management of AIDS patients and modification of the natural history of the disease, the incidence of previously rare opportunistic infections, such as nocardiosis, may increase.

Emerging *Nocardia* sp. infections. The heterogeneity within the species *N. asteroides* has been well documented by numerical phenetic (196, 204, 431, 512, 596, 598), genetic (174, 402), and immunologic (365, 449, 472) studies. In these studies, various *N. asteroides* subgroups have been found, but since few strains are common to all investigations, it is difficult to determine to what extent the defined groups overlap. However, isolates of one subgroup, containing ATCC 3318, a strain of the controversial *N. farcinica* taxon, were found to be biochemically, genetically, and immunologically distinct from other isolates identified as *N. asteroides* (174, 196, 204, 365, 402, 431, 449, 472, 512, 596, 598). Because the taxon appeared to merit species status, the Judicial Commission of the International Committee on Systematic Bacteriology has agreed to retain the name *N. farcinica*, with ATCC 3318 as the type strain (330). Recently, the work of Wallace et al. showed that *N. farcinica* has a specific drug resistance pattern and confirmed the previously described concept that drug resistance patterns of *N. asteroides* may be associated with specific taxonomic groups (626). In a later study, Wallace et al. showed that another drug resistance pattern was specific for *N. nova*, another subgroup of *N. asteroides* (621). While many clinical microbiology laboratories are still adjusting to the designation of isolates previously assigned as *N. asteroides* to these two newly recognized species, the remaining group of microorganisms now classified as *N. asteroides* continues to demonstrate considerable phenotypic heterogeneity. However, until further studies with modern molecular techniques can clearly establish whether there are additional subgroups that also may differ in their epidemiology, virulence, and pathogenesis, it seems appropriate to consider that *N. asteroides* isolates that do not belong to either of these two new *Nocardia* species belong to *N. asteroides* sensu stricto (600).

Schiff et al. (517) reported a case of cutaneous *N. farcinica* infection and included another 13 reported cases in their review of these unusual infections. Reports of infected patients were from Asia, Europe, and North America. *N. farcinica* may cause a variety of clinical presentations, including cerebral abscess and pulmonary and cutaneous infections. This study found the three most common sites of infection to be the lungs, brain, and skin. Underlying immunosuppressive conditions or therapies (leukemia or lymphoma and HIV infection) were found in 36% of these patients (517). As emphasized by this study, there is a clear need to differentiate between *N. farcinica* and other members of the *N. asteroides* complex because *N. farcinica* has a high degree of resistance to various antibiotics, especially to broad-spectrum cephalosporins, which may make drug treatment of the infection difficult (517, 626). *N. farcinica*

occurs more frequently than was previously recognized (626), and mouse pathogenicity studies have demonstrated that this species may be more virulent than other members of the *N. asteroides* complex (142).

N. nova was first isolated and characterized on the basis of numerical taxonomy by Tsukamura in 1982 (600). The clinical diseases associated with these isolates were similar to previously described diseases caused by *N. farcinica* and other *N. asteroides* complex microorganisms. In a report of an *N. nova* infection in an HIV-infected patient and a review, Schiff et al. emphasized that the bases for identifying these microorganisms are their susceptibility to erythromycin and broad-spectrum cephalosporins and their resistance to amoxicillin-clavulanate (518). Also, as suggested for *N. farcinica* infections, infections with *N. nova* may be more common than is presently suspected; however, the successful detection of these newly recognized species of microorganisms is dependent upon the performance of appropriate isolation and characterization techniques (621).

N. transvalensis was first described as a cause of mycetoma in an African patient in 1927 (450). Infections with this microorganism were recently reviewed by McNeil et al. (382). Initially recognized as a cause of mycetoma, *N. transvalensis* was also recently reported to cause life-threatening invasive and disseminated infections in severely immunocompromised patients (29, 382, 384). No environmental source for *N. transvalensis* has yet been identified. However, as has been found for *N. asteroides*, soil is a probable reservoir for this unusual actinomycete. Likely modes of transmission of *N. transvalensis* are primary inoculation, which may result in mycetoma, and inhalation, which in a predisposed immunocompromised patient may result in pneumonia or disseminated infection (or both). There is no known therapy of choice for *N. transvalensis* infections. Importantly, however, clinical isolates of this unusual species may demonstrate a high level of antimicrobial resistance, and therapy with trimethoprim-sulfamethoxazole may not always be effective (382).

Isolation

Since the aerobic actinomycetes are slowly growing microorganisms, their isolation from cultures of samples obtained from normally sterile sites, such as blood or cerebrospinal fluid, typically requires a prolonged incubation time (i.e., approximately 2 weeks). Isolation from tissue samples may take even longer (i.e., up to 3 weeks). These microorganisms grow satisfactorily on most of the nonselective media used for the isolation of bacteria, mycobacteria, and fungi. Blood specimens for culturing may be inoculated into either conventional two-bottle broth blood culture systems, biphasic blood culture bottles, or automated radiometric or nonradiometric blood culture systems; each of these systems supports the growth of *Nocardia* microorganisms (478). However, satisfactory isolation of these microorganisms by these methods must still take into account factors such as maintaining the incubating cultures for up to 3 weeks and performing frequent and terminal subculturing (616). Blood specimens processed by lysis-centrifugation, exudate, joint, and cerebrospinal fluid specimens, and homogenized tissue specimens should be inoculated directly into media such as thioglycolate broth, Trypticase soy broth, or chopped-meat glucose broth. Thioglycolate broth, in addition to supporting the growth of aerobic actinomycetes, also supports the growth of anaerobic actinomycetes if these are present (510). All specimens from sterile sites may be inoculated directly onto solid media; however, the plates must be sealed in a manner that prevents dehydration.

The isolation of aerobic actinomycetes from the complex mixed flora of the soil and clinical specimens from sites that are normally nonsterile (e.g., the respiratory tract and mycetomas) requires selective enrichment before these samples can be plated on selective isolation agar. Samples from soil and nonsterile sites usually need to be incubated for 48 to 104 h (186). In 1936, Gordon and Hagan introduced paraffin baiting, a simple technique based on the ability of aerobic actinomycetes, in particular, *N. asteroides*, to use paraffin as a sole source of carbon for energy and growth (219). However, successful isolation of these microorganisms by this technique may require up to 3 weeks of incubation for both soil samples (308) and clinical specimens (395, 434, 545, 547). Furthermore, the relatively slower growth of the aerobic actinomycetes than of other contaminating bacteria and fungi still makes successfully recovering them by this method very difficult. Because of these drawbacks, modifications of the paraffin baiting method have been suggested, and other selective enrichment procedures have been developed for isolating the aerobic actinomycetes from clinical specimens from normally nonsterile clinical sites and soil samples.

Several investigators have suggested adding various chemicals to a liquid broth to render the paraffin baiting technique more selective for the isolation of aerobic actinomycetes. Kurup and Schmitt (312) used a carbon-free broth developed by McClung (378) but supplemented it with 5% sodium chloride alone and in combination with 0.5 mg of cycloheximide per ml of medium for the isolation of *N. asteroides* and related genera from the soil. El-Nakeeb and Lechevalier (155) developed one of the first selective plating agars for the aerobic actinomycetes. Their formulation replaced paraffin with arginine-glycerol salts as nitrogen and carbon sources, and they further increased its selectivity by pretreating specimens with calcium carbonate. Ajello et al. (7) modified the El-Nakeeb–Lechevalier agar by incorporating the antibiotic pimaricin (Alcon Laboratories, Fort Worth, Tex.), which improved the selectivity of the agar for the isolation of actinomycetes from soil. Orchard and Goodfellow (431) described a selective method for the isolation of *Nocardia* spp. from soil that uses serial dilutions of the soil on diagnostic sensitivity test agar (CM261; Unipath Ltd. (Oxoid Ltd.), Basingstoke, United Kingdom) supplemented with the antifungal drugs nystatin (Mycostatin; Squibb) (50 μg/ml) and cycloheximide (Acti-Dione; Upjohn) (50 μg/ml) and the antibacterial drug demethylchlortetracycline (5 μg/ml) or methacycline (10 μg/ml).

It is not unusual for human colonizing or infecting aerobic actinomycete strains to be initially detected in clinical respiratory tract specimens submitted for mycobacterial culturing. The routine incubation of mycobacterial cultures for 2 or more weeks permits the detection of the slowly growing aerobic actinomycetes. However, respiratory tract specimens submitted for the isolation of mycobacteria undergo rigorous liquefaction and decontamination steps—procedures that also eliminate all but 10 to 20% of the mycobacteria contained in the specimens (303, 304). The observation that some *N. asteroides* isolates may not survive in these specimens (267) has prompted studies of procedures to facilitate the recovery of aerobic actinomycetes from these sources. In 1987, Murray et al. demonstrated that digestion-decontamination of respiratory tract specimens with *N*-acetyl-L-cysteine, sodium hydroxide, and benzalkonium chloride was toxic for *Nocardia* spp. (408). These same investigators studied modified Thayer-Martin medium containing vancomycin, colistin, and nystatin, a selective medium commonly used in clinical laboratories for the isolation of *Neisseria* spp. from contaminated specimens, and found that the mean plating efficiency for *N. asteroides* isolates was

55%, in comparison with that on brain heart infusion agar with 5% sheep blood, a nonselective medium (408). In addition, when sputum seeded with *Nocardia* spp. was inoculated onto modified Thayer-Martin medium, the normal oropharyngeal flora was inhibited and the growth of *Nocardia* isolates was obtained. Although this medium has not been evaluated with clinical specimens, the results of this study (408) encourage further studies with this selective medium.

In 1992, Vickers et al. (618) successfully isolated *N. asteroides* from buffered charcoal-yeast extract agar (BCYE) (Remel, Lenexa, Kans.) and selective BCYE, which contained polymyxin, anisomycin, and vancomycin; these media are commonly used in clinical microbiology laboratories for the isolation of *Legionella* spp. from respiratory tract specimens. However, these investigators found that pretreatment of the specimens with a low-pH KCl-HCl solution was necessary because the direct cultures became overgrown with normal flora, even on selective BCYE (618). In 1992, Garrett et al. (180) further investigated the use of selective BCYE for the recovery of *Nocardia* spp. from sputum specimens. Although it has not been firmly established that all clinical strains of *N. asteroides* can grow on selective BCYE, these researchers found that selective BCYE supported the growth of stock cultures of *N. asteroides*.

There are several reports of studies that have evaluated different selective enrichment and plating methods for the isolation of the aerobic actinomycetes. Ajello et al. (7) found that the paraffin baiting method developed by Kurup and Schmitt (312) was superior to the arginine-glycerol salts method used by El-Nakeeb and Lechevalier (155) for the selective recovery of *N. asteroides* from soil samples. However, despite its relatively poor performance for demonstrating the presence of *N. asteroides*, the arginine-glycerol salts method was shown to have a selective advantage for the detection of other known actinomycete pathogens, such as *A. madurae* and *N. brasiliensis*, and other potential pathogens, such as *Streptomyces* spp., *Saccharothrix* (*Nocardia*) *aerocolonigenes*, and *Amycolatopsis* (*Nocardia*) *orientalis*. Paraffin baiting was found to be twice as effective as direct streaking on conventional culture media (Sabouraud's dextrose agar [SDA] and glucose nutrient agar) for the isolation of *N. asteroides* from sputum specimens from patients with chronic chest diseases (395). Singh et al. (547) isolated 67 *N. asteroides* isolates by paraffin baiting of sputum specimens from patients with bronchopulmonary diseases and 30 by direct culturing on a conventional culture medium (SDA) ($P < 0.001$). Both groups of investigators demonstrated that paraffin baiting was more efficacious than conventional methods of culturing on SDA or glucose nutrient agar. In 1990, Shawar et al. (533) used the same technique; however, they used sputum specimens seeded with stock culture isolates of *N. asteroides*, rapidly growing group IV mycobacteria, and *Streptomyces* spp. Shawar and Clarridge (532) further amended this isolation procedure by adding an indicator for β-galactosidase activity to the paraffin agar; these investigators were able to differentiate most *Nocardia* spp. and *Streptomyces* spp. (β-galactosidase positive) from group IV mycobacteria (β-galactosidase negative) and rhodococci (β-galactosidase variable).

While no optimal method for isolating these microorganisms has yet been developed, the comparative studies discussed are in agreement that these methods all represent an improvement in the power of isolation relative to plating of respiratory tract specimens directly on conventional media, such as SDA, brain heart infusion agar, blood agar, or Lowenstein-Jensen agar.

After initial isolation, subcultures must be incubated at 25, 35, and 45°C to determine the optimal temperature for growth of the microorganisms. Most isolates of *N. asteroides* grow best at 35°C, while most *Streptomyces* isolates grow best at 25°C. Any specimen suspected of containing a thermophilic actinomycete must also be incubated at 50°C, since all thermophiles grow at this elevated temperature.

A scheme that allows for the isolation of the majority of the clinically important aerobic actinomycetes and related species is shown in Fig. 1. As a reference laboratory, the CDC Actinomycete Laboratory does not ordinarily accept primary clinical or environmental specimens; however, on rare occasions, when these are received, their isolation with this scheme has been successful.

Laboratory Diagnosis

Microscopic and macroscopic characteristics. In patients with a suspected nocardial infection and a compatible clinical picture, a definitive diagnosis usually depends on the demonstration of the organisms in smears or sections examined microscopically together with isolation and identification by microbiologic culturing. The importance of direct microscopic examination of stained preparations of clinical specimens in the diagnosis of aerobic actinomycete infections cannot be overemphasized. This examination may provide a rapid and specific diagnosis of the patient's infection, and the information that it yields may critically influence the clinician's choice of initial antimicrobial therapy.

The specimens most frequently received in a clinical microbiology laboratory for evaluation include sputum, bronchial washings, exudate, or cerebrospinal fluid. If possible, the material should be spread out in a petri dish and observed for clumps of microorganisms, which may resemble granules. If clumps or granules are present, they should be selectively removed and crushed between two glass microscope slides for microscopic examination. In addition, duplicate direct smears of the clinical material should always be prepared for staining; one smear should be stained with Gram stain, and the other should be stained by the modified Kinyoun method (49).

The diagnosis of a pulmonary nocardial infection is often delayed and, for some patients, may not be made until the postmortem examination (568). On primary isolation, typical colony growth may be slow to appear. Cultures should be kept for 1 week or more before being discarded as negative (568). The recovery of *Nocardia* spp. from sputum cultures may range up to 30% of cases if the cultures are maintained for 48 h and between 15 and 50% after 140 h (434, 544). Of importance, in some cases of pulmonary nocardiosis, repeated sputum cultures may not yield nocardial isolates; invasive procedures may be needed to make the diagnosis (657). In their case series, Georghiou and Blacklock found that bronchoscopic biopsy, lung biopsy, or thoracentesis was necessary to secure a diagnosis for 44% of primary pulmonary infections and that the interval between symptom onset and isolation of the microorganism ranged up to 12 months (186). The diagnostic yield of cultures of specimens obtained by invasive procedures such as transtracheal aspiration, bronchoscopic biopsy, and fine-needle aspiration has been reported to range between 85 and 90% (434, 544). Therefore, particularly in the management of high-risk patients, clinicians must maintain a high degree of clinical suspicion for the diagnosis and, as indicated, perform invasive procedures for these patients. If necessary, clinicians should alert clinical microbiology and pathology laboratory personnel to use special methods to seek and identify microorganisms suspected of being *Nocardia* spp. (248).

The well-known acid-fast nature of *Nocardia* spp. is often more pronounced in clinical material than in cultured material.

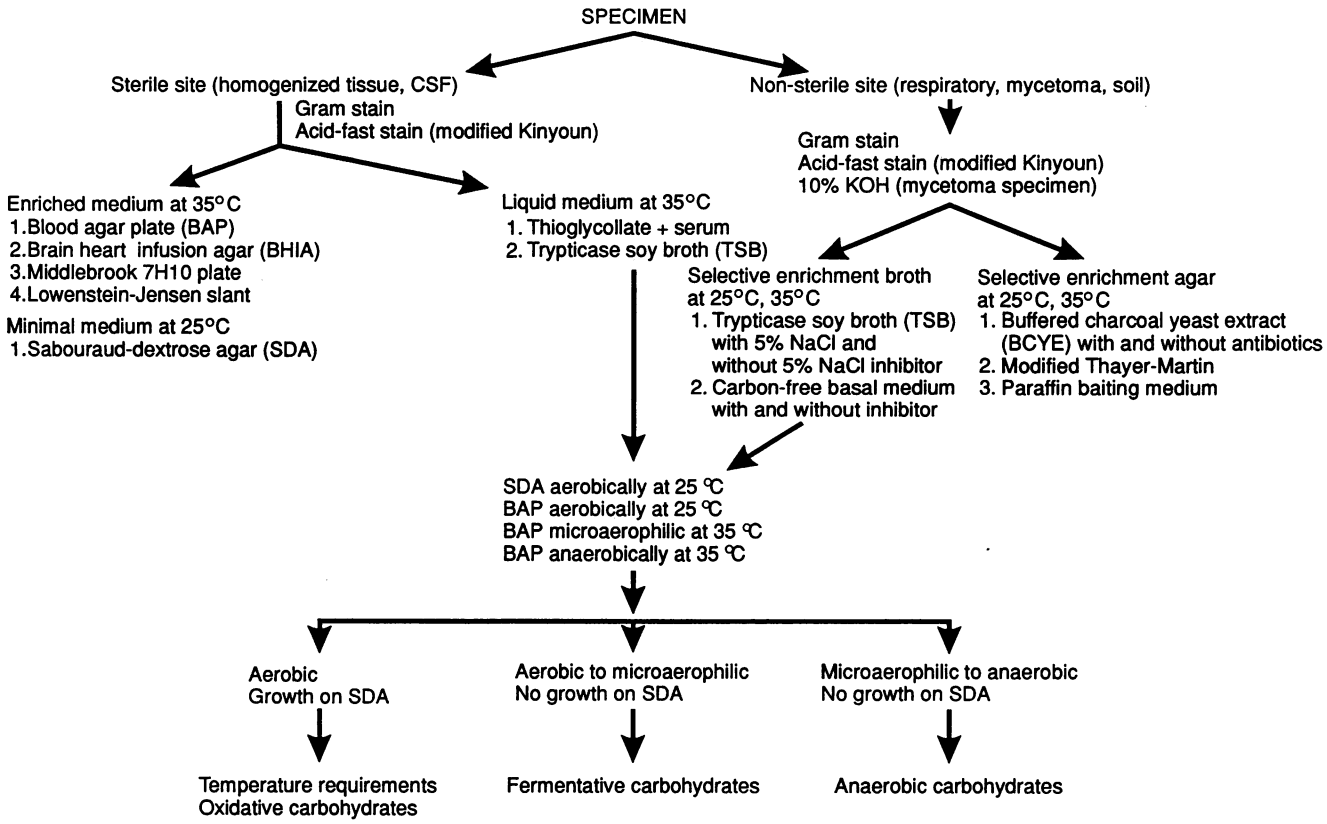

FIG. 1. Flow chart for the isolation of the actinomycetes. CSF, cerebrospinal fluid.

With the modified Kinyoun technique, *Nocardia* spp. may be found to be partially acid fast (showing both acid-fast and non-acid-fast bacilli and filaments). The presence of acid-fast branched filaments may be indicative of *Nocardia* spp.; however, in clinical material the results of acid-fast staining may be variable. Most isolates of *Nocardia* spp. in cultured material are found to be weakly acid fast by the modified Kinyoun procedure when 1% sulfuric acid is used as a decolorizing agent (49, 183). However, difficulties in the interpretation of acid-fast staining are frequently encountered. Variations in results may be dependent on the type of medium (SDA or a medium, such as Lowenstein-Jensen or Middlebrook 7H10, containing a high concentration of lipids) and the age of the culture. Of importance, isolates of *Streptomyces* spp. must be distinguished; they may show acid-fast coccoid forms and non-acid-fast hyphae but are considered non acid fast. Acid-fast staining, long considered of primary importance in the identification of *Nocardia* spp., is a difficult test to standardize, and this problem may affect its interpretation. For example, if all of an acid-fast-stained smear appears bluish pink, the stain must be repeated: there must be a contrast between the carbol fuchsin and the counterstain. Also, the demonstration of acid fastness by microorganisms grown in cultures should be used only in conjunction with other tests as a supportive test and not as an absolute diagnostic test (49). The microscopic morphology of these microorganisms in cultured material is similar to that in clinical material. They are gram positive, with short to extensively branched vegetative hyphae that are less than 1 μm in diameter and that may fragment into bacillary or coccoid, nonmotile forms. Aerial hyphae may be seen macroscopically in cultures, but in the early stages of growth they are seen only with a microscope. Short (two or three) chains of conidia may be found on the aerial hyphae but are rarely seen on the substrate hyphae.

The most satisfactory method for demonstrating the micromorphology of a nocardial culture is by direct in situ observation of a slide culture containing undisturbed colonies of the microorganism grown on a minimal medium, such as tap water agar or cornmeal agar without dextrose (471). Culture preparations on these minimal media are incubated at 25°C and examined periodically for 2 to 3 weeks. For examination of slide cultures under a microscope, it is important to recognize true branched substrate mycelium, aerial mycelium, and sporulation. The substrate hyphae of *Nocardia* spp. appear as very fine, dichotomously branched filaments. Movement of the objective up and down through several planes will reveal aerial hyphae (Fig. 2). The presence of aerial hyphae differentiates the genus *Nocardia* from other related genera, such as *Rhodococcus*, *Gordona*, *Tsukamurella*, *Corynebacterium*, and *Mycobacterium*. The rapidly growing mycobacteria, which phenotypically resemble the nocardiae, have simple, relatively short substrate hyphae that branch at acute angles; in contrast, the complex substrate hyphae of the nocardiae branch at right angles and usually have secondary branches (Fig. 3). Rhodococci grow as coccobacilli arranged in a zigzag fashion (Fig. 4). Of these microorganisms, only *Nocardia* spp. have aerial hyphae.

Macroscopic aerial hyphae may be lacking, sparse, or very abundant in *Nocardia* sp. cultures; therefore, colonies may have a smooth appearance or, more commonly, a chalky white appearance reflecting the growth of aerial hyphae. The gross morphology of the nocardiae is extremely variable and may

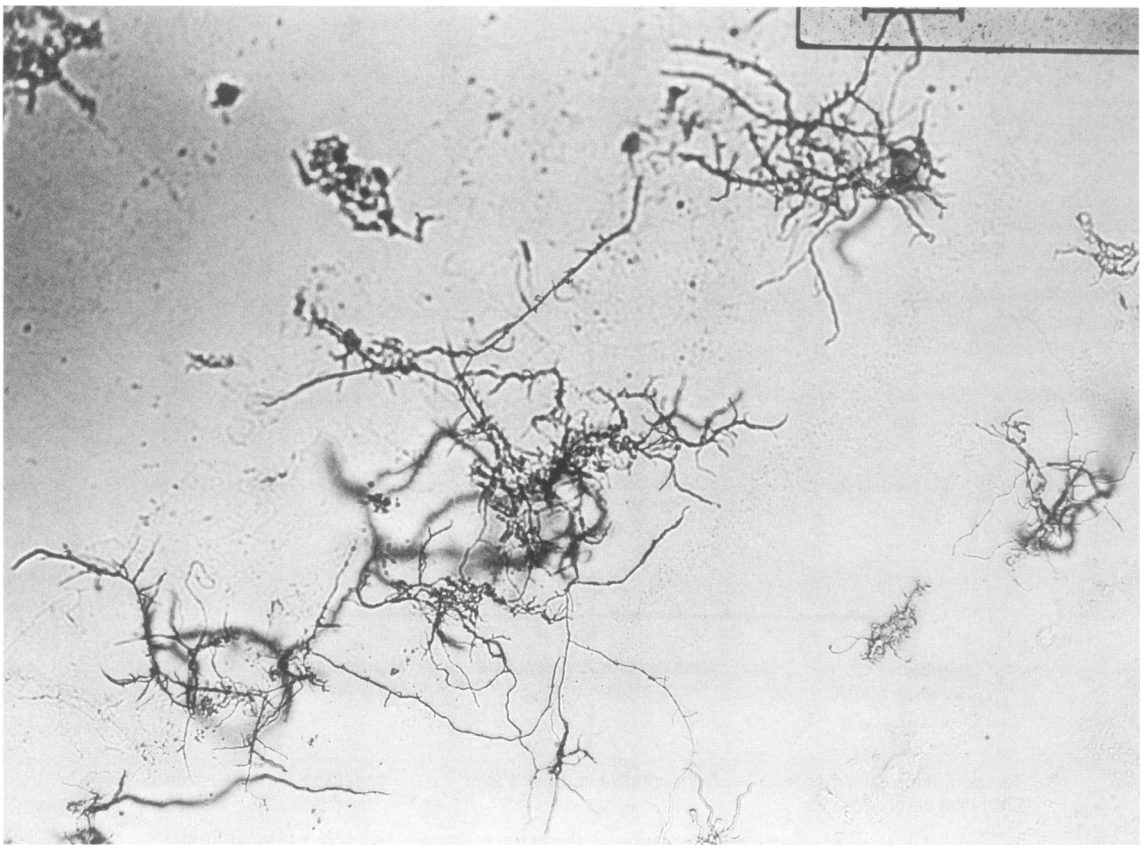

FIG. 2. *N. asteroides* substrate hyphae; aerial hyphae are out of focus. The slide culture preparation was grown on cornmeal agar without dextrose for 1 week at 35°C and was left unstained. Bar, 15 μm.

differ, depending on the medium or the incubation temperature used. The color of most colonies of the *N. asteroides* complex varies from salmon pink to orange on SDA and brain heart infusion agar slants. *N. brasiliensis* colonies are usually orange-tan; in contrast, *N. otitidiscaviarum* colonies are usually pale tan. *N. transvalensis* colonies may vary in color from pale tan to violet.

Identification. (i) Physiologic and biochemical methods. Two principal systems for testing the physiology of *Nocardia* spp. are the Gordon and Goodfellow methods. The former method, summarized by Berd (49), Gordon et al. (218), and Mishra et al. (394), has been found to be useful in differentiating the actinomycetes to the genus level on the basis of about 40 tests. However, Goodfellow, using 140 characters, found that some of the taxa recognized by Gordon, in particular, the *N. asteroides* complex, were heterogeneous (196). In 1980, Orchard and Goodfellow were able to distinguish five different clusters by using numerical taxonomic characters (431). Table 2 summarizes characteristics useful in differentiating the medically important *Nocardia* spp. (384, 394, 621, 626). Also, an identification scheme based on recent chemotaxonomic and antimicrobial resistance results is shown in Fig. 5.

Although the actinomycete nature of an aerobic isolate is often immediately obvious, particularly if an aerial mycelium is present, reliable differentiation of aerobic actinomycetes to the generic level is usually possible only when chemotaxonomic techniques are used. These include the examination of diagnostic cell wall components and testing for the presence or absence of mycolic acids, metabolism of glucose, and growth in lysozyme.

(ii) Cell wall composition. The examination of certain diagnostic cell wall components provides especially useful data. In whole-microorganism hydrolysates, the presence or absence of DAP and the differentiation of its isomers are of great diagnostic relevance. In addition, such hydrolysates may contain diagnostic sugars, which are also of diagnostic value in identification. For routine work, the simplified techniques of Becker et al. (46), Lechevalier et al. (332), Berd (49), and Staneck and Roberts (563) are recommended for the detection of diagnostic amino acids and sugars.

(iii) Mycolic acids. A qualitative evaluation of mycolic acid composition can be easily performed by thin-layer chromatography as described by Mordarska and Rethy (398) and Minnikin et al. (392) and further modified by Hecht and Causey (247), Minnikin et al. (393), and Hamid et al. (239). Mycolic acid methyl esters can be identified on thin-layer chromatograms because they are not removed when plates are subsequently washed with methanol-water (5:2 [vol/vol]) (393, 398). The presence or absence of mycolic acid methyl esters is one of the major tests used in presumptive identification to the genus level (Table 3). Mycobacterial mycolates can be recognized because they are precipitated from ether solutions by ethanol (247); mycolates from corynebacteria, nocardiae, and rhodococci remain in solution. However, Hecht and Causey (247) noted that many corynebacteria, nocardiae, and rhodococci yielded weak precipitates with this procedure. Recently, Hamid et al. described a more reliable and sensitive method for the precipitation of mycolic acid methyl esters that is based on the solubility of all mycolic acid methyl esters in acetonitrile-toluene (1:2 [vol/vol]) and the insolubility of these sub-

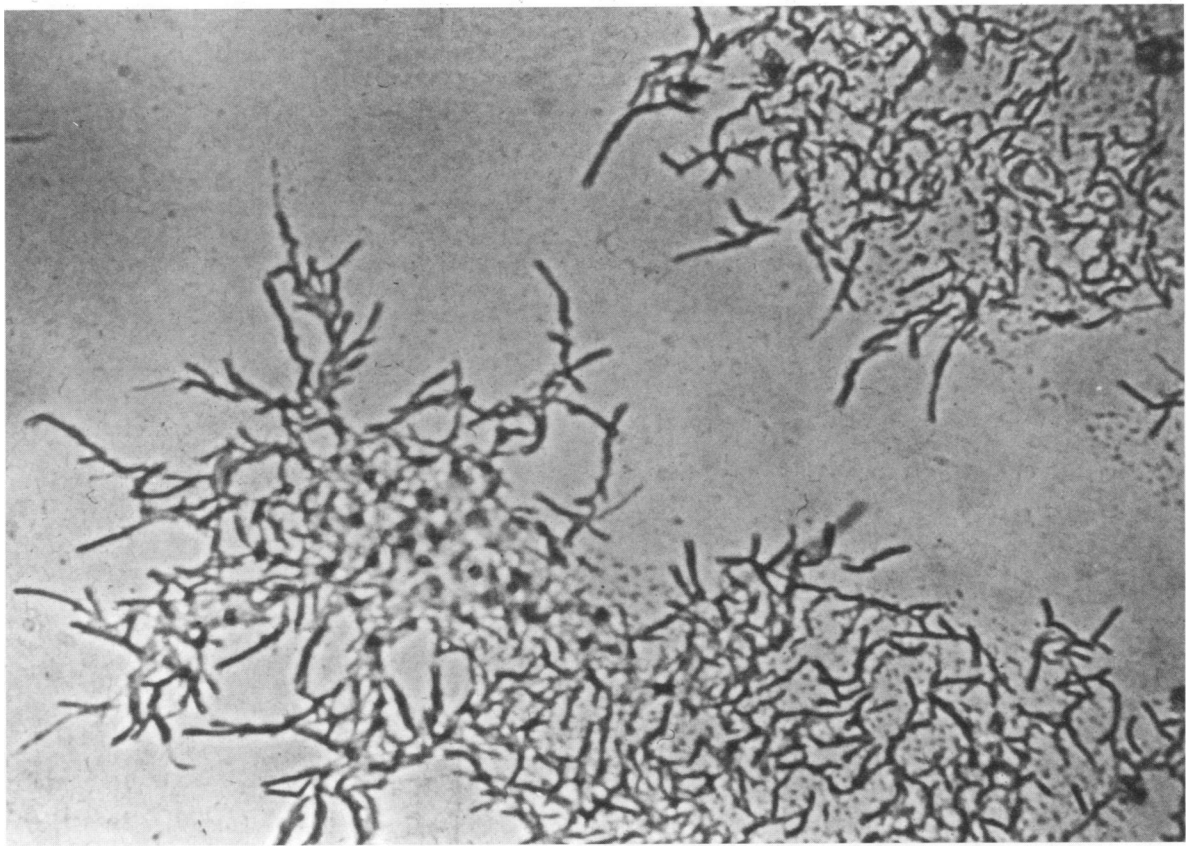

FIG. 3. *M. fortuitum*. Substrate hyphae branching at acute angles can be seen; no aerial hyphae are present. The slide culture preparation was grown on cornmeal agar without dextrose for 1 week at 25°C and was left unstained. Magnification, ×740.

stances from mycobacteria in acetonitrile-toluene (3:2 [vol/vol]) (239).

When thin-layer chromatography was performed on precipitates of mycobacteria and the remaining nonmycobacterial supernatants, the mobility of the mycolic acid spots reflected the chain length and structure of the constituent mycolic acid. Mycolate patterns with three spots were obtained for all mycobacteria, and patterns with two spots were obtained for *Tsukamurella paurometabola* strains. *Tsukamurella wratislaviensis* strains produced single spots with R_f values comparable to those of the single spots produced by gordonae and nocardiae. The corynebacteria and rhodococci also produced single spots, with slightly lower R_f values (239). Although the nonpathogenic nocardiae—*N. amarae*, *N. brevicatena*, *N. carnea*, and *Nocardia vaccinii*—have chemotaxonomic properties in common with the pathogenic species assigned to the genus *Nocardia*, the nonpathogenic species can be distinguished from the pathogenic species by use of a combination of biochemical and morphologic properties (330).

If the microorganisms do not form a macroscopic aerial mycelium readily, as is the case for some nocardiae, and if they contain *meso*-DAP and the characteristic cell wall sugars arabinose and galactose, they may be differentiated further from the related genera *Gordona*, *Rhodococcus*, and *Tsukamurella* and from the rapidly growing *Mycobacterium* spp. by quantitative mycolic acid analysis done by high-performance liquid chromatography (11, 88, 239). By combining information from this chemotaxonomic marker with other key characteristics, including arylsulfatase production and metabolism of glucose, differentiation can be made among *Rhodococcus*, *Mycobacterium*, *Corynebacterium*, *Gordona*, and *Tsukamurella* organisms (Table 4).

(iv) Fatty acids. The fatty acid profile is an additional useful chemotaxonomic characteristic and is determined by gas-liquid chromatography (118, 306, 336). This analysis includes determination of the composition and characterization of the majority of fatty acids (located in the cell membrane as glycolipid and phospholipid) and tuberculostearic acid, which is a useful marker that is not present in *Corynebacterium* spp. but is present in all the genera containing *meso*-DAP and the whole-cell sugars arabinose and galactose. The presence of tuberculostearic acid is characteristic of mycobacteria and the following genera of aerobic actinomycetes: *Nocardia*, *Gordona*, *Tsukamurella*, and *Saccharopolyspora*, as well as genera that lack mycolic acids—*Amycolata* and *Amycolatopsis*.

(v) Typing and subtyping. Typing systems are becoming increasingly important for characterizing microorganisms and have been applied in several epidemiologic investigations of disease outbreaks to identify a potential common source and determine likely mechanisms of disease transmission. Typing systems based solely on phenotypic tests have limitations because phenotypic traits of microorganisms may be inconsistently expressed. Therefore, additional stable phenotypic as well as genotypic assays are needed.

N. asteroides strains involved in human or animal infections produce one and, in some cases, two or more of four major antigens (448). These four antigens have been identified by gel diffusion and used to identify *N. asteroides* strains to the subspecies level. This technique for typing isolates was successfully applied in the investigation of an outbreak of nocardiosis

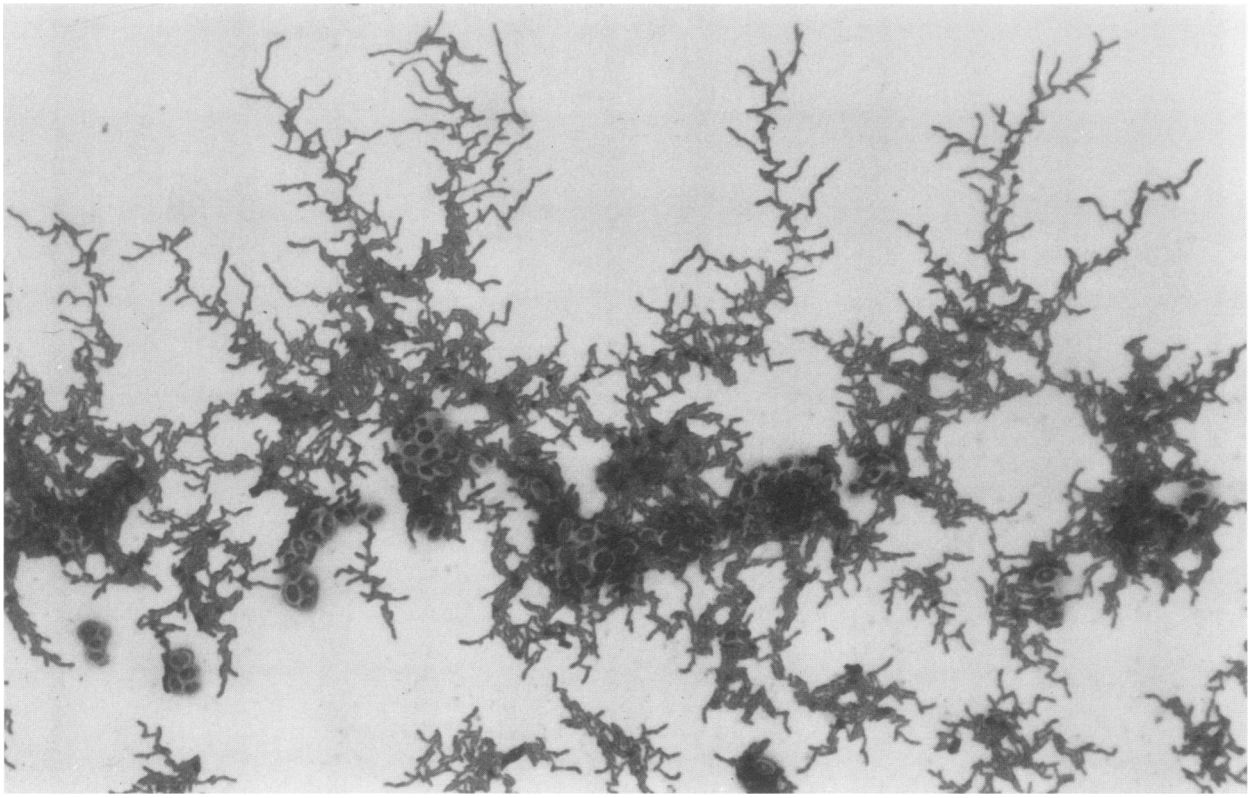

FIG. 4. *R. rhodochrous*. Coccobacilli are arranged in a zigzag fashion. The slide culture preparation was grown on cornmeal agar without dextrose for 1 week at 25°C and was left unstained. Magnification, ×740.

in immunocompromised patients in a renal transplant unit in the United Kingdom in 1981 (569). Isolates from these patients and the environment were shown to have a characteristic type III antigenic pattern. This pattern was one of the least common serotypes identified in two separate panels of isolates from the United States (15%) and the United Kingdom (25%). Although all the epidemic isolates also had identical antimicrobial susceptibility test results, this same antibiogram was found for all but one of the control isolates (569).

In 1986, Jonsson et al. (278) reported a case study of nocardiosis in an adult patient with chronic granulomatous disease. They studied the plasmid profiles of two *N. asteroides* isolates that had been obtained 2.5 years apart from the patient (278). These two isolates had identical plasmid profiles. However, the potential usefulness of plasmid analysis of *Nocardia* spp. as an epidemiologic tool is presently unclear, and only by a thorough evaluation of a significant number of epidemiologically related isolates will this usefulness be definitively established.

In 1988, Morace et al. (397) described a biotyping method that used 44 yeast strains belonging to the genera *Pichia*, *Candida*, *Saccharomyces*, and *Kluyveromyces* to test for their potential killer effect on 13 aerobic actinomycetes (*N. asteroides* [6 isolates], *N. brasiliensis* [1 isolate], *N. otitidiscaviarum* [1 isolate], and *A. madurae* [5 isolates]). They found that when nine killer yeast strains, grouped into triplets, were used to study eight *Nocardia* test isolates, each isolate had a different biotype pattern. These results suggested that the killer system may have promise as a highly discriminatory epidemiologic tool. However, this method also has yet to be evaluated with a significant number of epidemiologically related *N. asteroides* isolates. Until such a study is performed, the usefulness of this method will not be clear.

Restriction fragment length polymorphism analysis was successfully applied in the setting of one *N. asteroides* nosocomial pseudooutbreak (439). An epidemiologic investigation suggested that the increased rate of isolation of *N. asteroides* in the hospital represented a pseudoepidemic. As part of this investigation, a laboratory evaluation showed that 18 of 19 clinical isolates shared an identical restriction fragment length polymorphism profile when studied with the restriction enzyme *Pvu*II. This report suggests that a comparison of isolate restriction fragment length polymorphism profiles may prove to be an extremely useful technique for typing *N. asteroides* isolates in future epidemiologic studies.

Histopathology

In Gram-stained histologic sections, *Nocardia* sp. infections typically show evidence of an intense pyogenic tissue reaction in the form of small to massive, usually suppurative abscesses containing gram-positive branched filamentous hyphae (Fig. 6). The appearance of nocardiae is similar to that seen in cultures: they measure from 0.5 to 1 μm in diameter and as much as 20 μm in length. To be of diagnostic value, the branched hyphae must be at right angles. Although the hyphae of nocardiae may resemble those of *Actinomyces israelii* in width, they are usually much longer and more widely scattered throughout purulent material and the walls of the abscesses.

In mycetomas, compact granules that are similar to those observed for the anaerobic actinomycetes are formed (Fig. 7). Very rarely, clubbing is seen with *N. asteroides* (378), but most

TABLE 2. Characteristics differentiating the medically important *Nocardia* spp.[a]

Characteristic	Result for:					
	N. asteroides complex[b] (*n* = 137)	*N. farcinica*[c] (*n* = 40)	*N. nova*[d] (*n* = 20)	*N. transvalensis*[e] (*n* = 15)	*N. brasiliensis*[b] (*n* = 72)	*N. otitidiscaviarum*[b] (*n* = 43)
Decomposition of:						
Adenine	−	−	−	V	−	−
Casein	−	−	−	V	+	−
Hypoxanthine	−	−	−	V	V	+
Tyrosine	−	−	−	V	+	+
Xanthine	−	−	−	V	−	−
Growth on Trypticase soy agar (3 days) at:						
35°C	+	+	+	+	+	+
45°C	V	+	−	−	−	V
Growth in lysozyme (7 days)	+	+	+	+	+	+
Arylsulfatase production (14 days)	−	−	V	−	−	−
Acid production from:						
Adonitol	−	−	NT	V	−	−
Arabinose	−	V	NT	−	−	V
Cellobiose	−	NT	NT	−	−	−
Dulcitol	NT	NT	NT	−	NT	NT
Erythritol	−	NT	NT	V	−	−
Fructose	NT	NT	V	V	NT	NT
Galactose	NT	V	−	V	NT	NT
Glucose	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
Inositol	−	−	−	V	+	+
Lactose	−	NT	−	−	−	−
Maltose	−	NT	NT	−	−	−
Mannitol	−	−	−	V	+	V
Mannose	V	V	V	−	V	V
Melibiose	−	NT	NT	−	−	−
Raffinose	−	NT	NT	−	−	−
Rhamnose	V	V	−	−	−	−
Salicin	NT	NT	−	−	NT	NT
Sorbitol	−	−	−	V	−	−
Starch	NT	NT	NT	−	NT	NT
Sucrose	NT	NT	−	−	NT	NT
Trehalose	V	−	V	V	+	V
Xylose	−	NT	−	V	−	−

[a] Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; V, 11 to 89% of the strains are positive; NT, not tested.
[b] Data are from Mishra et al. (394).
[c] Data are from Wallace et al. (626).
[d] Data are from Wallace et al. (621).
[e] Data are from McNeil et al. (382).

often granules with clubbing are seen with *N. brasiliensis* and *N. otitidiscaviarum* (550). The hematoxylin and eosin stain is very useful for staining the tissue reaction and the granules but does not stain the individual filaments (105). A tissue Gram stain such as the Brown-Brenn procedure is recommended for demonstrating the gram-positive filaments of nocardiae. The Gomori methenamine-silver (GMS) stain may also be useful. However, with both of these procedures, the filaments may not stain uniformly. Acid-fast stains are also of value in the histopathologic diagnosis of infections caused by *N. asteroides*, *N. brasiliensis*, and *N. otitidiscaviarum*. These species are frequently, but not always, acid fast in tissue sections stained by either the modified Kinyoun method or the Fite-Faraco method (105). *Actinomyces* and related species are usually not acid fast.

Diagnostic Immunology

Because of the delay in the isolation and identification of *Nocardia* spp. by culturing, the use of other, more rapid, nonculture methods for diagnosis is desirable. However, serologic methods and skin testing with antigens derived from *Nocardia* spp. have remained experimental, and their potential clinical value is uncertain. Pier and Fichtner have pioneered work in this area with the preparation of an extracellular glycoprotein culture filtrate antigen for detecting precipitins and complement fixation antibodies and for skin testing (448). With this antigen and an immunodiffusion precipitin test, four major immunotypes were recognized within *N. asteroides*. Although the earlier study done by Pier and Fichtner described immunologic reactions in infected animals with nocardiosis, in 1978 a modification of their complement fixation test was reported to be useful with human clinical specimens (529). In this study, 13 of 16 patients with documented nocardiosis produced complement-fixing antibody to the previously described Neopeptone (Difco Laboratories, Detroit, Mich.)-derived extracellular antigen. However, cross-reactivity with sera from patients with leprosy (three of three patients) and tuberculosis (two of five patients) was still evident. Of interest,

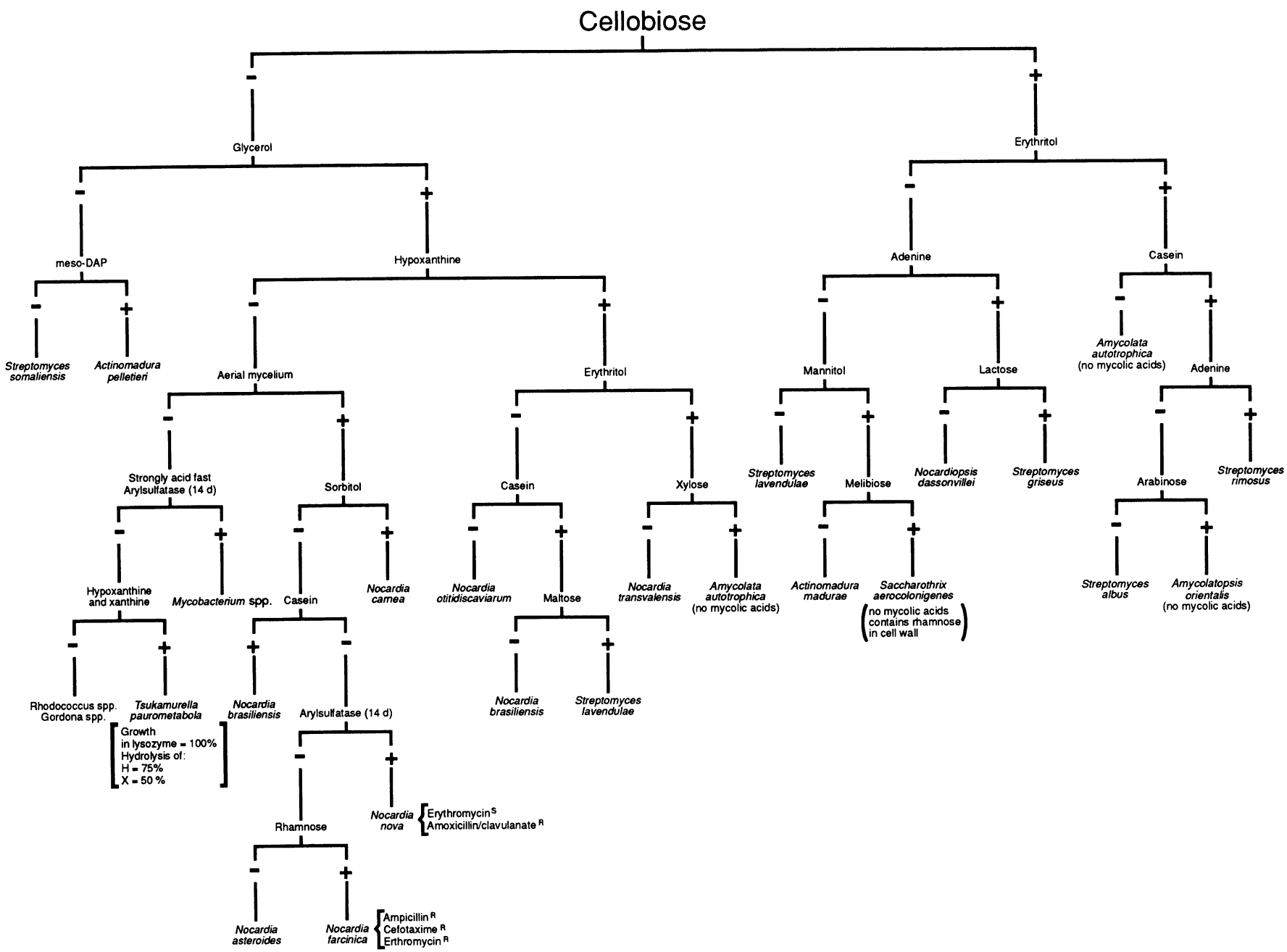
Cellobiose
Glycerol
Erythritol
meso-DAP
Hypoxanthine
Adenine
Casein
Streptomyces somaliensis
Actinomadura pelletieri
Aerial mycelium
Erythritol
Mannitol
Lactose
Amycolata autotrophica (no mycolic acids)
Adenine
Strongly acid fast Arylsulfatase (14 d)
Sorbitol
Casein
Xylose
Streptomyces lavendulae
Melibiose
Nocardiopsis dassonvillei
Streptomyces griseus
Arabinose
Streptomyces rimosus
Hypoxanthine and xanthine
Mycobacterium spp.
Casein
Nocardia carnea
Nocardia otitidiscaviarum
Maltose
Nocardia transvalensis
Amycolata autotrophica (no mycolic acids)
Actinomadura madurae
Saccharothrix aerocolonigenes
(no mycolic acids contains rhamnose in cell wall)
Streptomyces albus
Amycolatopsis orientalis (no mycolic acids)
Rhodococcus spp.
Gordona spp.
Tsukamurella paurometabola
Growth in lysozyme = 100%
Hydrolysis of:
H = 75%
X = 50 %
Nocardia brasiliensis
Arylsulfatase (14 d)
Nocardia brasiliensis
Streptomyces lavendulae
Rhamnose
Nocardia nova
Erythromycin S
Amoxicillin/clavulanate R
Nocardia asteroides
Nocardia farcinica
Ampicillin R
Cefotaxime R
Erthromycin R

TABLE 3. Tests used for the presumptive identification of the aerobic actinomycetes to the genus level[a]

Genus	Vegetative mycelium		Conidia	Growth in lysozyme	Metabolism of glucose	Presence[b] in whole cells of:		Motility	Mycolic acids	Acid fast nature
	Aerial	Substrate				DAP	Sugars			
Nocardia	+	+	V	+	O	*meso*	Arab, gal	−	+	w
Actinomadura	V	+	+	−	O	*meso*	Mad	−	−	−
Dermatophilus	−	+	−	NT	F	*meso*	Mad	+	−	−
Gordona	−	+	−	−	O	*meso*	Arab, gal	−	+	w
Rhodococcus	−	+	−	V	O	*meso*	Arab, gal	−	+	w
Amycolata	+	+	V	−	O	*meso*	Arab, gal	−	−	−
Amycolatopsis	+	+	V	V	O	*meso*	Arab, gal	−	−	−
Micromonospora	−	+	+	−	O	*meso*	Arab, xyl	−	−	−
Nocardiopsis	+	+	+	−	O	*meso*	None or gal	−	−	−
Oerskovia	−	+	−	−	F	None	Gal	+	−	−
Rothia	−	+	−	NT	F	None	Gal	−	−	−
Streptomyces	+	+	+	−	O	L	None	−	−	−
Tsukamurella	−	+	−	+	O	*meso*	Arab, gal	−	+	w

[a] Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; V, 11 to 89% of the strains are positive; NT, not tested; O, oxidative; F, fermentative; arab, arabinose; gal, galactose; mad, madurose; xyl, xylose; w, weakly or partially acid fast.
[b] As determined by the methodology of Lechevalier et al. (333) for whole-cell DAP and sugars.

the ability of the four immunotypes to identify *N. asteroides* to the subspecies level has been reported to be useful as part of an epidemiologic evaluation of an outbreak of nocardiosis in a renal transplant unit (569).

In 1979, Blumer and Kaufman (60) reported the development of a microimmunodiffusion test based on the culture filtrate antigen developed by Pier and Fichtner (448) and on disrupted whole-cell homogenates. The culture filtrate antigen proved to be superior to the whole-cell homogenate antigen by detecting 70 versus 49% of cases, respectively. However, the culture filtrate antigen also showed more cross-reactions in diseases, including tuberculosis, actinomycosis, histoplasmosis, and coccidioidomycosis.

More recently, various studies identified a major genus-specific extracellular antigen that may have promise in an immunodiagnostic test (14, 15, 63, 571). A 55,000-molecular-weight extracellular protein has been purified by Sugar et al. (571). This antigen forms the basis for a specific immunodiagnostic test for humans who have active disease caused by *N. asteroides*, *N. brasiliensis*, or *N. otitidiscaviarum*. Ninety-one percent of the patients with nocardiosis, including immunocompromised patients and patients with localized cutaneous disease, had positive antibody titers to this antigen (571). Currently, the enzyme immunoassay seems to be the most sensitive and specific serologic method under investigation.

In 1986, El-Zaatari and coworkers (156) separated *N. asteroides* culture filtrate antigens by electrofocusing and used these antigens to generate specific monoclonal antibodies. However, all but two of these cross-reacted with mycobacterial antigens. In 1990, Jimenez et al. (275) used whole-cell extracts of *N. asteroides* and *N. brasiliensis* to produce a library of monoclonal antibodies. Although they exhibited different degrees of cross-reactivity with *N. asteroides* and *N. brasiliensis*, these antibodies also exhibited cross-reactivity with culture filtrate antigens from mycobacteria.

In 1990, Boiron and Provost (63) reported the use of a 54-kDa immunodominant antigen for the diagnosis of nocardiosis. Boiron et al. took advantage of the cross-reactivity between nocardiae and mycobacteria to partially purify the 54-kDa *Nocardia*-specific antigen by immunoaffinity chromatography (67). For this study, an immunoaffinity column prepared with immunoglobulin G from the sera of patients with leprosy was used to purify the 54-kDa immunodominant antigen from culture filtrates of *N. asteroides* by removing antigens that were cross-reactive with mycobacteria. Using this purified 54-kDa antigen, these investigators generated monoclonal antibodies. The specificity of one monoclonal antibody was confirmed by demonstration in a dot immunobinding assay that the monoclonal antibody did not react with circulating antigens in sera from patients with *M. tuberculosis* infections and leprosy and uninfected persons. The dot immunobinding assay showed that a minute amount of the 54-kDa circulating antigen from nocardiosis patients can be detected in sera with the monoclonal antibody (67). This monoclonal antibody may provide the sensitivity and specificity needed for the development of a reliable diagnostic test for nocardiosis.

A rapid and reliable serodiagnostic test for nocardial infections in patients would provide a basis for instituting earlier chemotherapy and perhaps would represent a potentially useful method for monitoring the therapeutic efficacy of specific antimicrobial therapies in these patients. Both attributes might be expected to improve the prognosis for these patients.

Brownell and Belcher (84) have isolated a potential species-specific DNA probe for the rapid identification of *N. asteroides* isolates. Initially, the clones tested showed cross-reactivity with isolates of *N. brasiliensis* and *N. otitidiscaviarum* but were nonreactive with isolates of the related genera *Actinomadura*, *Mycobacterium*, and *Rhodococcus*. However, on additional testing of 50 of the recombinant clones, 2 that hybridized to 31% of *N. asteroides* isolates without cross-hybridizing to isolates of related species were identified. Additional libraries were then generated from five selected isolates of *N. asteroides* that had failed to hybridize to the initial clones. Four of five of these clones, when pooled, provided DNA probes specific for all of the *N. asteroides* isolates tested. However, further evaluation of the usefulness of these probes should be undertaken and should include well-characterized *Nocardia* strains.

FIG. 5. Flow chart for the tentative identification of aerobic actinomycetes and related genera. Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; H, hypoxanthine; X, xanthine; R, resistant; S, susceptible; d, days.

TABLE 4. Characteristics differentiating the genus *Nocardia* from related genera[a]

Genus[b]	Macroscopic aerial mycelium	Arylsulfatase production in 14 days	Metabolism of glucose	Mycolic acids (no. of carbons)[c]
Nocardia	+	V[d]	O	44–60
Corynebacterium	−	NT	F[e]	22–38
Mycobacterium	−	+	O	60–90
Rhodococcus	−	−	O	34–52
Gordona	−	−	O	48–66
Tsukamurella	−	−	O	64–78

[a] Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; V, 11 to 89% of the strains are positive; NT, not tested; O, oxidative; F, fermentative.

[b] All of the genera are cell wall type IV (contain arabinose and galactose as characteristic sugars).

[c] As detected by high-performance liquid chromatography (11, 88, 239).

[d] 75% of *N. nova* strains are positive, and 5% of *N. asteroides* strains are positive.

[e] "*Corynebacterium aquaticum*" oxidizes glucose.

All biotypes of *N. asteroides* sensu stricto should be tested, as should strains of the recently reclassified species, *N. farcinica* and *N. nova*.

Antimicrobial Susceptibility Testing and Therapy

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing of *Nocardia* spp. remains problematic. A major difficulty is the typically slow-growing nature of the aerobic actinomycetes. This slow growth allows time for spontaneous drug degradation, which may invalidate the results of attempted antimicrobial susceptibility studies, to occur. Thus, an essential requirement for a prospective method for susceptibility testing is that it facilitate the rapid growth of these microorganisms on test media. Many different antimicrobial susceptibility testing methods have been tried and found to be successful, but none has been accepted as the standard method. The National Committee for Clinical Laboratory Standards has recently organized a working group to evaluate and standardize antimicrobial susceptibility testing methods for the aerobic actinomycetes (506). When a standard method has been recommended, inter- and intralaboratory reproducibilities can be evaluated and the role of antimicrobial susceptibility profiles as predictors of clinical outcome can be better studied (506).

The most commonly used testing methods have been the modified disk diffusion method, the agar dilution method, and the broth microdilution method (62, 66, 96, 383, 622, 623, 653). The success of each of these testing methods is dependent upon the achievement of a satisfactory initial suspension of microorganisms, from which a standard inoculum (0.5 McFarland barium sulfate standard) is made. Two different techniques may be used to obtain a uniform microbial suspension. First, agitation (150 to 250 rpm) in a reciprocal shaker of a broth culture containing a few glass beads (3 to 5 mm in diameter) and supplemented with 0.05% Tween 80 (Difco Laboratories, Detroit, Mich.) is carried out, and then the culture is incubated at an optimal temperature (30 to 35°C) for 24 to 48 h until the desired homogeneous suspension of microorganisms, equal to a 0.5 McFarland barium sulfate

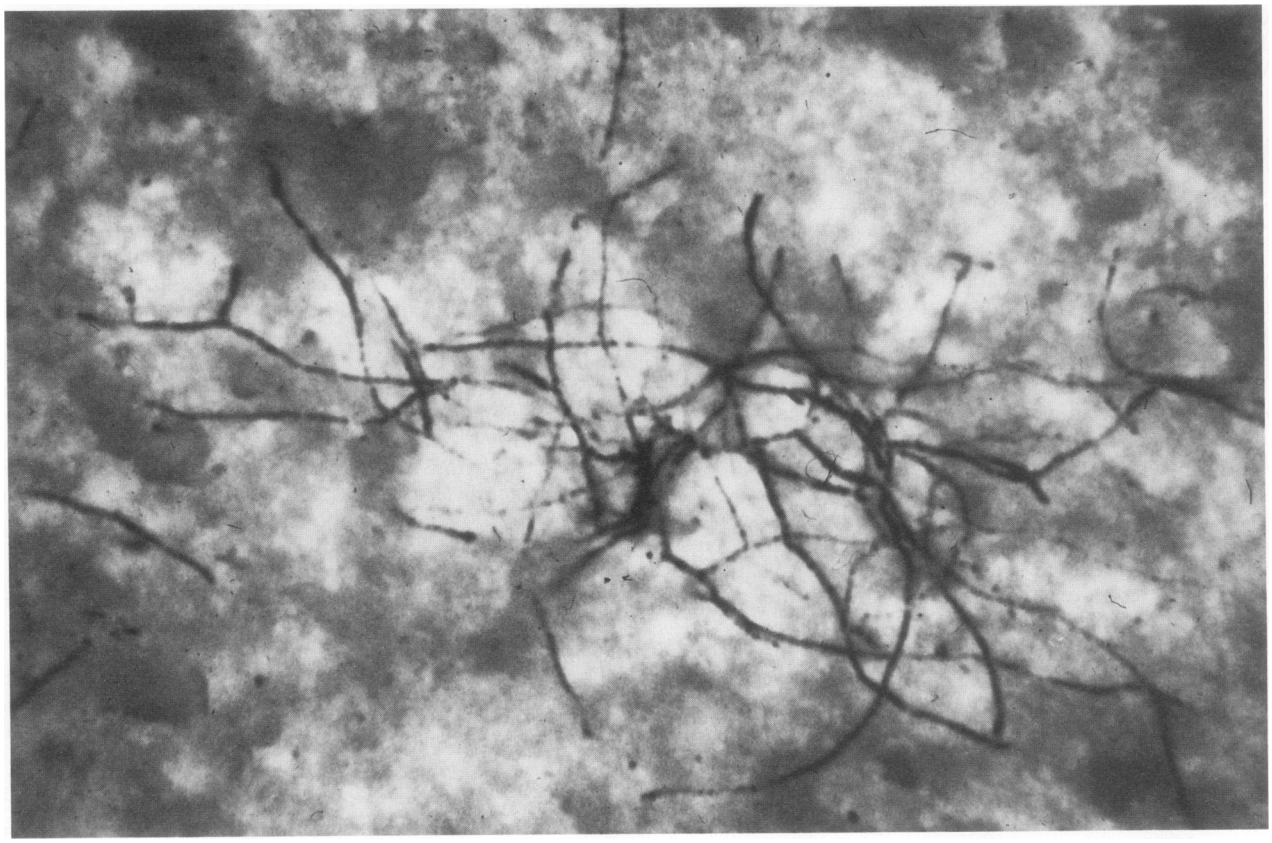

FIG. 6. *N. asteroides* branched hyphae in a section of human lung tissue. Brown-Brenn Gram stain. Magnification, ×840.

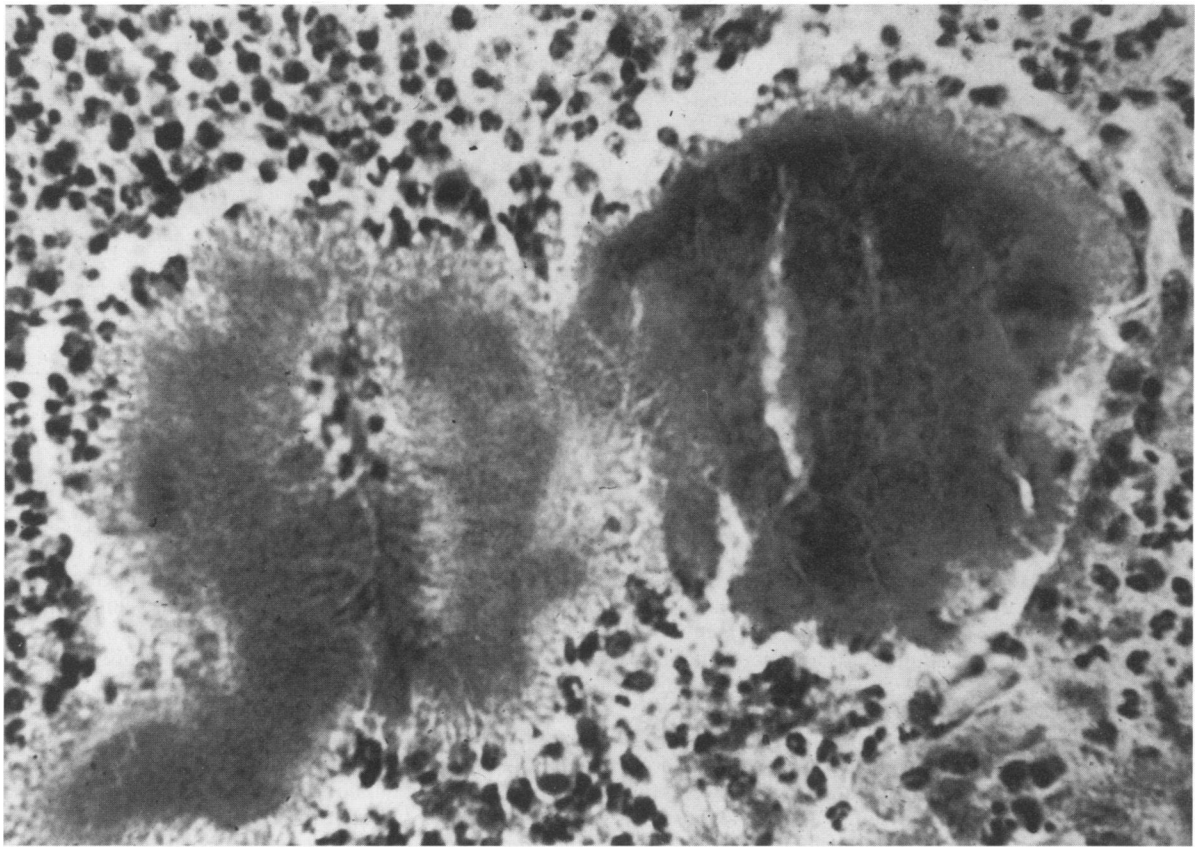

FIG. 7. *N. brasiliensis*. Scattered, fragmented microorganisms in granules in a section of a human mycetoma can be seen. Brown-Brenn Gram stain. Magnification, ×740.

standard, is obtained (411). Alternatively, a suspension of a growing colony from an agar plate is made in sterile water and agitated with sterile glass beads until a fine suspension of microorganisms that can be adjusted to the turbidity of a 0.5 McFarland standard is obtained.

The disk diffusion method currently recommended by both the U.S. Food and Drug Administration (161) and the National Committee for Clinical Laboratory Standards (411) is a slight modification of that described by Bauer et al. (40). In 1977, Wallace et al. were the first to report success using the disk diffusion method for antimicrobial susceptibility testing of *Nocardia* spp. (622). In 1988, Boiron and Provost used the disk susceptibility method to test 28 *Nocardia* clinical isolates against 16 antimicrobial agents (62). In 1992, Boiron et al. reported the results of another study that used this method to test 55 *N. asteroides* isolates; the results were read after 24 h of incubation at 37°C, as recommended by the Antibiogram Committee of the French Society for Microbiology (4, 66). All susceptibility tests were performed on Mueller-Hinton agar, except that for the sulfonamide disk susceptibility tests, the agar was supplemented with 5% hemolyzed horse blood containing thymidine phosphorylase. Although Boiron and Provost did not report any degree of inhibition with sulfonamide disks, the microorganisms may grow through several generations before inhibition occurs. In this situation, slight growth (80% inhibition) is disregarded, and the margin of heavy growth is measured. Sulfonamides and trimethoprim-sulfamethoxazole are the standard antimicrobial agents used for the therapy of patients with nocardial infections, and discrepancies have been noted between the results obtained by Boiron and Provost and those described in other published reports (383, 626). Recently, it was suggested that these discrepant results may be due to the use of different methods for antimicrobial susceptibility testing (McNeil et al. used a broth microdilution technique [383]). Alternatively, these discrepant results may reflect differences in the geographic origins of the particular isolates tested (42).

The agar dilution method of antimicrobial susceptibility testing has several advantages over the broth microdilution method, including its convenience for testing multiple isolates, its ability to detect microbial heterogeneity and contamination, and its slightly better reproducibility. Preparation of the inoculum is the same as for the disk diffusion and broth microdilution methods. The agar surfaces of the plates, containing twofold dilutions of the antimicrobial agents, and the control plate, containing no antimicrobial agent, are spot inoculated (without spreading) with a loop calibrated to deliver 0.001 to 0.002 ml (1 to 2 μl) or are inoculated with a replicating apparatus, such as that described by Steers (566). In each case, about 10^4 CFU is delivered to a spot 5 to 8 mm in diameter. The agar dilution antimicrobial susceptibility testing method has not been widely accepted because it necessitates the labor-intensive preparation and proper storage of prepared dilution plates. Both steps may be difficult to accomplish in a busy clinical microbiology laboratory. However, this method is ideal when a minimal number of antimicrobial agents is to be tested against multiple different test strains (96, 623, 653).

Recently, broth microdilution antimicrobial susceptibility

TABLE 5. Susceptibility test results for clinical isolates of the medically important *Nocardia* spp. and related genera

Antimicrobial agent(s)	% Resistant strains of:								
	N. asteroides[a] (*n* = 16)	*N. asteroides*[a] (*n* = 27)	*N. brasiliensis*[b] (*n* = 23)	*N. farcinica*[a] (*n* = 13)	*N. nova*[a] (*n* = 14)	*N. otitidiscaviarum*[c] (*n* = 6)	*N. transvalensis*[d] (*n* = 11)	*A. madurae*[b] (*n* = 42)	*S. griseus*[b] (*n* = 28)
Sulfamethoxazole	0	0	0	0	0	100	10	16	43
Erythromycin	100	100	0	100	0	100	50	7	14
Minocycline	0	33	25	10	0	0	46	10	10
Ciprofloxacin	87	100	70	0	100	NT[e]	40	3	57
Amikacin	0	0	0	0	0	0	18	0	0
Ampicillin	0	100	86	100	0	100	90	63	80
Cefotaxime	0	0	25	100	0	83	50	10	52
Imipenem	25	0	70	18	0	NT	10	0	19
Amoxicillin-clavulanate	NT	NT	35	53.3	97	100	70	19	43

[a] Data are from Wallace and Steele (624).
[b] Data are from McNeil et al. (383).
[c] Data are from Boiron and Provost (62).
[d] Data are from McNeil et al. (382).
[e] NT, not tested.

testing methods have become available. These are especially suited for clinical microbiology laboratories that have the capability to prepare their own reagents. Two factors in support of the routine use of these methods are their reliability and their economic feasibility. In 1975, commercially prepared microdilution trays became available; these are supplied and stored frozen. Several lyophilized systems have also been marketed; they differ only minimally in tray design, antimicrobial agents tested, and the type of inoculum and inoculation methods used. These commercial systems appear to provide very accurate and reproducible results when used in the manner specified by the manufacturers (125). Recently, the CDC Actinomycete Laboratory received several isolates of *N. asteroides* complex that did not grow in Mueller-Hinton broth. In a commercial lyophilized system (e.g., Sceptor; Becton Dickinson, Towson, Md.), different basal media may be readily substituted. However, such a substitution may invalidate results interpreted on the basis of the criteria promulgated by the National Committee for Clinical Laboratory Standards for use with Mueller-Hinton broth (411).

Antibiograms. Several studies have demonstrated different antimicrobial susceptibility patterns among isolates of the three major human pathogenic *Nocardia* species. This finding has been suggested to have taxonomic importance (62, 64, 204, 388, 621, 625–627). These studies have all shown relative uniformity in the results of antimicrobial susceptibility testing of *N. brasiliensis* and *N. otitidiscaviarum* isolates. However, considerable intraspecies variability has been demonstrated for the *N. asteroides* complex (627). It has been suggested that this variability may be due in part to the heterogeneity of this group because of the use of unreliable classical identification criteria.

In 1988, Wallace and Steele reported six different antimicrobial drug patterns for 78 clinical isolates of *N. asteroides* complex tested against 12 antimicrobial agents, including beta-lactams, aminoglycosides, ciprofloxacin, erythromycin, minocycline, and sulfamethoxazole (624). Four of these antimicrobial drug patterns predominated: together they accounted for 90% of the isolates tested in the study (624). Of interest, isolates of the two newly described taxa, *N. farcinica* and *N. nova*, were shown to be identical to two predominant groups of clinical isolates that were identified on the basis of their different susceptibility patterns in the 1988 study by Wallace et al. (625). Recently, the species status of these two microorganisms was also confirmed by mycolic acid patterns and DNA relatedness studies (651).

In 1990, Wallace et al. reported the typical antimicrobial resistance pattern of *N. farcinica* and correlated this pattern with the results of other phenotypic tests (626). In another study, Wallace et al. characterized the antimicrobial susceptibility pattern of *N. nova* and described some simple biochemical tests that separated this species from *N. farcinica* and other members of the *N. asteroides* complex (621). Antimicrobial susceptibility patterns found for *N. farcinica*, *N. nova*, *N. asteroides* (the two major sensu stricto patterns), *N. otitidiscaviarum* (62), *N. brasiliensis* (383), *N. transvalensis* (382), *A. madurae* (383), and *Streptomyces griseus* (383) are shown in Table 5. For ease of comparison, antimicrobial susceptibility test results—reported as the number of susceptible strains by Wallace et al. (broth microdilution method) (625) and Boiron and Provost (disk diffusion method) (62)—have been expressed here in terms of the percentage of resistant strains. Antimicrobial susceptibility test results may be taxonomically useful for *N. farcinica* and *N. nova*. The biochemical reactions associated with the *N. asteroides* complex, *N. farcinica*, *N. nova*, and the other medically important *Nocardia* spp. are given in Table 2.

Interpretative antimicrobial susceptibility test criteria are based on the assumption that MICs obtained for a particular antimicrobial agent, when within the range of achievable levels of that particular antimicrobial agent in serum, are evidence enough that therapy with the drug will engender a clinical response. However, reliable data correlating this interpretation with an in vivo clinical outcome are lacking. In addition, in the management of infected patients, complications may arise because of patient intolerance of the chosen drugs and the occurrence of resistant strains. Therefore, clinical laboratories need to become proficient in the isolation, identification, and susceptibility testing of these microorganisms.

Antimicrobial therapy. Antimicrobial therapy of nocardiosis remains problematic. Empiric recommendations only are available for the treatment of infected patients, since specific antimicrobial agents for the treatment of nocardial infections have not been evaluated in controlled clinical trials (166). For some patients, in addition to effective antimicrobial treatment, surgical intervention may also be indicated and may contribute to a favorable outcome. The clinical course for some patients on drug therapy for a primary infection may be complicated by treatment failure, which may be manifested as metastatic spread or late relapse. Byrne et al. reported that relapses occurred in patients with cerebral nocardiosis up to 1 year or

more following the cessation of antimicrobial therapy that was considered adequate for cure (90).

Sulfonamides (with or without trimethoprim) have been the mainstay of antimicrobial therapy for human nocardiosis (166, 552, 623). Trimethoprim-sulfamethoxazole has become a frequently used drug combination for this infection; however, this usage may not be as much related to properties of synergism or improved efficacy compared with that of sulfonamide treatment alone as to favorable pharmacokinetics (effective penetration of cerebrospinal fluid) and general familiarity among clinicians (568). For most patients with nocardiosis, clinical improvement is expected within 7 to 10 days after the initiation of empiric therapy with sulfonamides (with or without trimethoprim). For infected patients, the exact route of drug administration may be influenced by the assessment of their overall clinical status. In addition, a serum drug level estimation at least once following the institution of antimicrobial therapy may be useful to establish that adequate drug absorption is occurring and to provide a basis for any necessary adjustment of the patient's drug dosage to achieve recommended levels in blood of 100 to 150 μg/ml approximately 2 h after an oral dose. It is recommended that therapy with sulfonamides be given at a high dose (3 to 6 g/day) for extended periods (6 to 12 months). While primary cutaneous nocardiosis may be cured with a 1- to 3-month course of antimicrobial therapy and uncomplicated pulmonary nocardiosis may respond to therapy for 6 months or less, therapy for 12 months or more is usually required for disseminated infection or if the patient is immunocompromised (166). In addition, it is deemed advisable that HIV-infected patients receive long-term maintenance suppressive therapy.

A problem that is encountered not infrequently and that may complicate therapy for HIV-infected patients is patient intolerance of trimethoprim-sulfamethoxazole (214). Also, in organ transplant patients treated with the commonly used antirejection medication cyclosporine, trimethoprim-sulfamethoxazole may cause reversible cyclosporine-induced nephrotoxicity (22, 425, 475, 502, 585).

If a patient remains febrile on antimicrobial therapy, it is important to consider various factors, such as treatment failure, drug fever, a sequestered abscess (which may require surgical drainage), and a coexistent or secondary opportunistic infection with another pathogen. Antimicrobial susceptibility testing should be performed on all important clinical isolates, preferably at a specialized reference laboratory. The results of such testing not only alert clinicians to the presence of primary or acquired drug resistance, which may complicate a patient's drug therapy, but also provide a rational basis for the selection of alternative antimicrobial agents.

As alternatives to sulfonamides, antimicrobial agents of various classes have been reported either to possess significant in vitro activity against *Nocardia* spp. in laboratory studies or to have been proven successful in limited clinical case studies. Antimicrobial agents that may be of clinical benefit include broad-spectrum cephalosporins, fluoroquinolones (166, 568), clindamycin, erythromycin, ampicillin, aminoglycosides (in particular, amikacin), tetracyclines (including minocycline), and imipenem (42, 128). Broad-spectrum cephalosporins such as cefotaxime have also been shown to be very active in vitro and successful in the therapy of experimental infections (570). *Nocardia* sp. isolates have often been reported to be resistant to rifampin.

Beaman et al. recently reported on the development of an experimental model of murine nocardiosis that has provided data for evaluating various antimicrobial agents as treatments for this infection (42). Colony counts of *N. asteroides* in various target organs, assessed over time, have provided quantitative end points for assessing both the comparative bactericidal efficacy of these agents and the timing of bactericidal activity. Evidence from this animal model has shown that imipenem and amikacin are superior to other antimicrobial agents, including trimethoprim-sulfamethoxazole; this finding has also been supported by clinical observations for some patients (193, 194). In addition, in other experimental studies that used antimicrobial combinations that have been shown to demonstrate synergy in vitro, imipenem-amikacin, imipenem-cefotaxime, and amikacin-cefotaxime were found to be efficacious (42, 192, 195). In a study of central nervous system nocardiosis, these combinations were also proven to be statistically superior to single-agent therapy (193).

The results of a survey of clinicians who had successfully treated nocardiosis patients with nonsulfonamide antimicrobial regimens also provided additional information on antimicrobial agents used for the therapy of nocardiosis (42). For these patients, either primary sulfonamide therapy had failed or had been discontinued because of intolerance or primary nonsulfonamide therapy was prescribed to take potential advantage of a more rapidly bactericidal agent or a combination of agents, particularly for severe progressive disease in immunosuppressed patients with or without HIV infection. In this report, Beaman et al. (42) were able to distinguish two groups of patients and their respective therapies: a very ill group, who required parenteral therapy and were given imipenem, amikacin, or cefotaxime (or other broad-spectrum cephalosporins) or combinations thereof; and a less ill group, who were treated with oral antimicrobial agents, in particular, minocycline. In addition, three HIV-infected patients with pulmonary infections and a known hypersensitivity to trimethoprim-sulfamethoxazole were reported to have had a good initial response to primary therapy with imipenem (two patients) or imipenem and amikacin (one patient). However, as emphasized by the investigators, the results of this survey are not to be interpreted with the same weight as that accorded to a prospective trial designed to compare the efficacy of different antimicrobial treatments (42).

Mounting evidence from recent in vitro and in vivo studies, clinical observations, and taxonomic developments have all suggested that therapeutic decisions for patients with nocardiosis concerning the most appropriate drug treatment and the duration of therapy may be complicated. Such decisions may have to be individualized on the basis of knowledge not only of the site and type of infection involved and the nature and degree of any underlying immunocompromise in the patient but also of the particular *Nocardia* species causing the infection and the antimicrobial susceptibility test results for significant clinical isolates. In addition to the detection of primary drug resistance, the frequent requirement for very prolonged drug therapy, particularly in HIV-infected patients, makes subsequent antimicrobial susceptibility testing important, particularly if there is treatment failure or relapse of nocardiosis in the patient.

Pathogenicity Studies

Nocardiae elicit a very brisk inflammatory response in the host. Both polymorphonuclear leukocytes and activated lymphocytes are involved in the cellular response to the infection. Histologically, necrosis and abscess formation are common.

The pathogenicity of *N. otitidiscaviarum* soil isolates has been demonstrated for laboratory animals (311). Following intravenous injection into mice, both *N. otitidiscaviarum* and *N. asteroides* demonstrated equivalent degrees of pathogenicity

(553), and each of these species has also been shown to be more virulent than *N. brasiliensis* (396). Also, Mishra and colleagues found that pretreatment of the test animals with cortisone significantly reduced their resistance to infection with all three species when inoculated intravenously (396); the same was true for *N. transvalensis* (231). Also, mouse pathogenicity studies have suggested that *N. farcinica* clinical isolates are more lethal than *N. asteroides* clinical isolates (141).

ACTINOMADURA SPECIES

Historical Perspective: Introduction and Taxonomy

The pathogenic potential of the microorganisms now known as *Actinomadura* species was first recognized in 1894 by Vincent, who named the organisms "*Streptothrix madurae*" and described them as the causative agent of madura foot (619). In 1896, Blanchard transferred this species to the genus *Nocardia* as "*Nocardia madurae*" (58). In 1970, on the basis of cell wall studies, Lechevalier and Lechevalier proposed the genus *Actinomadura* to include this species and two other species, "*Nocardia pelletieri*" and "*Nocardia dassonvillei*" (331). However, the same authors also characterized the proposed species *A. madurae* and *A. pelletieri* as being closely related and emphasized the morphologic and chemical differences between them and "*Actinomadura dassonvillei*." In 1976, Meyer transferred "*A. dassonvillei*" to the new genus *Nocardiopsis*, as *N. dassonvillei* (387). In 1983, Fischer et al. determined by DNA-rDNA (genes coding for rRNA) cistron similarity and DNA relatedness that although the genus *Actinomadura* is genetically heterogeneous, it is clearly separated from *N. dassonvillei* (171). In 1985, Athalye et al. further confirmed the heterogeneity of the genus *Actinomadura* on the basis of numerical data involving 102 unit characters (23). Also, these authors were clearly able to distinguish the genus *Actinomadura* from *N. dassonvillei* by using numerical data.

At present, definition of species of the genus *Actinomadura* is based on a characteristic cell wall chemotype, the distinctive morphology of their aerial hyphae, a lack of growth in lysozyme, and a failure to demonstrate any constitutive mycolic acids in high-performance liquid chromatography. Their whole-cell hydrolysates have been found to contain *meso*-DAP and the sugar madurose, which was identified as 3-*O*-methyl-D-galactose by Lechevalier and Gerber (338). On the basis of the above-described findings, Lechevalier and Lechevalier proposed the genus *Actinomadura* in 1970 (331).

Twenty-six species were listed in 1989 in *Bergey's Manual of Systematic Bacteriology* (198). However, recent data obtained from molecular genetic and chemotaxonomic studies about intrageneric groupings are unaccounted for in this classification, since such studies are not considered to be helpful in the description of morphologically similar species. *A. madurae* and *A. pelletieri* are the only two *Actinomadura* species of clinical importance. However, numerous other *Actinomadura* spp. have been described and have been distinguished by less rigorous taxonomic methods, including spore chain morphology, spore wall ornamentation, the color of mature sporulated aerial hyphae, and the color of substrate hyphae. The only new species proposed since 1989 is *Actinomadura fibrosa*. Both physiologic characteristics and fatty acid composition differentiate this species from previously described species. This species produces a new polyether antibiotic (386).

Fischer et al. studied the phylogenetic relationship between the genus *Actinomadura* and the genera *Nocardiopsis*, *Streptomyces*, and *Streptosporangium* by determining the melting points of DNA-rRNA duplexes obtained with labeled 23S rRNA of *A. madurae* and DNA from the related species and genera (171). In this method, the genus *Actinomadura* formed two clusters, confirming the heterogeneity of this genus. These same investigators compared the rRNA clusters with DNA-DNA hybridization results and found that the highest values for relatedness occurred for *A. madurae* strains (96%) and for *A. pelletieri* strains (85 to 100%). Relatedness values for *A. madurae*, *A. pelletieri*, and other *Actinomadura* spp. ranged from 15 to 48%, implying a moderate degree of relatedness.

In another study, Stackebrandt and Schleifer examined the relatedness of the genus *Actinomadura* to other actinomycetes by comparative cataloging of 16S rRNAs (559). They compared *A. madurae* and four other species. These investigators found that the genus *Actinomadura* was unique among the actinomycete genera; however, they also found it to be genetically heterogeneous. Additional studies by Poschner et al., who compared additional species of the genus *Actinomadura* by using DNA reassociation and chemotaxonomy, also demonstrated heterogeneity within this genus (455).

Ecology

A. madurae seems to be widespread in soil, whereas *A. pelletieri* has been found only in clinical specimens.

Epidemiologic Aspects of Infection in Humans

Mycetoma. *A. madurae* is a frequent cause of actinomycetomas, superficial or deep suppurating tumefactions of the skin and subcutaneous tissues that result from soil contamination of a penetrating wound and usually involve the lower extremities. In mycetomas, the etiologic agents occur in the form of granules. The majority of the reports of infection by this species are from tropical and subtropical countries. However, the higher prevalence of such infections in warm climates may also be only a reflection of the increased tendency for exposure because of walking barefooted.

Other clinical infections. A recent review of the species of aerobic actinomycetes identified in clinical specimens by the CDC Actinomycete Laboratory from October 1985 through February 1988 found that *A. madurae* accounted for 42 (11.5%) of the total of 366 submitted isolates; this species was second in frequency only to *N. asteroides*, which accounted for 98 (26.8%) of these isolates (383). The majority of these isolates in this study were from sputum (24 isolates; 57.1%) and wounds (13 isolates; 31%). However, 1 (2.4%) of the 42 isolates was a blood isolate.

There are also a few recent reports of nonmycetomic infections caused by *A. madurae*. One case of *A. madurae* peritonitis developed in a patient undergoing long-term ambulatory peritoneal dialysis; the patient had no history of travel to tropical regions (648). Interestingly, this patient's infection responded to intraperitoneal therapy with amikacin. In another case, *A. madurae* was isolated in a pure culture from a hysterectomy wound infection, which was successfully treated with trimethoprim-sulfamethoxazole (186). In addition, in 1992, the first case of an AIDS patient with pneumonia caused by *A. madurae* was reported. The patient was a 39-year-old male with an 8-year history of intravenous heroin abuse. He was HIV type 1 antibody positive and had *Pneumocystis carinii* pneumonia, which responded initially to intravenous therapy with trimethoprim-sulfamethoxazole (385). For this patient, a histologic examination confirmed opportunistic disseminated cytomegalovirus infection, *A. madurae* pneumonia, and secondary bacteremia. There was also evidence of persistence of the *A. madurae* pneumonia despite antimicrobial therapy with many drugs to which the *A. madurae* blood isolate demon-

strated in vitro susceptibility. Thus, *A. madurae* is a potential pathogen in AIDS patients. In these highly immunocompromised patients, it may be a cause of a life-threatening infection.

Laboratory Diagnosis

Microscopically, actinomadurae form branched filaments, which may produce short chains of spores. The cultures may be white to pink to red in color, are usually mucoid, and have a molar tooth appearance after 2 days of incubation at 35°C. Aerial hyphae, if formed, are usually sparse and often are not produced until after 2 weeks of incubation. The physiologic characteristics helpful in differentiating the genus *Actinomadura* from related genera are given in Table 3.

Although the substrate hyphae of *A. pelletieri* isolates are usually deep pink to brownish red, the substrate hyphae of *A. madurae* isolates may also be red. However, *A. madurae* can be distinguished reliably from *A. pelletieri* on the basis of conventional biochemical tests (394). Both of these species hydrolyze casein and may hydrolyze hypoxanthine and tyrosine; however, only *A. madurae* hydrolyzes esculin. In addition, isolates of *A. pelletieri* are asaccharolytic, in contrast to isolates of *A. madurae*. The former produce acid only from glucose and trehalose, whereas *A. madurae* isolates usually produce acid from adonitol, arabinose, cellobiose, erythritol, glucose, glycerol, mannitol, mannose, rhamnose, trehalose, and xylose.

Morace et al. subtyped *A. madurae* isolates on the basis of their susceptibility to nine killer yeast strains, grouped into triplets (397). These investigators evaluated five *A. madurae* isolates; with their method, they were able to distinguish four different biotypes. However, this method of biotype differentiation needs to be further evaluated in studies with epidemiologically significant isolates before it can be recommended as a reliable epidemiologic tool.

Histopathology

A histopathologic examination may typically reveal intracellular and extracellular short filaments that are gram positive in Brown-Brenn-stained tissue preparations and that may also be readily seen in GMS-stained tissue sections.

Diagnostic Immunology

At present, there are only a few serologic procedures that are suitable for routine use in clinical laboratories either for the identification of actinomadurae or for the demonstration of antibodies in serum specimens from infected patients. An enzyme-linked immunosorbent assay was used to screen for antibodies to *S. somaliensis* and *A. madurae* in two regions of Sudan in which mycetoma caused by these agents was endemic (573). The results of this study suggested that a relatively large proportion of clinically asymptomatic individuals in one region were infected with *S. somaliensis* (6.8%) and, to a lesser degree, with *A. madurae* (573). Although no testing was performed for other species of actinomycetes, the relatively high degree of specificity of antibodies from patients infected with *S. somaliensis* and *A. madurae* suggested that cross-reactivity was improbable.

Antimicrobial Susceptibility Testing and Therapy

The method used for antimicrobial susceptibility testing of *A. madurae* isolates is generally similar to that used for *Nocardia* spp. However, *A. madurae* isolates have relatively slower growth rates: they may take up to 3 days to achieve an adequate inoculum; *Nocardia* spp. may take up to 2 days.

In the most extensive report of antimicrobial susceptibility test results for *A. madurae*, McNeil et al. tested 42 clinical isolates of *A. madurae* (Table 5) (383). Despite some intraspecies variability, all of these isolates were susceptible to amikacin and imipenem. However, 63% of the isolates tested were resistant to ampicillin. In another study, Boiron et al. used a disk diffusion method to determine the in vitro antimicrobial susceptibilities of 29 clinical and environmental isolates of *A. madurae* (61). The results obtained by these investigators showed good agreement with those in the earlier report (383); however, some major discrepancies were apparent with certain cephalosporins and penicillins and with trimethoprim-sulfamethoxazole. These antimicrobial agents were either only moderately effective or ineffective. Although β-lactamase production in *A. madurae* isolates or related isolates has not been studied, differences between the frequencies of isolates of *A. madurae* resistant to amoxicillin alone and resistant to amoxicillin-clavulanate suggest that β-lactamase activity may be a problem with some isolates (61).

The finding of intraspecies variability in antimicrobial susceptibility tests is in agreement with other evidence for heterogeneity within *A. madurae*, as characterized by chemotaxonomy and numerical taxonomy studies (198), and supports the need to perform antimicrobial susceptibility tests on clinically significant isolates so that more effective antimicrobial therapy may be instituted (383).

Pathogenicity Studies

Available information on the pathogenicity of *Actinomadura* spp. is contradictory. Pulverer and Schaal found it impossible to demonstrate any virulence factors for these microorganisms (464). However, Rippon reported that virulent strains of *A. madurae* produce a collagenase that has an important role in the pathogenicity of this microorganism (476). In humans, the route of infection is usually percutaneous inoculation, which results in a local skin infection that may progress to mycetoma. However, rarely, in severely immunocompromised patients, these microorganisms have been demonstrated to behave as invasive opportunistic pathogens (385).

DERMATOPHILUS CONGOLENSIS

Dermatophilosis, caused by the unusual aerobic actinomycete *Dermatophilus congolensis*, characteristically appears as an exudative dermatitis with encrustations. The few published reports of dermatophilosis have been from widely scattered geographic locations. While the overall number of reported cases is relatively small compared with that of nocardial cases, it is very likely that this diagnosis is overlooked by both clinicians and laboratory personnel (283). *D. congolensis* is unique among known microorganisms, and it may be easily recognized microscopically when viewed in all of its morphologic manifestations.

Historical Perspective: Introduction and Taxonomy

Dermatophilosis was first recognized as an infectious disease in 1915 by Van Saceghem, who described the disease in cattle in the former Belgian Congo (617). The disease was first reported in the United States in 1961 (77). It has a worldwide distribution and occurs in both temperate and tropical climates. In countries in which raising livestock is an important industry, the disease has the potential to cause considerable economic losses by damaging hides and wool of affected animals. Diagnosis has depended on culturing and recognition

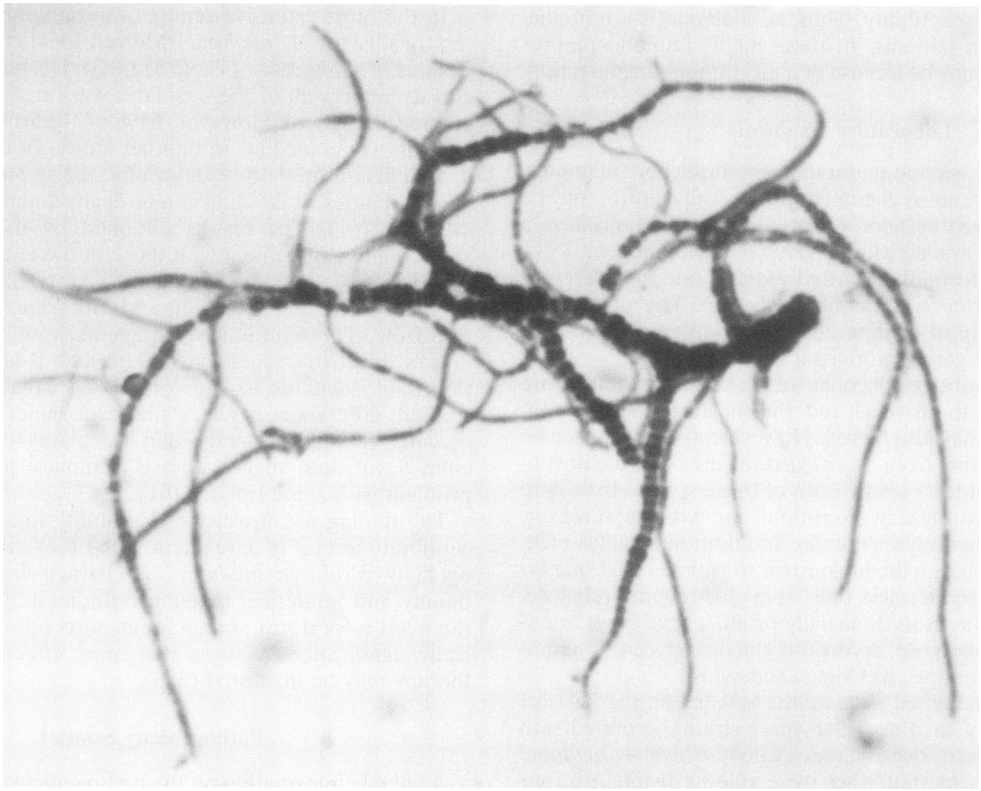

FIG. 8. *D. congolensis* branched filaments divided in transverse and longitudinal planes and with tapered fine hyphae. Direct preparation from a culture. Giemsa stain. Magnification, ×840.

of the characteristic microscopic morphology and biochemical reactions of the microorganism.

The only recognized etiologic agent of dermatophilosis is *D. congolensis*. In 1964, Gordon studied three accepted species—*D. congolensis* Van Saceghem 1915, *D. congolensis* Austwick 1958 ("*Actinomyces dermatonomus*" Bull 1929), and "*Dermatophilus pedis*" Austwick 1958 ("*Polysepta pedis*" Thompson and Bissett 1957)—and concluded that all three could be accommodated in the species *D. congolensis* (215).

Ecology

The natural reservoir for *D. congolensis* is presently unknown; however, contaminated soil is a likely source of this aerobic actinomycete (337).

Epidemiologic Aspects of Infection in Animals

Dermatophilosis may affect both wild and domestic animals and usually is manifested as severe skin lesions. In addition to cattle, the disease has been reported mainly for horses, goats, and sheep. Less commonly, the disease has been described for monkeys, polar bears, deer, other lower animals, and humans. Disease affecting wool-bearing areas of sheep has been variously known as mycotic dermatitis, lumpy wool, or rain rot and, when the distal extremities are affected, strawberry foot rot or proliferative dermatitis.

Epidemiologic Aspects of Infection in Humans

The acquisition of *D. congolensis* by humans is usually the result of contact with tissues of infected animals or contaminated animal products (217). Groups that have the greatest risk for acquiring the infection include abattoir workers, butchers, hunters, dairy farmers, and veterinarians (133, 655). However, in two reported cases, involving a physician and a patient with "hairy" leukoplakia, no such contact could be established (86). There has also been one report in which this microorganism was isolated from a patient's contact lens, but no association with ocular infection was established (51). Microorganisms indistinguishable from *D. congolensis* have been isolated from patients with pitted keratolysis, which characteristically attacks the soles of the feet (629, 646, 659). Such involvement of human keratinized tissue is not surprising, since the microorganism is known to liberate marked amounts of keratinase when cultured on appropriate substrate media (240).

Isolation

Isolation of *D. congolensis* may be difficult. Clinical material, preferably the underside of scabs, should be streaked onto a blood plate and incubated aerobically or in CO_2 at 35 to 37°C. Highly contaminated specimens may require animal passage for successful isolation. For this procedure, crusts and scabs are ground and applied to the shaved and scarified skin of a rabbit. Cutaneous lesions develop on the animal within 2 to 7 days. Isolation of the microorganism in a pure culture may be obtained by use of these infected sites (216).

Laboratory Diagnosis

Microscopic and macroscopic characteristics. Although *D. congolensis* may be seen at any stage of development by direct examination of clinical material, the appearance of branched filaments divided in their transverse and longitudinal planes is

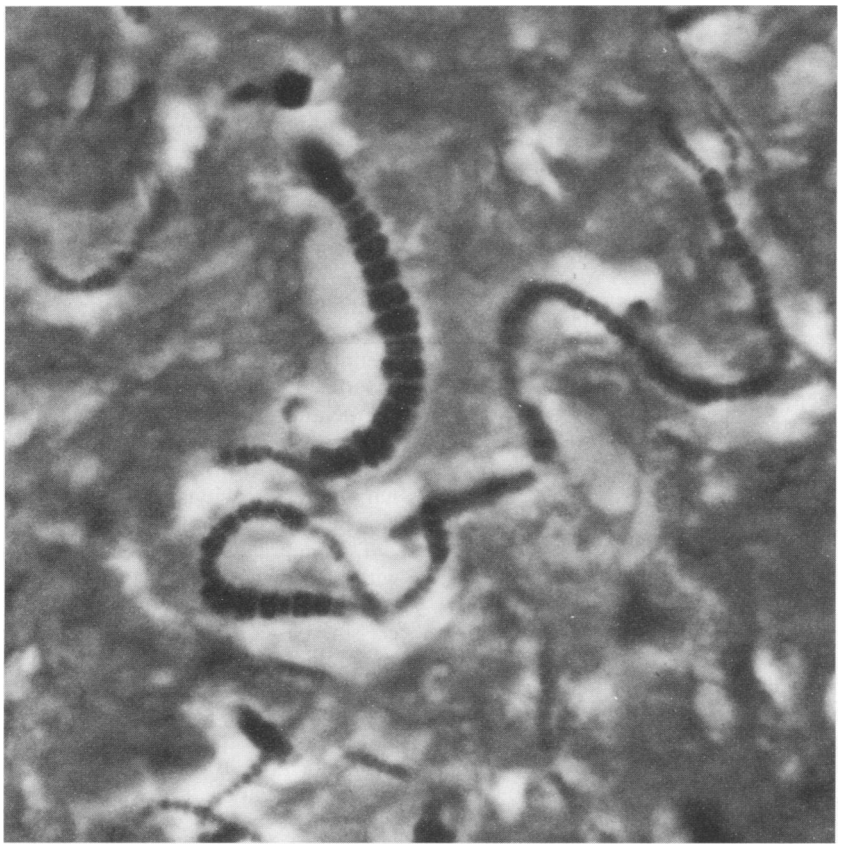

FIG. 9. *D. congolensis* subcutaneous tissue section from a cat, showing immature and mature microscopic morphologic forms. Giemsa stain. Magnification, ×840.

observed most frequently and is pathognomonic. This microorganism may be seen in wet mounts or in smears of clinical material stained with methylene blue or by the Giemsa stain method (Fig. 8). A Gram-stained preparation is not likely to be helpful for visualizing this microorganism because it is too dark and obscures crucial morphologic details.

The diagnosis of dermatophilosis depends upon the observation of *D. congolensis* in clinical material and the isolation and identification of the etiologic agent in a culture. The microscopic morphology of *D. congolensis* in cultures is similar to that in clinical specimens. Depending on the age of the isolate and the type of medium used for culturing, one may see completely coccal elements, many with flagellae or irregularly arranged cells in packets; germinating spores; or branched segmented or nonsegmented filaments. Motility is usually evident in isolates from fresh cultures. If cocci only are seen and *D. congolensis* is suspected, younger cultures should be examined for hyphae. At 24 h on brain heart infusion agar containing horse blood, tiny (0.5- to 1.0-mm), round colonies can be seen. The appearance of these colonies may vary, but they are usually gray-white and adherent and pit the medium. Later, in 2 to 5 days, they develop an orange pigment. Frequently, there is beta-hemolysis, which may be particularly prominent on medium containing horse blood. This beta-hemolysis is also more prominent on areas of the medium in which colonies are crowded. There is no growth on SDA.

Biochemical identification. *D. congolensis* is catalase positive. Urea is usually hydrolyzed in 24 h, but the hydrolysis of casein may take up to 7 days. Hypoxanthine, tyrosine, and xanthine are not hydrolyzed. Gelatin is liquefied, and milk is peptonized. Nitrate is not reduced. Acid but no gas is produced in fermentative basal medium from glucose in 48 h; acid may be produced transiently from galactose (acid in 48 h; negative in 2 weeks). Some strains produce acid from maltose in 1 to 2 weeks. No acid is produced from sucrose, lactose, xylose, dulcitol, mannitol, sorbitol, or salicin (215).

Although not an absolute requirement for the identification of *D. congolensis*, the presence of DAP and madurose in whole-microorganism hydrolysates may be an additional aid in the identification of this species (Table 3) (215).

Histopathology

For paraffin-embedded tissue sections, the Giemsa and GMS stains are the most satisfactory for visualizing *D. congolensis*. The forms seen in tissue resemble those seen in mature cultures (Fig. 9); often there is an accompanying dense infiltrate of eosinophilic leukocytes.

Diagnostic Immunology

Pier and associates evaluated the application of immunofluorescence for the diagnosis of dermatophilosis (449a). They were successful in preparing a specific conjugate for the detection of *D. congolensis* in exudate suspensions (449a). This serodiagnostic test is particularly useful when only nonspecific coccal forms are seen in smears made from cultures.

Antimicrobial Therapy

In in vitro disk susceptibility tests, *D. congolensis* is susceptible to many antimicrobial agents, including penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, and trimethoprim-sulfamethoxazole, and resistant to kanamycin and other sulfonamides (3, 477). Also, the combination of penicillin and streptomycin is more effective than either drug alone for the treatment of dermatophilosis in sheep (629).

Pathogenicity Studies

Isolates of *D. congolensis* are virulent for rabbits. Animal inoculation experiments may be useful for differentiating isolates of this species from filamentous forms of *Geodermatophilus obscurus*. The experimental inoculation of abraded rabbit skin results in acute ulcerative pustular dermatitis, mainly involving the hair follicles (216).

GORDONA SPECIES

Historical Perspective: Introduction and Taxonomy

In 1988, Stackebrandt et al. reintroduced the genus *Gordona* to accommodate microorganisms previously considered rhodococci on the basis of comparative analyses of 16S rRNAs (560). The species assigned to the genus *Gordona* include the former *Rhodococcus bronchialis*, *Rhodococcus rubropertinctus*, *Rhodococcus sputi*, and *Rhodococcus terrae*, which contain mycolic acids with between 48 and 66 carbon atoms and large amounts of a dihydrogenated menaquinone with nine isoprene units (239). The remaining *Rhodococcus* spp. contain shorter-chain mycolic acids (C_{34} to C_{52}) and a dihydrogenated menaquinone with eight isoprene units as the major isoprenoic quinone (119, 239). Of interest, it was recently shown that *Rhodococcus chubuensis*, listed as a species incertae sedis in *Bergey's Manual of Systematic Bacteriology* (198), contains a mycolic acid pattern similar to that of *G. bronchialis* (134).

In addition, in 1986, Hall and Rutledge found that the genus *Gordona* formed mycobactins and that the genus *Rhodococcus* failed to synthesize these compounds under growth conditions of a strictly limited availability of iron (237). The mycobactins of *Gordona rubropertincta* and *Gordona terrae* were found to be quite similar by thin-layer chromatography and high-performance liquid chromatography and could easily be distinguished from those of *G. bronchialis* (237).

DNA relatedness studies have shown that *G. bronchialis*, *G. rubropertincta*, *Gordona sputi*, and *G. terrae* form genetically distinct species (399–401, 403). The validity of the genus *Gordona* was supported by a numerical analysis of these microorganisms, which involved 116 unit characters (213).

The genera *Gordona* and *Rhodococcus* may be separated on the basis of characteristic mycolic acid patterns: mycolic acids of the genus *Gordona* contain 48 to 66 carbons, and those of the genus *Rhodococcus* contain 34 to 52 carbons (11, 239) (Table 4). However, despite recent advances in chemical and molecular methods for the separation of these genera, identification at the species level remains problematic as, with the possible exception of *G. bronchialis*, no simple and reproducible method for rapid identification and differentiation exists.

Epidemiologic Aspects of Infection in Animals

In 1988, Tsukamura et al. (604) reported a case of mesenteric lymphadenitis of the ileum in a 6-month-old pig. Microscopically, the lymph node showed granulomatous lymphadenitis that resembled tuberculosis. *G. sputi* was isolated in a pure culture from the lymph node, although it was not observed on histopathologic examination (604).

Epidemiologic Aspects of Infection in Humans

Primary cutaneous infection. Martin et al. described a 7-year-old nonimmunocompromised female with a granulomatous forearm skin lesion and axillary lymphadenopathy caused by a *Rhodococcus* sp. (373). The authors suspected that the isolate was *R. terrae* and that the production of acid from rhamnose might be a phenotypic marker for this microorganism. Subsequently, the use of ribotype analysis confirmed the identity of this patient's isolate as *G. terrae* (324).

Pulmonary infection. In 1971, when Tsukamura proposed the definition of *Gordona* as a new genus removed from the genus *Rhodococcus*, he also described the isolation of members of this genus from sputum specimens of patients with pulmonary disease (cavitary pulmonary tuberculosis or bronchiectasis) and from soil (597).

Tsukamura studied 101 isolates from the sputum of patients with pulmonary disease and from soil; 71 were classified into three species (597). Forty-one isolates, from sputum, were classified as *G. bronchialis*, and 14 resembled this species. The remaining 30 isolates, from soil, were classified as *Gordona rubra* (10 isolates) and later as *G. rubropertincta* and *G. terrae* (20 isolates) (597).

The first documented case involving *G. rubropertincta* was a lung infection in an immunocompetent 29-year-old woman (241). An important feature of this case was the initial diagnostic confusion with tuberculosis. However, mycobacteria were not detected in the sputum or bronchial washings, and the observation of numerous gram-positive bacilli in the Gram-stained smear of bronchial washings and the isolation of *G. rubropertincta* in a pure culture supported the pathogenic role of this microorganism. The patient responded to doxycycline and oral antituberculosis therapy that included rifampin (241).

Although *G. sputi* was observed in the sputum of patients with chronic pulmonary disease in another study by Tsukamura, it was not reported to be pathogenic for humans (599).

Catheter-associated sepsis. Recently, Buchman et al. reported that for two patients with long-term indwelling central venous catheters, *Gordona* sp. bacteremia complicated the receipt of home total parenteral nutrition (85). One patient's isolate was identified as *G. terrae* by conventional biochemical tests, and this identity was confirmed by use of a digoxigenin-labeled rDNA probe. Although the patient was treated empirically with vancomycin and gentamicin and his isolate was later found to be susceptible to both of these drugs in vitro, he remained febrile and the catheter was removed. The other patient's isolate could be identified only to the genus level despite extensive biochemical studies and rDNA ribotype analysis. The patient received intravenous vancomycin with the catheter left in situ, and subsequent blood cultures were negative.

Nosocomial transmission. An unusual nosocomial outbreak of sternal wound infections in patients following coronary artery bypass surgery was reported recently by Richet et al. (470). For this outbreak, epidemiologic and laboratory investigations (including molecular subtyping of isolates) implicated a single genetically distinct strain of *G. bronchialis* and traced the cluster of seven infected patients to a colonized operating room nurse. The epidemic strain of *G. bronchialis* was acquired during intraoperative exposure and was also isolated from the nurse's dogs and environment.

TABLE 6. Biochemical characteristics of the type strains of the genus *Gordona*[a]

Characteristic	Result for:			
	G. bronchialis	*G. rubropertincta*	*G. sputi*	*G. terrae*
Decomposition of:				
Adenine	−	−	−	−
Casein	−	−	−	−
Hypoxanthine	−	−	−	−
Tyrosine	−	−	−	−
Xanthine	−	−	−	−
Growth in lysozyme	−	−		
Acid production from[b]:				
Inositol	+	−	−	−
Mannitol	−	+	+	+
Rhamnose	−	−	−	+
Sorbitol	−	+	+	+
Trehalose	+	+	+	+

[a] Data are modified from those of Lasker et al. (324). Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative.
[b] In Gordon's carbohydrate basal medium (49).

Isolation

Isolation of the genus *Gordona* is similar to that of other aerobic actinomycetes (Fig. 1). However, for *G. sputi* from a swine lymph node, the microorganism was isolated only after 4 weeks of incubation on an Ogawa egg medium slant (604).

Laboratory Diagnosis

Microscopic and macroscopic characteristics. After 3 to 7 days of incubation, *Gordona* cultures appear as dry, wrinkled, raised beige colonies on blood agar plates. However, on further incubation these may become salmon colored, particularly on chocolate agar. *G. bronchialis* produces synnemata that may be confused with aerial hyphae. Microscopically, these microorganisms appear as gram-positive, weakly acid-fast, thin, beaded coccobacilli.

Identification. (i) Physiologic and biochemical methods. The identification of *Gordona* spp. is currently not possible on the basis of the characteristics listed in Table 6. In this summary of the carbohydrate panel used by the CDC Actinomycete Laboratory, the reactions for *G. sputi* and *G. rubropertincta* are identical and are separated from those for *G. terrae* only by the inability of *G. sputi* and *G. rubropertincta* to produce acid from rhamnose (324). Mycolic acid analysis by high-performance liquid chromatography can identify gordonae to the genus level; however, this technique fails to yield diagnostically distinct patterns for species. Therefore, simple and reproducible phenotypic markers for distinguishing the species of the genus *Gordona* are needed. Only ribotype analysis has been useful in separating *G. sputi*, *G. rubropertincta*, and *G. terrae*. The genus *Gordona* is separated from the genus *Nocardia* by the former's inability to produce aerial hyphae and inability to grow in the presence of lysozyme.

(ii) Typing and subtyping. Richet et al. demonstrated the utility of three methods of typing *G. bronchialis* isolates: plasmid profiling, restriction endonuclease analysis, and ribosomal typing (470). Lasker et al. and Buchman et al. both reported on the usefulness of a digoxigenin-labeled rDNA gene probe as an aid in the identification of clinical *Gordona* spp. when conventional physiologic and biochemical analyses proved unhelpful (85, 324).

RHODOCOCCUS SPECIES

Historical Perspective: Introduction and Taxonomy

The genus *Rhodococcus* has been considered a heterogeneous group of microorganisms more closely related to those of the genus *Nocardia* than to those of the genus *Mycobacterium*; however, rhodococci do not normally produce aerial hyphae. The members of this genus are gram positive, nonsporulating, and partially acid fast. *Bergey's Manual of Systematic Bacteriology* listed 20 different *Rhodococcus* species, four as species incertae sedis (198). Recently, taxonomic revisions that have reassigned certain members of the genus *Rhodococcus* to either of two new genera, *Gordona* (561) and *Tsukamurella* (121), have left the genus *Rhodococcus* as a more homogeneous taxon. However, despite these revisions, the differentiation and identification of clinical isolates as species of these three genera remain problematic. This difficulty is particularly true for microorganisms belonging to the genus *Rhodococcus*. By using 107 biochemical characteristics, Tsukamura was able to differentiate *Rhodococcus* spp. (600). More recently, in a 1990 taxonomic study, Goodfellow et al., using 125 unit characters, including rapid enzyme tests, found that numerical phenetic data strongly supported the homogeneity and the recognition of the newly revised genus *Rhodococcus* (207). However, this study of 99 *Rhodococcus* strains indicated that numerous multi- and single-membered clusters have yet to be characterized and named and that these may represent additional *Rhodococcus* spp. Although both of these proposed systems may accurately identify the majority of *Rhodococcus* spp., the number of biochemical tests involved limits their application in routine clinical microbiology laboratories (207, 600). The method used for routine identification at the CDC Actinomycetes Laboratory is based on a less extensive panel of phenotypic characteristics but has not provided for the definitive identification and adequate differentiation of all species of the genus *Rhodococcus* and related genera.

There have been a few reports of infections caused by unidentified species of the genus *Rhodococcus*, but only rarely has there been documentation of direct tissue invasion (106, 528, 556). In one of these reports, extensive biochemical testing and ribotype analysis, which compared a patient isolate to the 20 *Rhodococcus* type strains, failed to identify this patient *Rhodococcus* isolate to the species level (556). Of the species that have remained in the revised genus, *R. equi* appears to have the most clinical significance as a potential cause of infections in animals and humans. *R. equi* has been well known as a serious pathogen of livestock. In recent years it has been identified as the cause of potentially life-threatening infections in severely immunocompromised patients, in particular, patients with HIV infections. Much relevant information on *R. equi* was recently summarized by Prescott (458).

Ecology

The species of the genus *Rhodococcus* are widely distributed in the environment: *Rhodococcus rhodochrous*, *Rhodococcus coprophilus*, *R. equi*, *Rhodococcus erythropolis*, *Rhodococcus globerulus*, *Rhodococcus luteus*, *Rhodococcus maris*, and *Rhodococcus ruber* have been isolated from soil; *R. coprophilus* and *R. equi* have been isolated from herbivore dung; *Rhodococcus fascians* has been isolated from plants; *Rhodococcus luteus* and *R. maris* have been isolated from the intestinal tract of carp; *Rhodococcus rhodnii* has been isolated from the intestine of the reduviid bug; and *Rhodococcus aichiensis*, *R. chubuensis*, and *Rhodococcus obuensis* have been isolated from human sputum (198). In 1984, Barton and Hughes detected *R.*

equi in 54% of soil samples examined and the intestinal contents, feces from the rectum, and dung of all grazing herbivorous species examined (37). Using an enrichment broth, these investigators found a 10,000-fold increase in the rate of isolation of *R. equi* in dung from horses 1 to 2 weeks after its deposition. This multiplication of *R. equi* has implications for the study of disease caused by *R. equi* in foals. Crowded foaling paddocks may provide an environment conducive to massive challenge of foals with *R. equi* microorganisms at a time when they lack antibody or a functioning cell-mediated immune system.

Epidemiologic Aspects of Infection in Animals

R. equi was first isolated from suppurative lung lesions in foals in 1923 by Magnusson, and since then this microorganism has generally been considered to cause veterinary disease (364).

Clinical illness from and asymptomatic carriage of *R. equi* have been well documented in herbivorous animals, in particular, as a cause of pneumonia in foals (364) and ulcerative lymphangitis in cattle (412). In swine, *R. equi* may also be isolated as a commensal microorganism from the tonsils and the cervical lymph nodes (284) or as a pathogen causing lymphadenitis (284).

R. equi is endemic on some horse farms, causes only sporadic disease on others, and is unrecognized as a pathogen on the majority of such farms (486). Recently, a serologic survey of equine *R. equi* infection found that the microorganism was widespread on Japanese horse farms (501). The investigators tested 2,879 horse serum samples by using an enzyme-linked immunoassay technique and found that 11% were antibody positive. Although the rates of seropositive animals differed significantly between horse farms, it was shown that 100 of 160 horse farms had at least one antibody-positive horse. In 1993, Knottenbelt reported an outbreak of *R. equi* infection on a thoroughbred stud farm in Zimbabwe in which 24 foals became infected over a 2-year period (294). In this outbreak, six mares had foals that were infected in each of 2 years. The diagnosis was confirmed by clinical examination, culturing of the microorganism from tracheal aspirates, and thoracic radiography and was supported by significant elevations in the levels of plasma fibrinogen and the counts of neutrophils and platelets. Treatment with rifampin and erythromycin was administered until plasma fibrinogen levels and neutrophil and platelet counts remained normal for 2 weeks (294).

Epidemiologic Aspects of Infection in Humans

Reports of human infection with *Rhodococcus* spp. have been rare. The disease may have a variable clinical presentation, depending upon the underlying immune status of the host and possibly upon the site of inoculation and the virulence of the infecting microorganism. Also, any delay in making the diagnosis or commencing effective antimicrobial therapy in infected patients may be important in determining their outcomes. The first human infection was reported in 1967 and involved a patient who had *R. equi* pneumonia and who had received corticosteroid therapy for chronic hepatitis (191). Subsequent reports of human infection with *Rhodococcus* spp. have been rare and have emphasized the genus and *R. equi* in particular as the cause of invasive pulmonary infection in severely immunocompromised patients.

Rhodococcus spp. have been found as soil saprophytes in the environment, and in some patients soil contact may be important and may result in infection. *R. equi*-infected patients have frequently had a history of contact with farm animals, soil, or both (95, 191, 277, 613). In a recent review of *R. equi*-infected patients, 15 of 51 (29%) had a history of possible exposure to an animal source (149). There have also been isolated reports of localized *R. equi* infections that have resulted from probable soil contamination in patients without any known immunologic abnormality; *R. equi* has been the cause of posttraumatic cutaneous infections (100, 406), endophthalmitis (152, 255), and peritonitis, which developed in a patient on continuous ambulatory peritoneal dialysis (175).

Invasive pulmonary and fatal disseminated *R. equi* infections have been reported for patients who have hematologic and other malignancies and who have received chemotherapy (8, 50, 95, 179, 277, 370, 613); renal transplant recipients (277, 424, 493, 508, 612, 637); patients who have received corticosteroids (191, 254, 360); a patient with a history of chronic alcoholism (329); and, most recently, HIV-infected patients (57, 107, 146, 157, 165, 243, 309, 500, 509, 549, 554, 631, 644).

Cutaneous infection. *Rhodococcus* spp. have been reported to cause primary cutaneous infections, including actinomycetomas. Castor et al. described a female nurse who had repeated episodes of a factitious illness, which included a nonhealing leg ulcer, and repeated episodes of polymicrobial bacteremia, which included infection with *R. equi* (100). In addition, the microorganism isolated from a wound infection in a nonimmunocompromised host was characterized as being *R. equi*-like (406). Another report described a primary cutaneous infection with "*Rhodochrous* complex" in an 81-year-old nonimmunocompromised man. The infecting microorganism was found in tissue sections and cultured in vitro; the patient's infection was eradicated with doxycycline (106). Severo et al. reported a patient with an actinomycetoma caused by a non-*R. equi* *Rhodococcus* species (528). Antinori et al. reported an HIV-infected patient with a disseminated *R. equi* infection that presented initially as a foot mycetoma (21). Ellis-Pegler et al. described recurrent skin infections caused by a *Rhodococcus* species in an immunosuppressed patient (153). Also, Boughton et al. described a patient with septic arthritis caused by a *Rhodococcus* species (72).

Bacteremia. Bacteremia that is usually secondary to a pulmonary infection has been reported for patients with *Rhodococcus* infections. The patient described by Castor et al., who had a nonhealing leg ulcer, also had *R. equi* bacteremia (100). In addition, in a report of *R. equi* infections in three children, two of the three had bacteremia (359). Spark et al. reported a nonimmunocompromised patient with a fatal *Rhodococcus* infection and with secondary bacteremia (556). Also, in severely immunocompromised patients with primary lung or disseminated *R. equi* infection, including HIV-infected patients, secondary bacteremia frequently has been reported.

Invasive pulmonary infection. In severely immunocompromised patients, primary pulmonary *R. equi* infections (pneumonia and lung abscesses) have been reported most frequently. Acquisition is thought to occur by inhalation of the microorganism from the environment. As may be the case with other primary invasive aerobic actinomycete pulmonary infections in immunocompromised patients, the diagnosis of an *R. equi* infection is often delayed. In these severely ill patients, the clinical presentation may be nonspecific, and invasive biopsy procedures (bronchoscopy or open-lung biopsy) often may be required to make the diagnosis (146). Diagnostic difficulty may also be encountered in the clinical microbiology laboratory, since *R. equi* possesses a variable microscopic morphology (bacillary to coccoid forms) in clinical specimens, and members of the genus *Rhodococcus* may be misidentified and discarded as contaminants (diphtheroids) (146). In addition, on histopathologic examination of tissue specimens, unless there is a

high degree of suspicion for an *R. equi* infection, this species of rhodococci may go unnoticed because they often appear as nonspecific coccoid forms predominantly in macrophages.

Catheter-associated sepsis. Recently, Hinnebusch et al. described a patient who developed bacteremia while receiving long-term parenteral nutrition with an indwelling central venous catheter (256). Isolates from blood and catheter line tip cultures were initially identified as *R. equi* by use of the Rapid CORYNE kit method (Biomerieux, Marcy l'Etoile, France) but were identified as *Rhodococcus* sp. by conventional biochemical tests. The patient was treated effectively with a course of intravenous doxycycline and vancomycin.

AIDS. *R. equi* may cause life-threatening infections in HIV-infected patients. Since the first report of an *R. equi* infection in an AIDS patient in 1986 (500), more than 100 cases have been reported. In a recent review, Drancourt et al. reported 51 *R. equi* infections; of these, 20 (39%) occurred in HIV-infected patients, and 9 (18%) occurred in immunocompetent patients (149). Not included in this review, at least 50 additional patients with *R. equi* infections have been reported; the majority have been HIV-infected patients (57, 81, 114, 117, 136, 138, 146, 165, 172, 188, 236, 243, 309, 420, 503, 549, 554, 631).

In HIV-infected patients, *R. equi* most commonly causes invasive pneumonia, which may progress to cavity formation, pleural involvement, bacteremia, and dissemination to the brain, liver, spleen, skin, and other organs (57, 138, 172, 236, 309, 500, 503, 554, 631). Although uncommon, extrapulmonary *R. equi* infections have been described (165). The majority of *R. equi*-infected patients are severely immunocompromised, and the infection in HIV-infected patients may precede the development of an AIDS-defining diagnostic condition. The onset of the infection may be insidious, with symptoms of fever, productive cough and, in half of the patients, pleuritic chest pain. The course of the infection is subacute and marked by the later appearance of cavitary lung lesions, which frequently may be associated with empyema or pleural effusion.

In AIDS patients, *Rhodococcus* infections may precede the onset of AIDS-defining conditions, such as opportunistic infections or malignancies. These *Rhodococcus* infections may be unrecognized, and specific therapy may not be instituted early enough to affect the course of a patient's disease. Also, because of their tendency to cause cavitary pulmonary lesions, these infections may mimic mycobacterial, *P. carinii*, and fungal infections in AIDS patients (35, 452). In some patients, the detection of acid-fast bacilli in the sputum has resulted in a misdiagnosis of mycobacteriosis and the institution of antituberculosis drug therapy. The tendency to institute antituberculosis drug therapy, often followed by an interim response of the infection, may further delay making the underlying diagnosis (57, 172, 363). As suggested by some authors (236, 631), consideration should be given to including *R. equi* infections among the indicator conditions diagnostic of AIDS.

Isolation

The isolation of all species of rhodococci is similar to that of the other aerobic actinomycetes (Fig. 1) (458).

Laboratory Diagnosis

Microscopic and macroscopic characteristics. In stained smears of clinical specimens, in particular, purulent material and tissue (biopsy, surgical, and autopsy), the coccoid form of *R. equi* is usually seen. However, the bacillary form of *R. equi* has been reported for clinical specimens, such as blood, sputum, and bronchial lavage fluid (146, 277, 631). In contrast, for all of the reported non-*R. equi Rhodococcus* infections, smears prepared directly from clinical material have shown gram-positive coccobacilli (106, 528, 556); in smears prepared from sputum, the organisms were more filamentous than has been reported for *R. equi* (433).

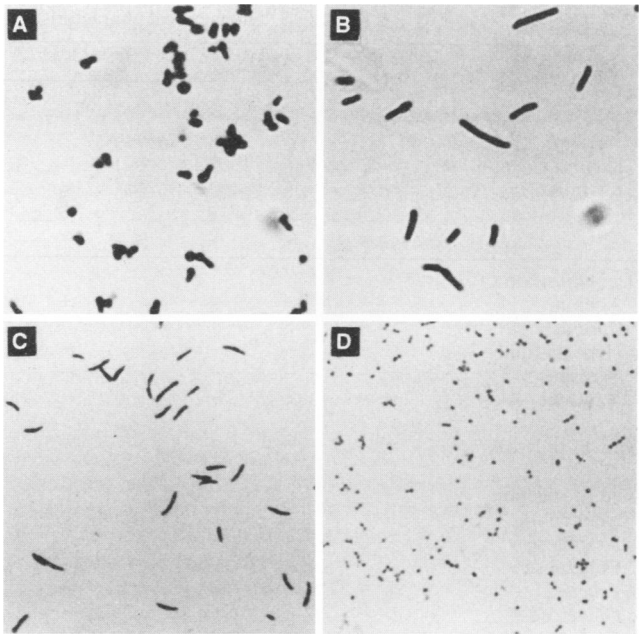

FIG. 10. *R. equi* cyclic microscopic morphology in cultures at 2 h (coccobacillary forms) (A), 4 h (bacillary forms with knobby ends) (B), 6 h (bacillary forms) (C), and 24 h (coccoid forms) (D). Gram stain. Magnification, ×1,022. (Reproduced from the *Journal of Acquired Immune Deficiency Syndrome* [363] with permission of the publisher.)

The microscopic morphology of *R. equi* in cultures is cyclic, varying from bacillary to coccoid, depending upon incubation time and growth conditions. At 6 h on heart infusion agar incubated at 35°C, this microorganism is completely bacillary, but at 24 h, it becomes completely coccoid (Fig. 10). Gram-positive rudimentary branched filaments have been observed for cultures in liquid medium, especially young cultures (458). The microscopic morphology of the non-*R. equi Rhodococcus* spp. is also cyclic, varying from a simple rod-coccus cycle in some species (*Rhodococcus chlorophenolicus* and *R. maris*) to a more complex hypha-rod-coccus cycle in others (*R. coprophilus*, *R. fascians*, *Rhodococcus marinonascens*, and *R. ruber*). *R. rhodochrous*, *R. coprophilus*, *R. erythropolis*, *R. globerulus*, *R. luteus*, and *R. rhodnii* are differentiated further into elementary branched hypha-hypha-rod-coccus cyclic forms (200). *R. coprophilus* and *R. ruber* may form a few aerial hyphae, whereas none of the other *Rhodococcus* spp. form aerial hyphae. All of the rhodococci from clinical specimens are generally weakly acid fast when stained by either the modified Kinyoun or the Ziehl-Neelsen method.

The colony morphology of *R. equi* is diverse and consists of three major varieties. The classic colony type is pale pink and slimy in 2 to 4 days on brain heart infusion agar or heart infusion agar containing 5% rabbit blood when incubated aerobically at 35°C. The second most frequent colony type is coral and nonslimy when grown on the same media under similar incubation conditions. The third and least common colony type is pale yellow, nonslimy, more opaque than the classic slimy type of colony, and identical to that of the *R. equi* type strain (ATCC 6939). Colonies of other members of the

TABLE 7. Biochemical characteristics of the type strains of the genus *Rhodococcus*[a]

Characteristic	Result for:														
	R. aichiensis	*R. chlorophenolicus*	*R. chubuensis*	*R. coprophilus*	*R. equi*	*R. erythropolis*	*R. fascians*	*R. globerulus*	*R. luteus*	*R. marinonascens*	*R. maris*	*R. obuensis*	*R. rhodnii*	*R. rhodochrous*	*R. ruber*
Decomposition of:															
Adenine	−	−	−	−	+	+	−	+	−	−	−	−	−	+	−
Casein	−														
Hypoxanthine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tyrosine	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Xanthine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth in lysozyme	−	−	+	−	−	+	−	+	−	+	−	−	+	−	−
Acid production from[b]:															
Arabinose	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−
Cellobiose	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Fructose	+	+	+	±	−	+	+	+	+	+	+	+	+	+	+
Galactose	−	−	−	−	−	−	±	−	−	+	−	+	−	−	−
Glucose	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	−	+	−	−	+	+	+	+	+	−	+	−	+	+
Inositol	+	+	−	−	−	+	−	−	−	+	−	+	−	−	−
Maltose	+	−	+	−	−	−	−	−	−	+	−	+	−	−	−
Mannitol	−	+	+	−	−	+	+	+	+	+	−	+	+	+	+
Mannose	+	−	+	−	−	+	+	+	+	−	−	+	−	−	−
Rhamnose	−	+	−	−	−	−	−	−	−	+	−	+	−	−	−
Salicin	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−
Sorbitol	−	+	+	−	−	+	+	+	+	+	−	+	+	+	+
Sucrose	+	−	+	−	−	+	−	±	+	+	−	+	−	−	+
Trehalose	+	−	+	−	−	+	±	+	+	−	−	+	−	+	−
Xylose	−	−	+	−	−	+	±	±	−	−	−	−	−	−	−

[a] Data are modified from those of Lasker et al. (324). Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; ±, weakly reactive in 28 days. All strains are negative for acid production from the following sugars: adonitol, dulcitol, erythritol, lactose, melibiose, raffinose, and starch.

[b] In Gordon's carbohydrate basal medium (49).

genus *Rhodococcus* may be rough, smooth, or mucoid and pigmented buff, cream, yellow, coral, orange, or red. Colorless colony variants may also occur, particularly for *R. equi.*

Identification. (i) Physiologic and biochemical methods. *R. equi* does not ferment carbohydrates in fermentative basal medium with Andrade's indicator (112), but in Gordon's basal medium (49), it produces acid via the oxidation of glucose in 14 days. A few strains produce acid from maltose after prolonged incubation. In oxidative-fermentative basal medium (112), acid production from glucose is variable. The majority of the classic pink, slimy isolates hydrolyze adenine. No isolates hydrolyze casein, hypoxanthine, tyrosine, or xanthine or grow in the presence of lysozyme. The criteria useful for differentiating *Rhodococcus* spp. are given in Table 7. However, this panel of phenotypic characteristics does not adequately differentiate all *Rhodococcus* spp., as can be seen from the similar patterns generated with this battery of biochemical tests for *R. maris* and *R. coprophilus*. Table 4 lists the characteristics that differentiate the genus *Rhodococcus* from related genera. Since *Rhodococcus* spp. may be acid fast, their differentiation from *Mycobacterium* spp. is based on the absence of arylsulfatase activity in the former after 14 days. Most *Corynebacterium* spp. are easily differentiated, since *Corynebacterium* spp., except for "*Corynebacterium aquaticum*," *Corynebacterium minutissimum*, and CDC group B-1, have a fermentative type of carbohydrate metabolism. *Nocardia* spp. are differentiated on the basis of the absence of the production of aerial hyphae in *Rhodococcus* spp.

Using numerical taxonomy, Goodfellow et al. (207) established the homogeneity of the recently redefined genus *Rhodococcus* in 1990. These investigators examined 99 test strains for 202 characters, including the rapid enzyme procedures based upon fluorophores (see "Recent Advances," below). For their final analysis, 77 of these characters were deleted because they contained no separation value, and 16 multimember and 23 single-member clusters were defined by a matching coefficient value at or above the 89% similarity level (207). Only 13 of these clusters corresponded to the validly described *Rhodococcus* species, including *R. aichiensis*. The remaining 26 clusters could not be assigned to a species. This study indicates the possibility that many of the rhodococci belong to taxonomic groups that have yet to be characterized and named.

In this numerical taxonomy study (207), *R. equi* was the only validly recognized species that was below the 96% similarity level, suggesting that this species may be more heterogeneous than the other described species. In an earlier study, Butler et al. (89) analyzed the mycolic acids of some *Rhodococcus* spp. by using high-performance liquid chromatography and found a single chromatographic pattern for both *R. erythropolis* and *R. rhodochrous* but two different chromatographic patterns for *R. equi*. The mycolic acid pattern with the shorter carbon chain length included *R. equi* type strain NCTC 1621 (ATCC 6939)

(89). In a subsequent analysis of human clinical isolates, type strain ATCC 6939, and some of the same reference strains as those used by Butler et al., McNeil et al. (380) found three mycolic acid patterns for *R. equi*. Two of these patterns corresponded to those described by Butler et al.; however, an additional pattern that was intermediate between the patterns previously designated by Butler et al. (89) was also found.

Gotoh et al. studied *R. equi* isolates from swine and also found marked differences in their mycolic acid chromatographic patterns (223). On the basis of the average length of the carbon chain in each isolate, *R. equi* isolates were classified into three patterns (223). Furthermore, these investigators found that the mycolic acids with relatively longer carbon chains were derived from *R. equi* isolates that caused abscesses in the lymph nodes of swine, while the mycolic acids with shorter carbon chains were derived from *R. equi* isolates from normal tonsils.

It is difficult to compare these three studies (89, 223, 380), since there were no common internal control strains in the study of Gotoh et al. (223). It is apparent that mycolic acid patterns constitute useful phenotypic markers for the rhodococci; however, representative strains from all of the 13 validly described *Rhodococcus* spp. must be characterized before the determination of mycolic acid patterns can be considered a potentially useful diagnostic procedure for these species in clinical laboratories.

The pathogenic potential of members of the relatively new genus *Rhodococcus* and of *R. equi* in particular may be underrecognized because the detection of these microorganisms in clinical specimens may be considered environmental contamination or skin colonization. Of particular importance in HIV-infected patients is the fact that these microorganisms contain mycolic acids in their cell walls, a fact which may result in their being misidentified as acid-fast, nontuberculous mycobacteria. In the majority of reported cases, the diagnosis of *R. equi* pulmonary infection may be established by direct microscopic examination and culturing of patient sputum. Clinical laboratories that perform acid-fast staining of sputum, bronchoalveolar lavage fluid, and stool specimens from AIDS patients for mycobacteria should be able to identify *R. equi* and thus exclude the latter whenever partially acid-fast coryneform or diphtheroid bacilli are isolated from clinical specimens.

(ii) Typing and subtyping. Ribotype analysis may provide a rapid and reliable adjunct to both currently used biochemical and DNA relatedness analyses in the identification of clinical and environmental *Rhodococcus* sp. isolates. In a recent study by Lasker et al., ribotype analysis of *Rhodococcus* spp. was found useful for species identification, and the results were strongly correlated with those of DNA hybridization (324). This study found that ribotyping was also a potentially useful epidemiologic tool for testing the relatedness of *R. equi* isolates, since there appeared to be considerable interstrain variation in the patterns of the rRNA gene bands. The implementation of new computer-imaging analysis techniques may also greatly assist in these applications. Finally, the currently used methods for isolating DNA, Southern blot analysis, and hybridization analysis, which are needed for ribotype analysis, are performed routinely only in molecular biology laboratories; modification of the technique will be necessary before it can be applied in routine microbiology laboratories.

Histopathology

On histopathologic examination, especially of lung tissue, there are often multiple abscesses, marked interstitial fibrosis, and fibrinous exudates. Typically, the lesion is densely infiltrated with macrophages with an eosinophilic granular cytoplasm that is periodic acid-Schiff stain and GMS stain positive; these macrophages resemble those seen in Whipple's disease (526). Intracellular and extracellular cocci may be evident in Brown-Brenn-stained tissue sections. These coccal forms may also be seen in GMS-stained preparations (Fig. 11).

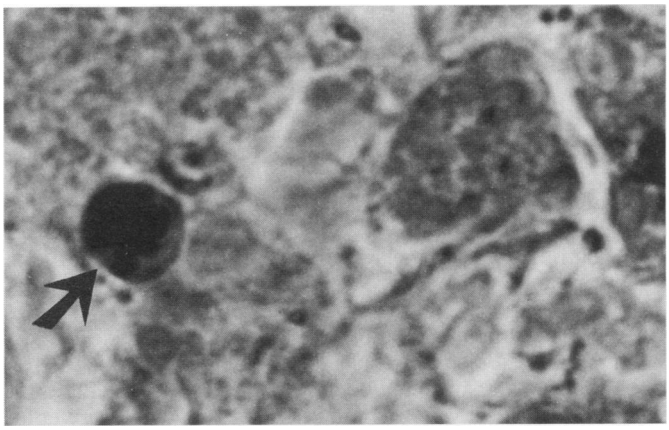

FIG. 11. *R. equi* intracellular coccal forms (arrow) in a macrophage in a section of human lung tissue. GMS stain. Magnification, ×840. (Reproduced from the *Journal of Acquired Immune Deficiency Syndrome* [363] with permission of the publisher.)

Antimicrobial Susceptibility Testing and Therapy

Susceptibility testing. The methodology used for antimicrobial susceptibility testing for *Rhodococcus* spp. is similar to that used for all other aerobic actinomycetes, except that the incubation time for *R. equi* isolates is usually only 1 day. The other *Rhodococcus* spp. require 2 days of incubation.

Antimicrobial susceptibility. Recent results of in vitro antimicrobial susceptibility studies of *R. equi* isolates are presented

TABLE 8. Antimicrobial susceptibility test results for *R. equi* patient isolates[a]

Antimicrobial agent(s)	MIC (μg/ml)[b]		No. (%) of resistant isolates (n = 98)[c]
	Median	Range	
Amoxicillin-clavulanate	4/2	≤2/1–>8/4	0 (0)
Ampicillin	4	≤0.12–>16	67 (68)
Ampicillin-sulbactam	8/4	≤8/4–>32/16	0 (0)
Cephalothin	32	1–>32	60 (61)
Ciprofloxacin	1	≤1–>4	18 (18)
Clindamycin	4	0.25–>8	49 (50)
Erythromycin	0.5	≤0.12–>8	3 (3)
Gentamicin	0.5	≤0.25–>16	0 (0)
Imipenem	≤2	≤2	0 (0)
Norfloxacin	8	≤4–>16	16 (16)
Oxacillin	>8	<0.12–>8	66 (67)
Penicillin	4	≤0.12–>8	58 (59)
Rifampin	1	≤1–>8	4 (4)
Tetracycline	4	≤2–>16	4 (4)
Trimethoprim-sulfamethoxazole	≤2/38	≤2/38–>16/304	1 (1)
Vancomycin	≤0.5	≤0.5–>32	1 (1)

[a] Reprinted from the *European Journal of Epidemiology* (381) with permission of the publisher.
[b] Broth microdilution method.
[c] National Committee for Clinical Laboratory Standards criteria.

in Table 8. In the largest of these studies, which tested 98 human clinical isolates against 16 antimicrobial agents, the isolates were usually found to be resistant to ampicillin, cephalothin, clindamycin, oxacillin, and penicillin (50% or more resistant isolates) (381). In addition, resistance to the quinolones was less frequent (ciprofloxacin, 18%; and norfloxacin, 16%), and a minority of the isolates (<5%) were resistant to erythromycin, rifampin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin (381). No isolates were resistant to amoxicillin-clavulanate, ampicillin-sulbactam, gentamicin, and imipenem.

Two other large antimicrobial susceptibility studies of *R. equi* isolates of soil and animal origins, by Barton and Hughes (66 isolates) (36) and Woolcock and Mutimer (100 isolates) (647), revealed a high degree of resistance to sulfonamides (58 and 50%, respectively). In contrast, in a study by Decre et al., in which five human isolates and three animal isolates were tested in vitro, sulfamethoxazole was found to be one of the most active antimicrobial agents for both human and animal isolates (136). Such discrepancies between the results of antimicrobial susceptibility studies are not readily explainable but may represent differences in the populations of isolates tested, variability in test methodology, or both.

Woolcock and Mutimer reported that all of the isolates that they studied were susceptible or moderately susceptible to penicillin (647). In contrast, 95.5% of the isolates studied by Barton and Hughes were resistant to penicillin (36), and 59% of the isolates studied by McNeil and Brown were resistant to penicillin (381). In addition, in a recent study of 13 *R. equi* isolates, Parquin et al. found that 5 of 6 patient isolates had acquired resistance to beta-lactams (437). Numerous other recent reports of antimicrobial susceptibility studies have included only a limited number of human clinical isolates (81, 114, 136, 188, 420). A meaningful comparison of the results of these studies is possible despite their use of different test methodologies.

Because of the successful treatment of *R. equi* infections in foals with the combination of erythromycin and rifampin (253, 294) and the ability of both drugs to penetrate macrophages and kill *R. equi*, the efficacy of these two antimicrobial agents alone and in combination has been studied. Clave et al. recently compared the antimicrobial susceptibilities of seven clinical and two reference *R. equi* isolates (114). Three of the clinical isolates were obtained sequentially from one patient. One isolate, obtained 3 months after the patient had begun treatment with the combination of rifampin and erythromycin, showed the emergence of resistance to rifampin. For the second isolate, obtained following a change in this patient's therapy to vancomycin-imipenem, there was a marked increase in the MIC of imipenem (114). Thus, this study indicates that erythromycin-rifampin may not be an effective therapeutic combination for the treatment of human infections.

In another recent study of four human *R. equi* clinical isolates, by Nordmann and Ronco, one isolate demonstrated resistance to rifampin and the quinolones (420). However, this resistance may have been selected for by monotherapy, as these antimicrobial agents were used successively in the therapy of the patient. Nordmann and Ronco studied MICs and MBCs of 36 antimicrobial agents for their *R. equi* isolates. The results of this study showed that for the beta-lactams that they tested, the lowest MICs were those of imipenem (0.25 μg/ml) and meropenem (0.5 μg/ml); however, no beta-lactams were bactericidal at clinically achievable levels in serum. In addition, the aminoglycosides amikacin, gentamicin, and netilmicin had both low MICs and low MBCs. The macrolides (clarithromycin, erythromycin, roxithromycin, and spiramycin) had low MICs but high MBCs. For the glycopeptides tested, the MICs were low, but only vancomycin was bactericidal. The MICs of rifampin were low, but the MBCs were high.

In another study, by Brown et al., the antimicrobial activity of erythromycin was compared with that of the newer macrolides—azithromycin, clarithromycin, and the major metabolite of clarithromycin, 14-hydroxyclarithromycin—against nine human *R. equi* clinical isolates; results were expressed as MBCs and MICs (81). This study found that clarithromycin, 14-hydroxyclarithromycin, and a 3:1 combination of the two were all highly active; the MIC for 90% of isolates was ≤0.125 μg/ml; that of erythromycin and azithromycin was 1 μg/ml. However, the MBC of clarithromycin for 78% of isolates was ≥16 μg/ml. In contrast, for eight of the nine isolates studied, the MIC and MBC of tobramycin were ≤4 μg/ml. Of interest, the finding of a low MBC for tobramycin was consistent with the results of the earlier study by Nordmann and Ronco, who obtained good bactericidal activity with other aminoglycosides (amikacin, gentamicin, and netilmicin) (420). One of the patients in the study by Brown et al. was successfully treated with the combination of clarithromycin and rifampin and surgery followed by long-term clarithromycin therapy (81).

Nordmann and Ronco also studied the frequencies of selection of mutants resistant to eight antimicrobial agents, including amikacin, ciprofloxacin, erythromycin, imipenem, minocycline, rifampin, trimethoprim-sulfamethoxazole, and vancomycin (420). The concentrations of the antimicrobial agents used were 5- and 10-fold their respective MICs. Antibiotic-resistant mutants were selected in vitro at a range of 2×10^{-8} for vancomycin to 5×10^{-4} for amikacin. There was no detectable frequency of mutation to erythromycin resistance and trimethoprim-sulfamethoxazole resistance. The high in vitro mutation frequency for resistance to the beta-lactam imipenem (range, 2×10^{-5} to 9×10^{-5}) plus the high MBCs (>256 μg/ml) of all the beta-lactams may be associated with treatment failure when these antimicrobial agents are used as monotherapy for *R. equi* infections.

In addition, Nordmann and Ronco determined fractional inhibitory concentration indices for combinations of antimicrobial agents (420). Criteria for the selection of the antimicrobial agents tested in their study included low MIC, low MBC, high maximum concentration/MIC ratio, and the availability of oral and intravenous preparations. The synergistic combinations tested were rifampin-erythromycin, rifampin-minocycline, erythromycin-minocycline, and imipenem-amikacin. Erythromycin-amikacin was the only antagonistic combination of antimicrobial agents tested. The frequencies of selection of antibiotic-resistant mutants were determined at concentrations 5- and 10-fold the MICs and ranged from $<1 \times 10^{-8}$ for erythromycin and trimethoprim-sulfamethoxazole to 5×10^{-4} for amikacin. However, combinations of antimicrobial agents, even at fivefold the MICs, reduced the mutation frequencies for resistance to all antimicrobial agents to $\leq 1 \times 10^{-8}$. This result illustrates why combination antimicrobial therapy is more beneficial than monotherapy, which is associated with frequent mutations in vitro (420).

Since infections with *R. equi* have been successfully treated with antimicrobial agents with a low level of uptake by macrophages, such as vancomycin, imipenem, and aminoglycosides (157, 243, 613), and relapses have occurred in patients who have received therapy with antimicrobial agents with a high level of uptake by macrophages, such as rifampin, erythromycin, doxycycline, and trimethoprim-sulfamethoxazole (157, 243,420), Nordmann and Ronco concluded that both the in vitro and the in vivo properties of potential antimicrobial agents are important considerations in the selection of appro-

priate therapy for *R. equi* infections (420). In addition, these researchers suggested that the frequent requirement for long-term therapy in the treatment of *R. equi* infections makes the development of a more effective oral antimicrobial agent(s) imperative (420).

Antimicrobial therapy. In 1992, Nordmann et al. (419) investigated the in vivo activity of several antimicrobial agents and antimicrobial combinations against *R. equi* inoculated intravenously into nude mice that were congenitally T-lymphocyte deficient. The efficacy of the antimicrobial agents was determined on the basis of the reduction of bacterial CFU per gram in the lungs and spleen after 4 and 11 days of therapy. Of the selected antibiotics with low MICs, amikacin, ciprofloxacin, erythromycin, imipenem, minocycline, vancomycin, imipenem, and rifampin were the most effective agents in monotherapy. Amikacin, ciprofloxacin, erythromycin, and minocycline alone were not effective in this model. The most effective drug combinations were those including vancomycin. The combinations of imipenem and vancomycin and of imipenem and teicoplanin (another glycopeptide) were recently used successfully in the treatment of *R. equi* infections (107, 491). Nordmann et al. considered that trimethoprim-sulfamethoxazole and clarithromycin, which were not included in their study, may be interesting to study with this nude mouse model (419). These antimicrobial agents have low MICs against *R. equi* and antimicrobial activity against other pulmonary opportunistic pathogens (*P. carinii* and rapidly growing mycobacteria) that may coexist with *R. equi* in AIDS patients.

Experience in the therapy of non-*R. equi Rhodococcus* infections is extremely limited. Therapy with various antimicrobial agents has been reported to be successful in a few cases (106, 234, 528, 556). In these infected patients, the choice of a specific drug therapy is perhaps best guided by the results of antimicrobial susceptibility testing of the isolates from these patients (234).

Opportunistic *R. equi* infections in HIV-infected patients may be very difficult to treat. The drugs of choice for aerobic actinomycete infections have been the sulfonamides (or trimethoprim-sulfamethoxazole). It has been recommended that therapy be given for a period of up to 1 year. However, on the basis of some limited reviews of the antimicrobial susceptibility of *R. equi*, it has been suggested that these drugs may not be effective therapy for *R. equi*-infected patients. Frequent severe reactions to this class of antimicrobial agents have been described, in particular, for HIV-infected patients (214). Drug therapy for *R. equi* infections in HIV-infected patients may also be complicated by the development of acquired resistance to beta-lactam antimicrobial agents (437, 500, 631).

Several antibiotics are active in vitro against *R. equi* (243). Erythromycin and rifampin are the most effective because they produce high concentrations inside phagocytic cells (463), in which *R. equi* appears to be capable of multiplying. Moreover, these drugs act synergistically when used together (459). However, despite its in vitro susceptibility to various antimicrobial agents, *R. equi* is difficult to eradicate even after prolonged therapy (172, 309, 363, 500, 613, 631). Thus, relapses are frequently observed and may occur even after surgical resection of the affected lung segments (57, 236, 500). Several measures are important in the effective therapy of infected patients. Therapy with antimicrobial agents that are active against intracellular microorganisms or possess in vitro activity against the infecting microorganisms needs to be instituted rapidly. Resection of lung tissue as a therapeutic adjunct should be considered. Following such therapy, patients should be observed on a continuing basis for evidence of relapse.

There is no optimal antimicrobial therapy for *R. equi* infections. In some immunocompromised patients with localized pulmonary infections, antimicrobial therapy has been supplemented by surgical resection of the affected lung segments (95, 637). Also, effective antimicrobial therapy of *R. equi* infections in HIV-infected patients, other immunocompromised patients, and nonimmunocompromised patients has been hindered by the lack of an accepted, standardized method for antimicrobial susceptibility testing of *R. equi* or other aerobic actinomycetes.

Pathogenicity Studies

Although several *Rhodococcus* spp. have been isolated in studies of human infections, like most actinomycete pathogens, *Rhodococcus* spp. are opportunistic rather than invasive. In 1978, Haburchack et al. showed that steroid-treated guinea pigs inoculated with *Rhodococcus* spp. developed visible granulomas over the peritoneum and liver capsule (234). In their study, mycelial forms of these microorganisms were demonstrated on smears of the liver and lungs; *Rhodococcus* spp. were isolated from these tissues (234). In 1989, Osoagbaka (433) inoculated immunosuppressed white mice with *R. ruber* (*Rhodococcus pellegrino*) and found the pathologic appearance of the lungs to be compatible with that shown by Haburchack and colleagues (234). However, Osoagbaka was able to demonstrate on impression smears of the lungs and liver of guinea pigs (433) coccoid forms intracellularly in the macrophages and extracellular coccobacillary forms but not the filamentous forms seen by Haburchack et al. (234).

Variations in the virulence of *R. equi* isolates in experimentally infected mice and foals have been seen. The type strain does not cause pneumonia in foals (371, 575) and is avirulent in mice (409, 577). The virulence of *R. equi* isolates for mice infected intravenously is related to the ability of the isolates to resist clearance from the liver and spleen by resisting phagocytosis and intracellular killing by mouse macrophages (409, 577).

On the basis of data from experimental infections, clinical isolates appear to be more pathogenic than environmental isolates; however, the reason for this difference in virulence has not yet been determined (73). Variations in the pathogenicity of *N. asteroides* for mice have been attributed to the presence of particular mycolic acids (44). The more virulent microorganisms have a higher percentage of long-chain mycolic acids in their cell walls, whereas the less virulent microorganisms have a higher percentage of short-chain mycolic acids (44).

Two distinct mycolic acid patterns have been described for a limited number of *R. equi* isolates, including the avirulent type strain (89). In a recent study, McNeil et al. identified three distinct mycolic acid patterns among a collection of *R. equi* reference strains and clinical isolates; these patterns were also associated with differences in the antimicrobial susceptibility and colony morphology of these strains and isolates (380). In another study, Gotoh et al. associated three different mycolic acid patterns of *R. equi* isolates with their virulence in vivo in a mouse model (223). In this study, *R. equi* isolates having longer carbon chains for mycolic acid-glycolipid showed a higher degree of virulence, as determined by lethality and granuloma formation in mice, than did those having shorter carbon chains (223). In addition, when purified glycolipid was injected into mice, granuloma formation and liver damage were most prominent with the glycolipid having longer-carbon-chain mycolic acids. These results suggested that glycolipid may be a potential virulence factor of *R. equi* or may be a marker for an underlying virulence factor.

Other candidate virulence factors are the variable antigenic polysaccharide capsule and the exoenzymes, or "equi factors," cholesterol oxidase and phospholipase C. The polysaccharide capsule has been used as the basis for a serotyping system. With this system, 27 or more different capsular serotypes have been identified; of these, capsular serotype 1 is the most prevalent worldwide (410). Although no direct correlation between capsular serotype and virulence has been demonstrated, the polysaccharide capsule may inhibit phagocytosis of *R. equi* (458). In 1982, Linder and Bernheimer (348) used an in vitro model to demonstrate the synergistic lysis of sheep erythrocytes with the exoenzymes cholesterol oxidase and phospholipase C, produced by *R. equi*, and phospholipase D, produced by *Corynebacterium pseudotuberculosis*. These equi factors appear to exert their effects on components within the cell membranes of erythrocytes (348). The significance of these equi factors with respect to the virulence of *R. equi* is not known. It is possible that the action of equi factors on the liposomal membrane contributes to the cell degeneration that occurs after foal alveolar macrophages ingest viable *R. equi* (660).

In 1991, Takai et al. analyzed antigens from *R. equi* by immunoblotting with naturally infected foal sera (576). Culture supernatants and whole-cell antigens of clinical isolates of *R. equi* revealed major protein bands with molecular masses of 15 to 17 kDa, and all isolates containing these antigens were virulent for mice (576). In contrast, the 15- to 17-kDa antigens were not present in type strain ATCC 6939, which was avirulent for mice. In addition, 77 of 102 environmental isolates lacked the 15- to 17-kDa antigens and were avirulent for mice (576). Later in that year, Takai et al. (578) further evaluated 10 isolates of *R. equi* by comparing the immunodot assays for the 15- to 17-kDa antigens, plasmid profiles, and murine pathogenicity. All of the isolates containing the 15- to 17-kDa antigens contained a large plasmid of approximately 85 kb and were virulent for mice.

At the same time, Tkachuk-Saad and Prescott (588) isolated plasmids from 54 isolates of *R. equi* from different clinical sources. A plasmid of approximately 80 kb was isolated from 21 of 22 isolates from horses and 20 of 28 isolates from pigs. A larger plasmid, of approximately 105 kb, was isolated from 7 of 28 pigs. The type strain, ATCC 6939, consistently failed to yield a plasmid. There was a significant but not exact association between the presence of the 80-kb plasmid and the production of a diffuse 17.5-kDa thermoregulated virulence-associated protein described by Takai et al. (578). In 1991, Takai et al. showed that virulent *R. equi* contained a large plasmid and that curing of this plasmid coincides with a loss of detectable 15- to 17-kDa antigens and a striking decrease in lethality in mice (578).

In 1992, Takai et al. further characterized the virulence-associated 15- to 17-kDa antigens of *R. equi* (574). The expression of these antigens was regulated by temperature: cells grown at a low temperature (25 to 32°C) did not express them; however, cells grown at a higher temperature (34 to 41°C) expressed these antigens. These antigens were located on the externally exposed surface, as shown by their susceptibility to digestion by an exogenously added protease, trypsin. In addition, when intact cells were biotinylated, labeled 15- to 17-kDa antigens were detected with avidin-peroxidase and a color indicator (574).

Recently, Takai et al. (579) surveyed for plasmid DNA among isolates obtained from 23 different *R. equi*-infected foals that were diagnosed as having the infection at the time of postmortem examination. Of the 23 clinical isolates, 19 contained an 85-kb plasmid and the remaining 4 contained a 90-kb plasmid. All of the isolates contained the 15- to 17-kDa antigens and were virulent for mice. When these 85- and 90-kb plasmids were examined by restriction enzyme and Southern blot analyses, there were large regions of DNA homology, which suggested that they have a common origin (579). To date, no virulence studies with human isolates have been reported.

In 1992, Hondalus et al. developed an *R. equi* radiobinding assay that uses bacteria labeled with [^{3}H]uracil (265). This radiolabeling technique was used to quantitate the attachment of *R. equi* to both murine peritoneal and foal alveolar macrophages adherent to glass coverslips. Binding was dose dependent, saturable, and specific for macrophages and was enhanced in the presence of fresh serum. This assay represented an initial important step in identifying the macrophage receptors that are involved in the recognition of *R. equi* and may help to provide information on how macrophages recognize intracellular bacteria in general (265).

In 1993, Hondalus et al. (264) identified the macrophage receptors that mediate the binding and ingestion of *R. equi*. Binding to mammalian cells required complement and was mediated primarily by the leukocyte complement receptor Mac-1, also termed complement receptor type 3. *R. equi* does not bind to fibroblastoid or epithelial cells that lack this receptor. *R. equi* binds poorly to macrophages unless exogenous complement is added to the incubation medium. Heat inactivation of complement or immunologic depletion of complement component C3 reduced the binding of *R. equi* to Mac-1 receptors (188). Other intracellular microorganisms (e.g., *Leishmania major* [404], *Legionella pneumophila* [440], *Cryptococcus neoformans* [345], *Listeria monocytogenes* [150], *M. tuberculosis* [520], and *Mycobacterium leprae* [521]) fix complement by activating the alternative pathway and then bind to macrophages via their complement receptors. In most of these cases, however, these microorganisms have an alternative method of binding to macrophages that is independent of complement and often adhere to other, nonmacrophage cell types (150, 404, 426, 440). In contrast to these microorganisms, *R. equi* bound only to cells expressing complement receptor Mac-1 in vitro and bound only in the presence of a functionally opsonic complement system (264). This dependence of *R. equi* on a specific complement receptor for cell attachment is unique and may explain the in vivo observation that *R. equi* binding is limited to macrophages that express complement receptor Mac-1 (264).

The immunohistochemical features of pneumonic lesions caused by *R. equi* infection in foals were studied by Ishino et al. (273) with a biotin-streptavidin indicator system (Biogenex Laboratories, San Ramon, Calif.). This study used specific antibodies prepared against *R. equi*, lysozyme, α_1-antitrypsin, α_1-antichymotrypsin, and *Mycobacterium bovis* BCG and immunoglobulins to clarify the roles of these agents in the infection. In comparison with tissue examination by the Gram stain method, immunostaining with anti-*R. equi* serum was found to be more sensitive for the demonstration of intracellular *R. equi* in macrophages. Ultrastructural examination showed that most of the *R. equi* microorganisms appeared intact. These findings suggest that microbial surface components may be important factors in the protection of these microorganisms from the effects of intracellular enzymes of macrophages. Anti-BCG antibody also demonstrated reactivity with *R. equi* microorganisms. This result may have been due to a relative similarity between the surface components of mycobacteria and those of *R. equi*. Although this study did not include a detailed analysis of surface components, the investigators speculated that the surface components of *R. equi* may

resist lysozyme action or otherwise affect the activity of lysozyme within macrophages (273). Lysozyme and the antienzymes α_1-antitrypsin and α_1-antichymotrypsin have been demonstrated in the cytoplasm of macrophages (286). In the study by Ishino et al., these enzymes were found to be activated in macrophages that contained *R. equi*; an especially intense stain was shown with antibody to lysozyme (273). In addition, cells containing intracytoplasmic immunoglobulin M, immunoglobulin G, and immunoglobulin A were noticeably few in number and scattered predominantly around the lesions in *R. equi*-induced pneumonia (273). However, Hietala and Ardans showed that lymphocyte factors derived by in vitro incubation of sensitized peripheral blood lymphocytes with *R. equi* surface antigens enhanced macrophage bactericidal activity (252).

The role of humoral antibodies in *R. equi* infections is considered to be weak in comparison with that of phagocytosis by macrophages. However, the relative contributions of cell-mediated immunity and humoral immunity in resistance against *R. equi* infections are controversial (458). The immunoprophylactic capacity of specific immune plasma in foals with an experimentally induced *R. equi* infection has been reported (372). In 1989, Martens et al. established that the administration of immune plasma before a challenge with *R. equi* decreased the severity of pneumonia in foals (372). They speculated that the protective effect of passive immunization might result from the presence of specific opsonizing antibodies to surface structures, cholesterol oxidase, and phospholipase C or from nonspecific humoral factors, such as lymphokines and complement. However, in 1991, Machang'u and Prescott showed that the antibody responses of foals to immunizations with partially purified cholesterol oxidase and phospholipase C were poor and that antibodies to these exoenzymes did not provide protective immunity against *R. equi* pneumonia in foals (361). The higher incidence of *R. equi* infection in very young foals than in older equines may be explained by the lack of maturity of mechanisms of humoral immunity, alveolar macrophage function, and cell-mediated immunity in the former (252). In 1992, Nordmann et al. studied the mechanism of acquired resistance to *R. equi* infection in the murine model by using the intravenous route of inoculation (421). They found that vaccination with live bacteria conferred a degree of immunity, while a killed vaccine was not protective (421). This finding was in agreement with those of similar reports for other intracellular pathogens (e.g., *L. monocytogenes*) (297). Adoptive transfer of resistance was obtained with spleen cells but not immune serum from mice immunized 30 days earlier with live bacteria, suggesting that humoral immunity was not involved in resistance (297). However, these results are contrary to the previous finding that immune plasma can protect foals against a fatal *R. equi* infection (372). Whether this discrepancy results from species-specific differences in the infected animals (foals versus mice) or differences in the route of inoculation (respiratory versus intravenous) is yet to be resolved. For normal mice, clearance from the spleen, liver, and lungs within 3 weeks of an intravenous challenge with 5×10^6 CFU of *R. equi* was obtained, whereas athymic nude mice were unable to clear the bacteria (421). In addition, in vivo depletion by monoclonal antibodies showed that both $CD4^+$ (helper) and $CD8^+$ (suppressor) T-cell subsets participate in the clearance of bacteria (421). However, in vivo depletion of the $CD8^+$ T-cell subset alone led to a marked increase in CFU in the liver and spleen, suggesting that $CD8^+$ T cells play a more important role in eradicating *R. equi* than do $CD4^+$ T cells (421). These studies emphasize the importance of both humoral immunity and cell-mediated immunity in protection against *R. equi* infections and reinforce the conclusions from various reports that in AIDS patients it is cell-mediated immunity in particular that provides resistance to pulmonary and disseminated *R. equi* infections (149, 613).

OTHER OPPORTUNISTIC PATHOGENIC ACTINOMYCETES

Taxonomy and Clinical and Epidemiologic Aspects

Amycolata autotrophica. *A. autotrophica* is a rare human pathogen that has recently been removed from the genus *Nocardia*. The reclassification was done on the basis that the genus *Amycolata* lacks mycolic acids; however, its cell wall composition is remarkably similar to that of true nocardiae (339).

During the last 20 years, one published report has suggested that this unusual aerobic actinomycete is a potential human pathogen (103). The authors reviewed the case histories of eight patients. No patient in the review had histopathologic evidence of infection. However, there was strong evidence supporting this actinomycete as a pathogen in one patient with purulent pericarditis and suggestive evidence for three other patients. Of these three, one had meningitis, one had a leg wound, and one had sepsis associated with bone marrow hypoplasia. For the four remaining patients, *A. autotrophica* was judged to have probably not been a significant pathogen.

When the methods described by Gordon (49) were used, the morphologic, physiologic, and biochemical studies of *A. autotrophica* isolates confirmed the homogeneous nature of this species. *A. autotrophica* was found to be gram positive, with branched filaments that were not acid fast, as determined by the Kinyoun method. Aerial hyphae were abundant. All isolates decomposed esculin, hypoxanthine, tyrosine, and xanthine but did not decompose casein. None of the isolates grew in the presence of lysozyme. All isolates produced acid from adonitol, arabinose, fructose, galactose, glucose, maltose, mannitol, mannose, sorbitol, sucrose, trehalose, and xylose. No acid was produced from dulcitol, lactose, raffinose, rhamnose, or starch. Acid production from inositol and salicin was variable.

In the same study (103), pathogenicity in mice was tested by intraperitoneal inoculation. Only 50% of the isolates produced grossly visible peritoneal, hepatic, or splenic abscesses within 7 days. Although definite proof of the pathogenicity of *A. autotrophica* remains to be shown, this study (103) strongly inferred that, in isolated instances, this species may be the cause of significant disease.

A. orientalis. *A. orientalis* (*N. orientalis*; "*Streptomyces orientalis*") is primarily known for the production of the antimicrobial agent vancomycin (453). In 1978, Gordon et al. reviewed five groups of isolates that resembled nocardiae and compared these groups with the recognized species of the genus *Nocardia* (221). One of these groups, comprising 21 isolates, was initially identified as *N. orientalis* (221). Although these isolates had a cell wall composition similar to that of true nocardiae, they did not contain mycolic acids. Subsequently, *N. orientalis* was removed from the genus *Nocardia* and assigned to the genus *Amycolatopsis* (339) (Table 1).

The genus *Amycolatopsis* is characterized by the presence of *meso*-DAP, arabinose, and galactose but not mycolic acids. The predominant menaquinone is MK-9 (H_2,H_4). This species does not grow in the presence of lysozyme. When formed, aerial hyphae produce cylindrical, occasionally ovoid conidia in straight to flexuous chains. Substrate hyphae branch frequently and appear to zigzag in places. Casein, hypoxanthine, xanthine, and tyrosine are decomposed, but adenine is not. Esculin is hydrolyzed. Acid is produced from adonitol, arabinose, cello-

biose, erythritol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, trehalose, and xylose, as determined with Gordon's carbohydrate basal medium (49).

A. orientalis grows at 10 to 42°C. Of the 21 isolates studied by Gordon et al. (221), most were isolated from soil and vegetable matter. The sites of isolation for two isolates were reported to be cerebrospinal fluid and an unknown clinical source (221). Nothing more is known about the pathogenicity of this microorganism.

***Micromonospora* spp.** The members of the genus *Micromonospora* have rarely been encountered in human clinical specimens. In a recent review of clinical isolates received by the CDC Actinomycete Laboratory during a 29-month study period, only 6 of 366 were identified as members of this genus (383). To our knowledge, no descriptions of human infections have been published.

This genus is known primarily as the microbiologic source of clinically significant antimicrobial agents, especially the aminoglycosides and macrolides. In 1989, Bibikova et al. studied 500 isolates of *Micromonospora* spp. and were able to subdivide this genus into nine groups on the basis of culture and morphologic characteristics, the ability to produce certain chemical classes of antimicrobial agents, and susceptibility to 18 different antimicrobial agents (56). Groups designated V and VI, i.e., black-pigmented *Micromonospora* isolates, produce mostly aminoglycoside antimicrobial agents, while brown-pigmented groups II and III form macrolide antimicrobial agents (56).

In clinical microbiology laboratories, the identification of these species is based upon the results of morphologic, physiologic, and biochemical studies obtained by use of methods initially described by Gordon (221) and subsequently modified by Berd (49). These microorganisms are gram positive, have branched filaments, and do not stain acid fast. Nonmotile spores are borne singly, in a sessile manner, or on short or long sporophores that often occur in branched clusters. The spores occur only on substrate hyphae; aerial hyphae are absent. The cell walls contain *meso*-DAP, its 3-hydroxy derivative, or glycine. The characteristic cell wall sugars are xylose and arabinose. No mycolic acids are found.

Colonies on agar media are initially pale yellow to light orange. With maturity, the colonies become progressively darker because of the production of brown to black spores. The production of single spores on substrate hyphae is one of the well-defined characteristics of this genus. Although some species have been reported to have maroon-purple and blue-green pigments, they have not been observed in clinical laboratories.

Most *Micromonospora* isolates are strongly proteolytic; all isolates from human clinical specimens have hydrolyzed casein. In addition, they have hydrolyzed esculin, and 75% have hydrolyzed tyrosine. No isolates hydrolyze xanthine or hypoxanthine. None of the isolates grow in the presence of lysozyme. The isolates may show differences in the production of acid in the carbohydrate basal medium of Gordon, as described by Berd (49), from the carbohydrates glucose, rhamnose, and trehalose; however, all of the isolates produce acid from arabinose, fructose, galactose, lactose, and xylose.

Before the analysis of whole-cell hydrolysates was introduced as a diagnostic tool, many *Micromonospora* isolates were likely discarded as unidentified actinomycetes. The application of this test, together with the characteristic formation of orange colonies that turn black and the production of single spores, may result in the more frequent identification of these rare species in clinical laboratories (49).

***N. dassonvillei*.** The microorganism now known as *N. dassonvillei* was first isolated in 1904 from mildewed grain and named "*Streptothrix dassonvillei*" by Brocq-Rousseu (78). In 1911, Liegard and Landrieu isolated from a subject with conjunctivitis a microorganism that they considered identical to "*S. dassonvillei*" but that they assigned to the genus *Nocardia* (347). Later, Gordon and Horan reported on the similarity between the macroscopic and physiologic characteristics of "*N. dassonvillei*" and *S. griseus* (220). Subsequently, Lechevalier and Lechevalier proposed the genus *Actinomadura* to harbor "*N. madurae*," "*N. pelletieri*," and "*N. dassonvillei*" (331). In 1976, Meyer, on the basis of morphologic and biochemical characteristics, removed "*N. dassonvillei*" from the genus *Actinomadura* and placed it in the new genus *Nocardiopsis*, of the order *Actinomycetales* (387). This author described the microorganism as an aerobic, gram-positive, non-acid-fast, catalase-positive actinomycete.

Colonies on organic media are abundant, coarsely wrinkled, and folded, with a well-developed substrate mycelium. Hyphae are long and branched and fragment completely into spores. The aerial mycelium is usually well developed and abundant. The cell walls contain *meso*-DAP but no diagnostically important carbohydrate (type IIIc). Whole-cell hydrolysates do not contain either madurose or nocardia-type mycolic acids. It is important to distinguish this genus from *Actinomadura* spp. and other closely related genera (Table 3).

Whole-cell hydrolysates of this aerobic actinomycete were found to contain the *meso* form of DAP; in contrast, whole-cell hydrolysates of *Streptomyces* spp. contain the *levo* form of DAP. Specific identification is made on the basis of the lack of diagnostically important carbohydrates in whole-cell hydrolysates of the former organism and the failure of this organism to grow at 45°C and in lysozyme broth. Moreover, this actinomycete produces long zigzagging chains of arthroconidia, which are characteristic of this species (387). The sporulation process was studied in detail by Williams et al. (639). They observed that the process is initiated by a single ingrowth of the hypha wall, resulting in a cross wall. The first elements delimited are often long and are sometimes subdivided by further cross wall formation. The process is completed by the disruption of the sheath between the spores. From these observations, it seems that the characteristic zigzag arrangement of developing spore chains is caused by lateral displacement of spores within the sheath (Fig. 12).

There have been few reports of clinical disease caused by *N. dassonvillei*. There have been three reports of cutaneous infections caused by this microorganism: one was a case of mycetoma (546), the second was a familial cluster of skin infections (548), and the third occurred in an elderly man, who developed a hand abscess after a soil-contaminated injury that required surgical debridement and skin grafting; this last infection responded to oral treatment with trimethoprim-sulfamethoxazole (447). There has been a single report of a patient in whom *N. dassonvillei* was suggested to be the causative agent of extrinsic alveolitis (54). To date, there have been no reports of disseminated *N. dassonvillei* infections.

***Oerskovia* spp.** The genus *Oerskovia* has been reported to comprise two distinct species, *Oerskovia turbata* and *Oerskovia xanthineolytica*, and two types (A and B) of nonmotile *Oerskovia*-like strains (NMOs) (334). Most strains are yellow and have extensively branched substrate hyphae that grow on the surface of and penetrate into agar media. The hyphae break up into either motile (*Oerskovia*) or nonmotile (NMOs), very short or long, rod-shaped elements. The colony morphology observed with a light microscope under low power (magnification, ×100) consists of dense centers with filamentous fringes.

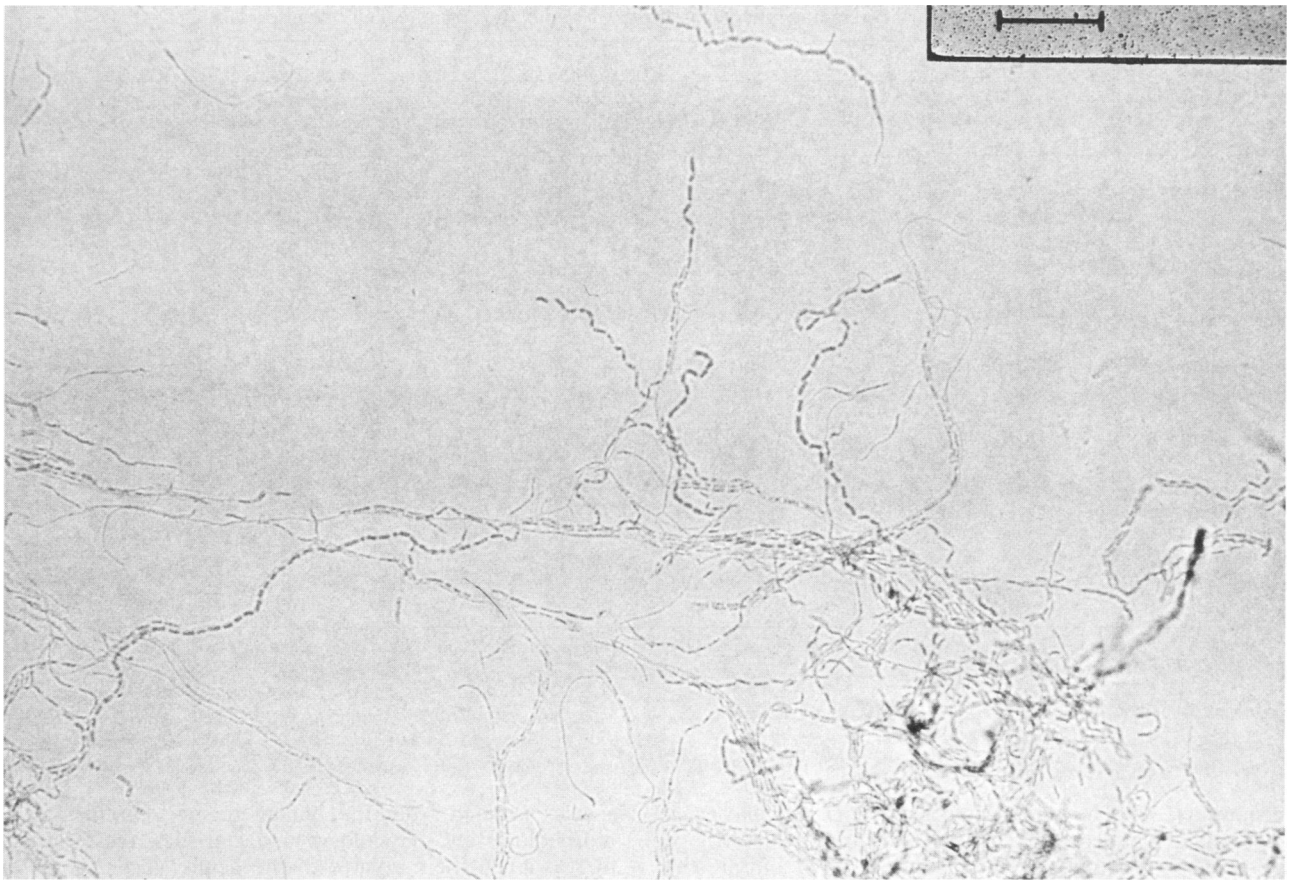

FIG. 12. *N. dassonvillei*. Long chains of oblong conidia within a sheath, often distributed in a zigzag fashion, can be seen. The slide culture preparation was grown on cornmeal agar without dextrose for 1 week at 25°C and was left unstained. Bar, 15 μm.

No aerial hyphae are formed after 7 days of incubation at 25 to 35°C. Table 9 lists the properties that differentiate the two described *Oerskovia* species, the NMOs, and related species.

Recently, the oerskoviae have evoked considerable controversy among taxonomists. On the basis of DNA-DNA relatedness studies, supported by comparative analyses of 16S rRNAs, Stackebrandt et al. (558, 561) have proposed that the oerskoviae be removed to the genus *Cellulomonas*. The basis for this proposal is that *Cellulomonas cellulans* is more closely related to members of the genus *Oerskovia* than to other members of the genus *Cellulomonas* (558, 561). These investigators argue that the first description of the genus *Cellulomonas*, in 1923, has precedence over the description of the genus *Oerskovia* Prauser in 1970 and therefore that the correct name of the genus formed by the union of *Cellulomonas* and *Oerskovia* should be *Cellulomonas*. Furthermore, as the DNA relatedness between *O. turbata* and the other species of the genus *Oerskovia* is only moderate, *O. turbata* probably does not belong in this genus.

Sottnek and associates (555) reported on the characteristics of 35 clinical isolates of the genus *Oerskovia* that were formerly included in group A-1 and A-2 *Corynebacterium* spp. Nine of the isolates were identified as *O. turbata*, and 25 were identified as *O. xanthineolytica*. These were all isolates submitted to a reference laboratory, and no clinical histories of the patients were available (555). Nine were blood isolates, five were from heart valves or cardiac tissue, one was from cerebrospinal fluid, and the remainder were from sources such as urine, sputum, wounds, eye drainage, liver, and lung biopsy. Rihs and colleagues (474) reported a case of *O. xanthineolytica* peritonitis in a patient receiving peritoneal dialysis and reviewed other reported *Oerskovia* infections. Several other cases of infection caused by *O. xanthineolytica* have been described. This species was cultured from the vitreous humor of a 47-year-old man diagnosed with traumatic endophthalmitis and from the cerebrospinal fluid of a 38-year-old woman with an indwelling ventricular shunt and associated meningitis (269, 281). In 1992, Truant and colleagues (593) reported the first case of *O. xanthineolytica* bacteremia. In 1975, Reller and colleagues published the first report of a human infection caused by *O. turbata*; the patient had endocarditis complicating a homograft of a cardiac valve (468). In 1989, LeProwse et al. reported a case of a 3-year-old child who had *O. turbata* bacteremia associated with an indwelling central venous catheter (340). In each of these patients, removal of the indwelling foreign prosthetic device was necessary for cure.

In two cases, the *Oerskovia* strains were not identified to the species level. Cruickshank et al. described a case of pyonephrosis caused by a microorganism that resembled NMO type B (126). The source of the infection was unknown, and the patient recovered following a nephrectomy. In addition, catheter-related sepsis developed in a woman receiving home total parenteral nutrition therapy; the causative microorganism was identified as an *Oerskovia* sp. For this patient, the source of the infection was thought to be contaminated parenteral nutrition fluid, and the bacteremia resolved following therapy with

TABLE 9. Characteristics differentiating the genus *Oerskovia* and NMOs from related organisms lacking DAP[a]

Characteristic	Result for:					
	O. turbata	*O. xanthineolytica*	NMOs		*C. cellulans*[b]	*"C. aquaticum"*
			Type A	Type B		
Pigment	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Motility	+	+	−	−	−	+
Metabolism of glucose	F	F	F	F	F	O
Branched substrate hyphae	+	+	+	+	+	−
Decomposition of:						
Casein	+	+	−	−	NT	NT
Xanthine	−	+	+	−	+	NT
Hypoxanthine	−	+	+	−	NT	NT
Tyrosine	−	−	NT	NT	NT	NT
Production of cellulase	−	−	+	+	+	−
Cell walls containing:						
meso-DAP	−	−	−	−	−	−[c]
Galactose	+	+	+	+	+	+

[a] Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; NT, not tested; F, fermentative; O, oxidative, as determined with King's oxidation-fermentation medium (112).
[b] Type A NMO (330).
[c] Contains diaminobutyric acid (147).

vancomycin (233). The last case and a case of *O. xanthineolytica* bacteremia in a patient with cirrhosis and variceal hemorrhage were cured by treatment with vancomycin (593) and are the only two reported cases of *Oerskovia* bacteremia for which antimicrobial therapy was successful. Despite specific antimicrobial therapy with penicillin and rifampin in one case of meningitis (281) and trimethoprim-sulfamethoxazole combined with ampicillin and amoxicillin in an endocarditis case (468), these drugs being the antimicrobial agents to which these patient isolates demonstrated in vitro susceptibility, respectively, the infections were unresponsive. Most *Oerskovia* infections are associated with an indwelling prosthetic device and are resolved following the removal of the devices. With the extended survival of severely compromised patients, the increased use of long-term indwelling central venous catheters, the widespread use of home total parenteral nutrition therapy, the difficulty in sterilizing homografts with antimicrobial agents, and the poor response to antimicrobial therapy (despite demonstrated in vitro susceptibility) without removal of the foreign foci, it is highly probable that these microorganisms will be encountered more frequently.

Rothia dentocariosa. In 1949, Onisi was the first to isolate *R. dentocariosa* from human carious dentine (429). Onisi considered that this microorganism belonged in the order *Actinomycetales* and proposed the name *"Actinomyces dentocarious"* because of its morphologic and physiologic similarities to *Actinomyces* species found in the mouth (429). Onisi described this species as a pleomorphic group of microorganisms that were facultatively anaerobic and had both coccoid and branched filamentous structures (429). In 1957, Roth (489), while studying isolates from carious dentine, discovered a similar species. Although Roth considered these isolates to be identical to *"A. dentocarious"* (429), their preference for aerobic growth conditions led her to place them in the genus *Nocardia* as *"Nocardia dentocariosus."* In 1960, in England, Davis and Freer (131) independently described a similar species isolated from the human mouth. On the basis of morphologic and physiologic characteristics, these investigators assigned their isolates to the genus *Nocardia* and suggested the name *"Nocardia salivae."* However, these authors commented that since the cell walls of these microorganisms lacked DAP, they differed significantly from other *Nocardia* spp. In 1967, Georg and Brown (184) compared strains of *"N. salivae"* and *"N. dentocariosus"* and concluded that they were identical. Since neither the genus *Actinomyces* nor the genus *Nocardia* was appropriate for these microorganisms, the new genus *Rothia* was created.

R. dentocariosa, a commensal of the oral cavity, is frequently isolated from throat and sputum cultures. In 1969, in one of the first reports of the isolation of this microorganism from clinical specimens, 39 of 50 isolates studied by Brown et al. (83) were from the throat, sputum, and carious teeth. Also, in a study of 565 isolates identified in the clinical microbiology laboratory of a large general hospital during a 5-year period, 503 isolates were recovered from patients (59). Of these, 275 were from sputum and bronchial washings, 164 were from the throat, and 64 were from miscellaneous sources (59). The other 62 isolates were recovered from the throats of hospital employees who had visited the hospital's health service (59). For approximately 50% of the cultures studied, *R. dentocariosa* was the predominant organism (59). Since these reports, other studies (262, 343, 541) have mostly implicated this microorganism as a cause of human dental plaque. However, this organism has rarely been recognized as an etiologic agent in invasive human clinical infections. In 1975, the first report of *R. dentocariosa* as a primary pathogen involved a 19-year-old woman with an abdominal infection (515). Clinical findings were typical of actinomycosis, and following surgery the patient was cured with penicillin treatment. Other reported infections associated with *R. dentocariosa* included one patient with a pilonidal abscess (356), one with an infected postoperative maxillary cyst (391), and five with endocarditis (79, 271, 435,

TABLE 10. Differential characteristics of *R. dentocariosa*, *C. matruchotii*, and *A. viscosus*[a]

Characteristic	Result for:		
	R. dentocariosa	*C. matruchotii*	*A. viscosus*
Coccal forms	+	−	−
Esculin hydrolysis	+	−	+
Acid production from[b]:			
Glucose	+	+	+
Mannitol	−	V	−
Lactose	−	−	+
Sucrose	+	+	+
Maltose	+	+	+
Cell walls containing *meso*-DAP	−	+	−

[a] Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; V, 11 to 89% of the strains are positive.
[b] In an enteric fermentative base with Andrade's indicator (112).

513, 514). Although a specific source of bacteremia was not identified in these cases, a dental source was implicated in three of them. Some cases have involved immunocompromised patients. Two patients had leukemia. In one of these patients, a woman with chronic lymphatic leukemia and toxic bone marrow depression, bacteremia followed treatment with clomipramine and zuclopenthixol. The other patient, an 84-year-old woman with acute myelocytic leukemia, presented with a left upper lobe infiltrate on a chest radiograph. *R. dentocariosa* was isolated from both bronchoalveolar fluid and a transthoracic needle aspirate. The pneumonia resolved after prolonged treatment with clindamycin (445, 516). In addition, *R. dentocariosa* has been suggested as a possible etiologic agent of cat scratch disease (187, 235, 443). These cases emphasize the fact that *R. dentocariosa* is a potential human pathogen.

The method of isolation of *R. dentocariosa* is similar to that of *Nocardia* spp. (Fig. 1). Selective media may be needed to isolate *R. dentocariosa* from heavily contaminated sources, such as supragingival plaque. Routinely used blood culture media are satisfactory for primary isolation from patients with septicemia or endocarditis.

R. dentocariosa is a gram-positive, extremely pleomorphic microorganism that varies from predominantly coccoid and bacillary forms in broth cultures to predominantly branched filaments on solid media (1.0 μm in width to 2.5 μm in length). The colony morphology at 35°C varies also, from smooth to slightly rough on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) in 24 h to strikingly rough, highly cerebriform, and irregularly edged in 7 days. *R. dentocariosa* grows well on enriched media, such as Trypticase soy agar and brain heart infusion agar, but little growth is obtained on minimal media, such as SDA. Characteristics used for the differentiation of the genus *Rothia* from other actinomycetes are given in Table 3. The definitive characterization of morphologically and physiologically similar bacteria (*Corynebacterium* [*Bacterionema*] *matruchotii* and *Actinomyces viscosus*) is summarized in Table 10. The genus *Rothia* differs significantly from the genus *Nocardia* by having a fermentative metabolism, failing to grow on inorganic nitrogen sources, and having a different cell wall composition. *Nocardia* cell walls contain DAP and arabinose, both of which are absent from *R. dentocariosa*. *R. dentocariosa* more closely resembles *A. viscosus* and *C. matruchotii*; however, the former has different end products of glucose metabolism, and the latter contains DAP as a cell wall component.

A moderately specific fluorescent-antibody test has been used successfully to distinguish microorganisms belonging to the genus *Rothia* from similar genera. However, this serologic identification technique occasionally yields false-negative results because some *Rothia*-like isolates fail to react with the currently available battery of fluorescein isothiocyanate-conjugated antisera. This fact may indicate that there is heterogeneity within the genus. Additional support for heterogeneity is provided by reports of microorganisms that differ biochemically from the type strain (279, 342, 550). Lesher and colleagues (342) recognized four biotypes and three serotypes of *R. dentocariosa* and suggested that one of the former merited the rank of species.

The drug of choice for *Rothia* infections seems to be penicillin, but a beta-lactam-resistant strain was isolated from a clinical infection of a postoperative maxillary cyst (391). Dzierzanowska et al. tested 90 *Rothia* strains against 18 antimicrobial agents (151). The strains were susceptible to cephaloridine, amoxicillin, doxycycline, erythromycin, ampicillin, chlortetracycline, cefazolin, benzylpenicillin, cefamandole, methacycline, cephalothin, flucloxacillin, cephalexin, cephradine, cloxacillin, and carbenicillin. They were resistant to clindamycin and highly resistant to lincomycin.

Abscess formation caused by *R. dentocariosa* in mice has been demonstrated experimentally (390, 490, 515).

***Streptomyces* spp.** Members of the genus *Streptomyces* are gram positive and form extensive, branched, stable substrate and aerial hyphae bearing long chains of conidia. The cell wall is a peptidoglycan that contains L-DAP but no characteristic sugar (331). From 1940 through 1957, over 1,000 *Streptomyces* spp. were described (461). By 1970, the total had increased to 3,100, although many species had been inadequately described or cited in patent literature (592). Identification to the species level was based on a limited number of subjectively chosen features, with a significant emphasis on morphology and pigmentation. However, after recent and exhaustive phenetic studies, Williams et al. (638, 640) suggested that the significant weighting given to morphologic characters in prior classifications was no longer justified. They suggested that there should be relatively few cluster groups (or species) within the genus and assigned over 300 *Streptomyces* and *Streptoverticillium* spp. to 41 minor clusters (2 to 5 strains) and 22 single-membered clusters, which corresponded to species, and 20 major (6 to 71 strains) clusters, which were provisionally considered species groups. These species groups were named after the earliest validly described species that they contained according to the principle of priority; in most cases, the other species within a cluster were listed as subjective synonyms of this name. For example, since *Streptomyces anulatus* has precedence over *S. griseus*, *S. anulatus* is the name of the species group containing *S. griseus* as a subjective synonym (640).

In 1986, Goodfellow et al. (209–212) studied 475 strains, which included 394 *Streptomyces* type cultures from the International *Streptomyces* Project (536–539), by using 139 unit characters. This study led to the transfer of the genera *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa*, and *Microellobosporia* into the same genus, *Streptomyces*. The *Streptomyces* type strains were assigned to nine cluster groups, 23 major clusters (6 to 71 strains), 20 minor clusters (2 to 5 strains), and 25 single-membered clusters (209–212).

Most recently, Goodfellow et al. (202) studied representative strains studied by Williams et al. (640) to evaluate the original numerical classification and clarify the taxonomy of

species groups, in particular, the *Streptomyces albidoflavus* species group. These investigators used characters usually used for streptomycetes and newly applied rapid enzyme tests based upon fluorophores (described in detail in "Recent Advances"; see below). From their studies, these authors concluded that, at present, the genus *Streptomyces* includes too few species and that *S. albidoflavus* encompasses at least three taxospecies, two of which, *S. albidoflavus* and *S. anulatus*, can be equated with genomic species.

Because most members of the genus *Streptomyces* are saprophytes, it has been traditional to minimize their significance in clinical microbiology. A report of *Streptomyces paraguayensis* associated with black-grained mycetoma has doubtful taxonomic significance (132). The widely distributed strain with this name has physiologic reactions that indicate that it is a strain of *S. griseus* (394). One species, *S. somaliensis*, has been identified as one of the etiologic agents of actinomycetoma occurring in many countries, including Saudi Arabia, Nigeria, Niger, Sudan, Somalia, South Africa, Venezuela, India, Mexico, Malaysia, Algeria, India, and the United States (224, 245, 366, 586). In these countries, this species has been associated in particular with mycetomas affecting the head and neck (craniocervical, cranial, and epidural), causing madura skull, and involving the cranial vault (cephalic). However, several reports in recent years suggest that it is no longer acceptable to consider this species the only human pathogen of this genus. Several reviews that describe clinical isolates from animals or humans have been published (48, 383, 394). In 1973, Berd studied 25 clinical isolates of *Streptomyces* species; four were identified as *S. somaliensis*, 2 were identified as *Streptomyces fradiae*, and the remaining 19 were not identified to the species level (48). Mishra and coworkers (394) studied 110 clinical isolates of *Streptomyces* species from humans and animals. The majority of their isolates were identified as either *S. griseus* (53%) or *S. somaliensis* (25%). However, they also identified other *Streptomyces* species as *Streptomyces albus*, *Streptomyces rimosus*, and *Streptomyces lavendulae*. These researchers considered all of these species to be of potential medical importance. In that study, *S. griseus* was the third most represented species after *N. asteroides* and *N. brasiliensis* as strains received by medical laboratories. In another review of the distribution of these and other aerobic actinomycetes in 1990, McNeil et al. found that of 366 isolates received during the 29-month study period, *S. griseus* (7.7%) was the third most isolated species after *N. asteroides* and *A. madurae* (383). In order of frequency, the sources of *S. griseus* identified by these investigators were sputum, wounds, blood, and brain. In addition, there have been a few isolated case reports of *Streptomyces* spp. causing nonmycetomic infections. However, other *Streptomyces* species isolated from humans include *Streptomyces violaceoruber*, *Streptomyces coelicolor*, and *S. albus*, from pulmonary streptothricosis, dental caries, blood, tonsils, skin, and sputum (316). Strains identified as "*Streptomyces candidus*" from the purulent exudate of a fractured patella, "*Streptomyces gedaensis*" from sputum and abscesses, "*Streptomyces horton*" from pus, and "*Streptomyces willmorei*" from streptothricosis of the liver have also been reported (316). The only case of a nonmycetomic *S. somaliensis* infection was reported in 1970 for a patient with a perforated peritoneum (230). In another patient with chronic pericarditis, an operative pericardial specimen grew a *Streptomyces* sp. in culture, and the microorganism was clearly identified in large numbers throughout histopathologic pericardial tissue sections (530). Reports of other infections have included septicemia and primary lung involvement (298), panniculitis (92), brain abscess (113), and cervical lymphadenitis in an AIDS patient (263). In another recent report, by Caron et al., a *Streptomyces* sp. was the only microorganism identified in a bronchoscopy culture and was proposed to be the cause of nodular pneumonia in a 30-year-old HIV-infected male patient (94).

T. paurometabola. *T. paurometabola* is a gram-positive, weakly or variably acid-fast, psychrophilic, nonmotile, nonspore-forming, rod-shaped obligate aerobic actinomycete without aerial hyphae; the organism is notable for very-long-chain, highly unsaturated mycolic acids and other novel glycolipids and menaquinones in the cell wall (205, 467, 589, 590). It was first discovered in humans in 1971 by Tsukamura and Mizuno after being isolated from the sputum of patients with tuberculosis in Japan (605). Subsequently, this microorganism was found primarily in soil and sludge. Until 1988, *T. paurometabola* was referred to variously as *Rhodococcus aurantiacus* or *Gordona aurantiaca* (560, 608). A recent sequence comparison of 16S RNAs (121) suggested that this microorganism is identical to the original isolate of *Corynebacterium paurometabolum* obtained from the mycetomes and ovaries of bedbugs (567). Therefore, on the basis of chemical and molecular systematics, *R. aurantiacus* has been removed from the genus *Rhodococcus*, renamed *T. paurometabola*, and assigned a new type strain. The genus *Tsukamurella* accommodates gram-positive, weakly acid-fast, aerobic actinomycetes that contain mycolic acids with 64 to 78 carbons, major amounts of unsaturated menoquinones with nine isoprene units, *meso*-DAP, and the characteristic cell wall sugars arabinose and galactose (Tables 3 and 4).

A new species of this genus was proposed in 1991 by Goodfellow et al. (213). The taxon was assigned to the genus as *T. wratislaviensis*. This new taxon has chemical, enzymatic, nutritional, and tolerance properties consistent with its assignment to the genus *Tsukamurella*. In addition, the new taxon formed a DNA hybridization group corresponding to the one formed by *T. paurometabola*. Both numerical taxonomic and DNA relatedness data showed that *T. wratislaviensis* was most closely related to *T. paurometabola*; however, the members of the genomic species of *T. wratislaviensis* have DNA that is less rich in guanine plus cytosine than the DNA of the members of the genomic species of *T. paurometabola*. The new type strain of *T. paurometabola* (ATCC 8368) was not used in that study (213).

T. paurometabola is a psychrophilic microorganism that grows best at temperatures cooler than that of the human body. It is found naturally in soil, sludge, and arthropods. It is only rarely a pathogen in humans, perhaps only under special conditions. In a review of seven previously reported cases (99, 462, 602, 603), Shapiro et al. (531) suggested that contamination might have accounted for at least three of the seven (462, 602, 603). These instances involved patients from whom multiple microorganisms were isolated in addition to *T. paurometabola*. The patients had histories of recurrent antimicrobial treatment. No microorganisms had been isolated from prior cultures. The other four cases involved indwelling catheters. In one case, in which the association of *T. paurometabola* and disease was strong, a person undergoing peritoneal dialysis developed peritonitis. A single microorganism was isolated from an initial culture before the start of antimicrobial therapy; it had a morphology consistent with that of *T. paurometabola* upon Gram staining. The patient recovered after appropriate therapy.

Shapiro et al. (531) recently reported catheter-associated sepsis in three unrelated oncology patients who had hematologic malignancies or solid tumors and who were receiving chemotherapy. All three patients had long-term, indwelling central venous catheters. *T. paurometabola* was isolated from

cultures of blood as well as samples from the tips of the intravascular devices. After removal of the catheters and treatment of one patient with trimethoprim-sulfamethoxazole and another with ceftriaxone, the infections resolved. For the third patient, the catheter was also removed and therapy was begun with erythromycin and gentamicin. Although this regimen proved to be effective for the therapy of the patient's infection, the patient's isolate was shown to be resistant to erythromycin in vitro, and susceptibility to gentamicin was not tested.

Recently, Auerbach et al. (24) reported a common-source outbreak of a pseudoinfection caused by *T. paurometabola*. A hospital reported obtaining 12 isolates of this organism from 10 patients during a 17-month period. Case-control studies found that the positive specimens were more likely to have been processed in the tuberculosis or fungal room, to have been tissue samples, and to have been handled by one technician. Epidemiologic and laboratory investigations indicated that this outbreak of a *T. paurometabola* pseudoinfection resulted from laboratory contamination, probably in the tuberculosis or fungal room. Contamination may have been promoted by the reuse of extrinsically contaminated saline solutions over a prolonged period to prepare specimens for culturing. Typing on the basis of biochemical, antimicrobial resistance, DNA fingerprinting, and ribotype profiles showed that the isolates from the outbreak were essentially identical and that they were distinguishable from each of the two isolates obtained after the outbreak and from two type strains. These findings support the hypothesis of a common-source outbreak of a pseudoinfection.

The new type strain (ATCC 8368) differed from all of the other isolates identified as *T. paurometabola* (12 outbreak-associated isolates, 2 isolates obtained after the outbreak, and the original type strain, ATCC 25938) in terms of colony morphology, biochemistry, antimicrobial susceptibility, and ribotype. These differences suggest that type strain ATCC 8368, proposed in 1988, may be a species different from the other type strain and the isolates from the outbreak and that the nomenclature of *T. paurometabola*, thought to have been resolved recently (121), may require further clarification.

The method for isolating *T. paurometabola* from clinical specimens is shown in Fig. 1. At 24 h on heart infusion agar containing 5% rabbit blood, colonies are usually 0.5 to 2.0 mm in diameter. The colonies are circular, usually have entire but may have rhizoid edges, are dry but easily emulsified, and are white to creamy to orange in color. Rough colonies are produced after prolonged incubation for up to 7 days. These colonies are characteristically cerebriform and do not produce aerial hyphae. They superficially resemble the rapidly growing mycobacteria, especially when creamy in color. However, mycobacteria can be differentiated from *T. paurometabola* on the basis of arylsulfatase production within 14 days. Most strains of *T. paurometabola* are acid fast, as determined by the Kinyoun method. Hypoxanthine (75%), xanthine (50%), and tyrosine may be decomposed, but casein and adenine are not decomposed. Acid is regularly produced from galactose, glucose, inositol, mannitol, mannose, sorbitol, trehalose, and xylose but not from rhamnose.

Comparative immunodiffusion studies and thin-layer chromatography analyses of whole-cell methanolysates were performed on representative test strains of *T. paurometabola* and *Mycobacterium album* as well as 23 reference strains, including species of the genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, and *Streptomyces* and other, related genera (473). Strains of *M. album* and *T. paurometabola* had six precipitinogens in common with each other but few precipitinogens in common with the 23 reference strains. The characteristic mycolic acid and menaquinone profiles distinguished members of the *M. album* and *T. paurometabola* taxa from all the established mycolic acid-containing taxa that were tested (473). In 1991, *M. album* was transferred to the genus *Tsukamurella* as *T. wratislaviensis* (199, 213).

T. paurometabola is susceptible to amikacin, imipenem, sulfamethoxazole, trimethoprim-sulfamethoxazole, and ciprofloxacin. It is resistant to amoxicillin-clavulanate, ampicillin, doxycycline, and erythromycin.

THERMOPHILIC ACTINOMYCETES

Clinical and Epidemiologic Aspects

The taxonomy of thermophilic actinomycetes is complex; however, only a relatively few species have been recognized to cause human disease. These microorganisms are ubiquitous and can be found in water, air, soil and compost piles, home and industrial air-conditioning systems, house dust, hay, and bagasse (solid plant residue left after sugar cane has been crushed to extract sugar). Repeated inhalation of dust containing these microorganisms or their spores may result in hypersensitivity pneumonitis or extrinsic allergic alveolitis, a serious, disabling, immunologically mediated pulmonary disease that may affect agricultural, office, and industrial workers.

Various names have been given to different forms of hypersensitivity pneumonitis. These names indicate the high-risk occupational group affected, the substrate that gives rise to the particular antigen, or a specific type of environmental exposure. The best-characterized form of the disease is farmer's lung, which was first described in 1932 for a group of United Kingdom farmers and which was associated with exposure to the dust of moldy hay (91). In 1963, the predominant sensitizing agent from this source was identified as *Saccharopolyspora rectivirgula* (441); however, two other species, *Thermoactinomyces vulgaris* and *Thermoactinomyces candidus*, may also be present and induce the disease. Respiratory diseases resembling farmer's lung may also occur naturally in cattle and horses exposed to moldy hay and in experimental animals (rabbits, hamsters, and mice) (260, 428, 633). Other forms of the disease and their associated sensitizing agents include mushroom worker's lung, induced by exposure to moldy compost (*S. rectivirgula*, *T. vulgaris*, and *T. candidus*); bagassosis, induced by exposure to moldy sugar cane (*Thermoactinomyces sacchari* and *T. vulgaris*); air conditioner-associated lung disease, resulting from contaminated ventilation ducts (*Saccharomonospora viridis*, *T. vulgaris*, and *T. candidus*); and humidification system-induced disease, caused by contamination of the system's reservoir (*T. vulgaris* and *T. candidus*). There is also a need to differentiate other thermophilic actinomycete species, such as *Thermoactinomyces intermedius*, *Thermoactinomyces putidus*, *Thermoactinomyces dichotomica*, *Thermomonospora* spp., and *Pseudonocardia* spp. Although these presently are not known to be etiologic agents of hypersensitivity pneumonitis, they may be isolated from the same environments and thus may be confused with pathogenic thermophiles.

Importantly, in addition to inhalation of dust containing actinomycetes, hypersensitivity pneumonitis may also result from inhalation of dust containing various fungi, avian serum proteins, and other substances. All of these particulate allergens are characterized by a small particle size (1 to 5 μm) that facilitates their penetration into lung alveoli, upon which they can impinge and in which they can persist for long periods (322). A compilation of fungi and organic dust that have been

implicated in causing the disease can be found in each of several reviews (169, 261, 352, 479). Despite the variety of settings and multiple possible etiologies, similar presenting symptoms and signs in the patients and the typical findings from histopathologic examinations of pulmonary tissues are consistent with a singular pathologic process or disease entity, which has been termed hypersensitivity pneumonitis.

Characteristically, the onset of acute hypersensitivity pneumonitis follows within 4 to 6 h after exposure to the triggering agent, with malaise, sweating, chills, anorexia, dyspnea, chest tightness, cough, and fever. These symptoms typically resolve within 18 to 24 h unless the exposure persists. Rarely, however, recurrent acute episodes or long-term, low-dose exposure may contribute to a more chronic form of the disease. Characteristics of the chronic form of hypersensitivity pneumonitis include progressively increasing dyspnea, cough, weight loss, airway obstruction and, ultimately, irreversible lung fibrosis. In the acute form of the disease, the typical appearance of a patient's chest radiograph is micronodular pulmonary shadowing. Pulmonary function tests may be of limited diagnostic value for the acute form of the disease; however, for the chronic form, these tests may be extremely useful in assessing both the severity and the progress of the disease.

Pathologically, hypersensitivity pneumonitis exhibits a lymphocyte-dependent granulomatous inflammatory reaction, predominantly of the lung parenchyma. In the acute disease, the typical histopathologic appearance of a lung biopsy is mainly mononuclear interstitial pneumonitis (alveolitis), together with evidence of bronchiolitis, noncaseating epithelioid granulomata, and vasculitis. In the chronic disease, diffuse interstitial fibrosis is common, and cystic changes may be evident. In addition, experimental animal models have been developed to elucidate potential pathogenic mechanisms involved in the disease (428, 633). Depending upon the particular animal model system used and the method of disease induction, cytotoxic (type II), immune complex (type III), and cell-mediated (type IV) immune mechanisms have all been proposed to be important in the pathogenesis of the disease. Also, supporting evidence for each of these immune mechanisms has been provided by clinical studies of patients with hypersensitivity pneumonitis. Recently, a unifying hypothesis of the pathogenesis of hypersensitivity pneumonitis was proposed by Schorlemmer et al. (523), Salvaggio and Karr (499), and Hollick et al. (260).

A majority of patients with acute farmer's lung demonstrate in double-gel diffusion tests precipitating serum antibodies (precipitins) to an antigenic extract of *S. rectivirgula* (285, 315, 375, 480, 632). Following a single attack, these antibodies may persist for variable periods; however, in most patients, these antibodies are lost after approximately 3 years. Also, in patients with chronic disease, the results of such serologic tests are variable. Thus, the diagnostic value of such tests is not optimal, and apparently healthy farmers exposed to moldy hay may often be found to have positive test results for precipitating serum antibodies directed against thermophilic actinomycetes. The low sensitivity of these serum precipitin tests is most likely a result of the nonspecificity of the antigens used (375). The use of serum precipitin tests has also been complicated by the finding that the sera of patients with positive test results may react to more than one thermophilic actinomycete, as was found by Wenzel et al. for 44.2% of the patients that they tested (632). Serum precipitin tests with positive results for more than one thermophilic actinomycete have also been reported by other investigators (32, 313). Whether these results are due to immunologic cross-reactivity of the antigens or to exposure to several thermophilic actinomycetes remains unclear.

The concentrations and types of actinomycete spores present in plant material are influenced not only by the nature of the substrate but also by other factors, especially the water content and temperature of the plant material. Environments in which hypersensitivity pneumonitis occurs have been reported to have more than 10^6 spores per m^3 in the atmosphere (322). A specific environmental source(s) of actinomycete spores may be suggested by a patient's clinical history; avoidance of further exposure to the source(s) is usually advisable. A specific environmental investigation may also be indicated. It should include culturing of environmental samples to isolate particular pathogenic thermophilic species. However, since these microorganisms are widespread in the environment, the interpretation of environmental sample culture results may be problematic. In addition, specialized selective isolation procedures may be required to detect these and other thermophilic actinomycetes in moldy fodder, compost, and airborne dust.

Criteria useful for making the diagnosis of hypersensitivity pneumonitis induced by thermophilic actinomycetes include (i) a history of exposure to a potential environmental source of an antigen in a patient's home or work environment, which may require an environmental investigation, air sampling for further delineation, or both steps; (ii) characteristic clinical, radiographic, and lung function findings, which may have an onset or may worsen within hours following exposure to the antigen; (iii) demonstration in the patient's serum of precipitating antibodies to the causal antigen; and (iv) evidence in chest radiographs of pulmonary infiltrates compatible with hypersensitivity pneumonitis (322).

Steroid therapy has been widely used for the treatment of acute hypersensitivity pneumonitis. In patients with this disease, disodium cromoglycate may also have a role as supplemental or prophylactic therapy; however, to date there have been no reports of comparative evaluations of this drug. Environmental control measures, such as modern agricultural practices, including silaging (storing fodder in silos), prevent molding and are considered effective in limiting exposure of farm workers to the disease (349). Other proposed environmental protective measures have included wearing of masks and spraying of hay with substances to prevent molding.

Taxonomy and Microbiologic Aspects

Three genera of thermophilic actinomycetes are considered of medical importance: *Saccharopolyspora* spp. (or *Faenia* spp.), *Saccharomonospora* spp., and *Thermoactinomyces* spp. Within these three genera, there are three important *Thermoactinomyces* species (*T. vulgaris*, *Thermoactinomyces thalpophilus*, and *T. sacchari*), one important *Saccharopolyspora* species (*S. rectivirgula* [*Faenia rectivirgula*]), and one important *Saccharomonospora* species (*S. viridis*). Tolerance of a temperature of 50°C or above is a pathognomonic characteristic for all of the thermophilic species. In addition, all of these species form *meso*-DAP and lack mycolic acids in their cell walls. Also, as a group, they are gram positive and are not acid fast. Individual thermophilic species are differentiated on the basis of their microscopic and macroscopic morphologies—in particular, number of spores and type of spore production, ability or inability to produce aerial hyphae, the color of their colonies, and the presence or absence of arabinose and galactose in their cell walls. Additional criteria that are useful in separating these species include hydrolysis of the substrates casein, xanthine, hypoxanthine, adenine, tyrosine, and esculin (259, 314, 319–321) (Table 11).

One of the first practical schemes for identifying the thermophilic actinomycetes was developed in 1975 by Kurup and

TABLE 11. Physiological characteristics of thermophilic actinomycetes[a]

Characteristic	Result for:				
	S. rectivirgula[b] (*n* = 10)	*S. viridis*[b] (*n* = 6)	*T. vulgaris*[c]	*T. thalpophilus*[c]	*T. sacchari*[c]
Morphology					
Substrate hyphae	+	+	+	+	+
Aerial hyphae	+	+	+	+	Transient
Spore type	On aerial and substrate hyphae	On aerial hyphae	Endospores on sphorophores on aerial and substrate hyphae	Endospores sessile on aerial and substrate hyphae	Endospores on sphorophores on aerial and substrate hyphae
Spore number	Short chains	Single	Single	Single	Single
Colony color	Yellow	Blue-green	White	White	Colorless to white
Growth at:					
50°C	+	+	+	+	+
60°C	+	−	+	+	+
Cell wall composition					
meso-DAP	+	+	+	+	+
Arabinose and galactose	+	+	−	−	−
Mycolic acids	−	−	−	−	−
Hydrolysis of:					
Casein	−	+	+	+	+
Xanthine	V (7)	−	−	−	−
Hypoxanthine	− (10)	−	−	−	−
Adenine	−	−			
Tyrosine	V (3)	V (2)	−	+	−
Esculin	+ (9)	−	+	−	+
Growth in the presence of lysozyme	V (8)	−	+	+	NT

[a] Symbols: +, positive; −, negative; V, variable; NT, not tested. Numbers in parentheses indicate numbers of isolates.
[b] Data are from Kurup and Fink (314).
[c] Data are from Unsworth and Cross (609).

Fink (314). Although the methods used for the identification of these species in the laboratory have remained almost unchanged, this group of microorganisms has undergone numerous taxonomic changes. For example, isolates of *T. vulgaris* that Kurup and Fink studied in 1975 corresponded to the aggregate cluster for which, in 1980, Unsworth and Cross proposed reviving the name *T. thalpophilus* Waksman and Corke 1953 (314, 609). In addition, the isolates designated *T. candidus* by Kurup and Fink were synonymous with the original *T. vulgaris* strains isolated by Erikson in 1953 and called "*Micromonospora vulgaris*" by Tsiklinsky in 1899 (160, 314, 594). Furthermore, "*Micropolyspora faeni*" (314) has undergone two additional taxonomic changes; "*Micropolyspora faeni*" and "*Micropolyspora rectivirgula*" were considered synonymous and, on the basis of a ruling by the Judicial Commission of the International Committee on Systematic Bacteriology (628), *Faenia* was established as the official genus name, with *F. rectivirgula* as the type species (628). In 1989, *F. rectivirgula* was transferred to the genus *Saccharopolyspora* as *S. rectivirgula* Krasil'nikov and Agre 1964 by Korn-Wendisch et al. (301). This transfer was established on the basis of the results of extensive biochemical and molecular tests (fatty acid, phospholipid, and menaquinone patterns) (301).

All forms of hypersensitivity pneumonitis are characterized by the presence of humoral antibodies against the causative antigen. Dust extract, water samples, or antigens from cultured microorganisms may be used to detect precipitating antibodies in a patient's serum. However, comparisons of studies are very difficult to interpret because investigators have failed to examine well-characterized representative control isolates of all validly described species of these microorganisms. For example, the importance of *T. vulgaris* as a causative agent of hypersensitivity pneumonitis has been underestimated because isolates used in testing patient sera have often been *T. thalpophilus* instead (321).

WHIPPLE'S DISEASE

Whipple's disease, or intestinal lipodystrophy, is a systemic disorder first described by George Whipple in 1907 (635). The disease predominates in middle-aged white males and is characterized by arthralgia, diarrhea, abdominal pain, and weight loss. Lymphadenopathy, fever, and increased skin pigmentation may be additional findings. The diagnosis is made on the basis of the histologic demonstration of "foamy" macrophages infiltrating the lamina propria of the small intestine and containing periodic acid-Schiff stain-positive inclusions. These inclusions are gram-positive bacilli (Whipple's bacilli), which may also be found extracellularly. They possess a unique cell wall structure. Therapy with antimicrobial agents ultimately effectively eradicates the disease. Patients with Whipple's disease show evidence of impaired cell-mediated immunity before and after successful treatment. However, infection with these bacilli has yet to be documented in a patient with AIDS. Wilson et al. reported a 16S rRNA sequence that they amplified from a duodenal biopsy specimen

from a patient with Whipple's disease and concluded that the Whipple's disease bacillus was an uncharacterized gram-positive bacterium most closely related to *R. equi* (642). Recently, Relman and colleagues reported a technique in which oligonucleotide primers specific for the Whipple's disease bacillus were used in a PCR to amplify sequences of bacterial 16S rRNA directly from infected tissues (469). Following a comparative 16S rRNA sequence analysis, they concluded that the disease is caused by an as-yet-uncultured and uncharacterized actinomycete, which they named *Tropheryma whippelii*. Their study also makes possible a diagnostic test, a specific PCR-based assay, which should have improved sensitivity over histologic examination of infected tissues.

RECENT ADVANCES

Rapid Identification

In contrast to the use of conventional, growth-dependent procedures for identifying bacteria, chromogenic enzyme substrates can rapidly detect constitutive enzymes produced by microorganisms. These enzymes allow specific organism identification when considered with the results of conventional tests and observations, such as microscopic and macroscopic morphologies and Gram stain characteristics.

The API ZYM system (Societe Analytab Products Inc., La Balme Les Grottes, France) was evaluated for the rapid identification of members of the family *Actinomycetaceae* and related bacteria by Kilian (288). Data from 162 strains of these microorganisms were screened for activity with 19 different enzymes. Twelve enzymes proved to be of value for differentiating the species studied. In combination with the β-xylosidase test and information on catalase activity and oxygen requirements, this series of tests provided a basis for identifying species of the family *Actinomycetaceae* and some related species within 4 h. One enzyme, valine aminopeptidase, was useful in separating *R. dentocariosa* and *C. matruchotii*, which were almost identical in all other reactions. Although the reactions with 12 strains of *N. asteroides* were consistent, the author recommended that additional strains of *N. brasiliensis*, *N. otitidiscaviarum*, and *A. pelletieri* be evaluated before this system is used for the identification of these taxa.

In 1990, Boiron and Provost further evaluated the API ZYM system for the detection of constitutive enzymes on chromogenic substrates with 62 isolates belonging to the genus *Nocardia* and related bacteria (65). Results from this study were consistent with those of Kilian's study (288), except for the reactions with *N. brasiliensis*. Two enzymes, α-glucosidase and α-mannosidase, were negative in Kilian's study but positive in the study of Boiron and Provost. However, these studies used different media for growth of the inocula and different incubation times. Also, the possibility of different geographic biovariants cannot be excluded. Although *N. asteroides* showed a consistent profile of enzymatic activity distinct from that of *N. brasiliensis*, *N. otitidiscaviarum*, and *N. farcinica*, it would be informative to restudy these isolates from the viewpoint of some of the newer taxonomic criteria, in particular, antimicrobial susceptibility and mycolic acid patterns within the *N. asteroides* taxon (625, 651).

A related application of rapid chromogenic substrates is the use of fluorogenic enzyme substrates for the detection of bacterial enzymes and the identification of microorganisms. The use of fluorescence is a much more sensitive method for the detection of enzymatic activity than is the chromogenic method. In addition, because bacterial enzymes are preformed by the bacterial inoculum and test instrument detection systems are highly sensitive to low levels of fluorescence, prolonged incubation of unknown bacteria to produce a detectable endpoint is not required, and readings are possible in as soon as 2 h. Goodfellow and colleagues (206) used the fluorogenic indicator 7-amino-4-methylcoumarin (7AMC) linked to peptide hydrolase substrates to characterize the rhodococci. In a more extensive study of the rhodococci, Goodfellow and coworkers (207) used the fluorophore 7AMC and another fluorophore, 4-methylumbelliferone (4MU), to improve the taxonomy of the rhodococci by biochemical means. In addition, in a study of the numerical taxonomy of *Streptomyces* spp., Goodfellow et al. (202) tested the ability of strains to cleave 22 4MU-conjugated and 41 7AMC-conjugated derivatives by using a protocol and an automated data capture system developed in collaboration with Sensititre U.K. Ltd. (East Grimstead, United Kingdom).

Although these rapid, miniaturized methods can be helpful for identifying well-defined aerobic actinomycete species, many of these organisms do not fit into established genera, so these methods are more suitable for use in specialized reference or research laboratories.

Molecular Techniques

Recent advances in molecular strain typing techniques, such as the development of rDNA analysis, have shown promise as rapid and specific aids in the identification and epidemiologic typing of many bacteria that are difficult to identify. Although such techniques are not immediately applicable in clinical microbiology laboratories, correlation of the genetic data obtained with phenotypic results of conventional biochemical identification tests may permit a more accurate identification of aerobic actinomycete species by a limited number of biochemical tests.

Strain- and species-specific ribosomal restriction endonuclease patterns have been visualized after hybridization of labeled 16S and 23S rRNAs of *Escherichia coli* to genomic blots of DNAs from several bacterial genera (227). Species-specific differences in ribotype patterns have also been used successfully to identify isolates of several different bacterial species for which conventional laboratory identification methods may often be unreliable, technically demanding, labor-intensive, or time-consuming. Recent reports on the application of ribotyping to bacteria of public health importance have included *Campylobacter* (405, 485), *Legionella* (228, 507), *Staphylococcus* (135), and *Leptospira* (444) species.

In a recent study, plasmid, DNA fingerprinting, and ribotype analyses were used to reliably identify epidemiologically related clinical isolates of *G. bronchialis* (470). In another study, Lasker et al., using two restriction endonucleases, determined the rRNA gene restriction endonuclease patterns for the type strains of the 20 recognized species in the genus *Rhodococcus* (324). They also analyzed *G. bronchialis* and *R. equi* clinical isolates by ribotype analysis. A large number of restriction fragment polymorphisms in the rRNA genes of *R. equi* allowed the observation of potential differences in the ribotype patterns of *R. equi* isolates from patients who have AIDS and those who do not. Finally, the utility of ribotype analysis was demonstrated by use of rRNA gene patterns in identifying three of five biochemically atypical *Rhodococcus* clinical isolates.

Diagnostic histopathology continues to be an essential counterpart of clinical microbiology because it serves to define the cellular configuration of host-tissue reactions. It also enables one to judge whether a given host-cell reaction is in accord with its designated causative agent. Concordance between microbiology and histopathology provides a strong inference of

infectious disease causality, especially when the suspected microbiologic agent has been visualized within the tissue itself. In 1991, Brown et al. demonstrated *R. equi* in situ in the tissue of HIV-infected patients by hybridization with a biotinylated DNA probe (82). Application of in situ hybridization methods may be particularly beneficial when putative microorganisms are rare or have a nonspecific (coccoid) morphology, as is the case with *R. equi* (82). An important and even more recent technologic advance has been the PCR analysis of 16S rRNA, which has further increased the sensitivity of the detection of microorganisms that cannot be cultured on artificial media.

CONCLUSIONS

Recently, the aerobic actinomycetes have emerged as unusual but important potential human pathogens that may be the cause of significant morbidity, affecting not only immunocompetent hosts but also severely immunocompromised patients. The number of susceptible immunocompromised patients continues to increase as a result of modern technologic advances that have enabled the survival of critically ill patients and, to a lesser extent, the steady increase in the number of HIV-infected patients. The more widespread use of improved noninvasive medical diagnostic techniques has resulted in an increased understanding of the pathogenic potential of many of these microorganisms. However, it must be emphasized that invasive biopsy techniques often may be needed to obtain appropriate clinical specimens for culturing or histopathologic examination to make a clinical diagnosis. Also, in a clinical microbiology laboratory, the isolation of many of these microorganisms may be difficult. Steps to facilitate isolation include alerting the laboratory to the suspicion of the disease so that special methods may be employed to facilitate the isolation and identification of these microorganisms. Unfortunately, no approved standardized antimicrobial susceptibility test method currently exists. It has been suggested, however, that antimicrobial susceptibility testing of all clinically significant isolates may be useful not only in choosing initial therapy for patients but also in monitoring for the development of antimicrobial resistance, which may complicate drug therapy for patients. There is a frequent requirement for prolonged therapy for the initial infection. In addition, maintenance or prophylactic therapy may be needed, especially for patients with HIV. Also, there are major limitations with the presently available oral antimicrobial agents. Thus, there is an urgent and compelling need for the development of new, effective, alternative, safe oral drugs.

The medically important aerobic actinomycetes that have been associated with human disease have undergone considerable recent taxonomic revisions. Further clarification of the taxonomy of these microorganisms is needed and should be done by use of recently developed, more reliable molecular methods. Taxonomic groupings for these microorganisms have so far been based primarily on phenotypic characteristics and simpler, genetic analyses, which have not always proved to be satisfactory. There is an urgent need to apply new genetic techniques that definitively establish the taxonomy of the aerobic actinomycetes. Once the taxa of aerobic actinomycetes have been reliably distinguished on the basis of cell wall analyses and nucleic acid hybridization or sequencing or both, biochemical tests that may be useful for routine identification purposes may be selected. Until such taxonomic revisions are complete, the full potential of epidemiologic tools for use with these microorganisms, such as molecular subtyping, and highly accurate and specific diagnostic methodologies, such as probe identification and PCR detection, will not be realized.

Molecular subtyping techniques have been successfully applied in the investigation of a nosocomial pseudooutbreak of *N. asteroides* (439) and a nosocomial cluster of *G. bronchialis* wound infections (470). These techniques have also been shown to be useful for the epidemiologic evaluation and identification of *R. equi* and other *Rhodococcus* spp. However, comparative studies of the available methods including well-characterized clinical and reference isolates are needed to determine which technique is best for each major pathogen. The application of improved computerized methods for data storage, analysis, and comparison, in particular, techniques for comparing results with previously obtained data, will considerably enhance the usefulness of molecular subtyping techniques.

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