Aerobic Actinomycetes of Clinical Significance

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ABSTRACT The group of Gram-positive bacillary organisms broadly known as “aerobic actinomycetes” consists of heterogeneous and taxonomically divergent genera. They are found in a wide variety of natural and man-made environments but are rarely considered a part of the normal human flora, with infections normally originating from exogenous sources. An extensive number of genera have been described, but only a minority of these has been associated with human or veterinary health. The association with human disease is usually of an opportunistic nature, either through accidental means of inoculation or through involvement with immunocompromising conditions in the host. They cause a wide spectrum of diseases in humans, which may differ greatly between the genera and even between species, but which also may have a great amount of overlap. The occurrence of such infections is probably greater than appreciated, since many may go unrecognized. Etiologic prevalence of specific genera and species varies geographically within the United States and worldwide. Traditional phenotypic identification methods for separation of the many genera and species of aerobic actinomycetes have found great difficulties. Recent use of chemotaxonomic analyses and emerging technologies such as molecular analysis of nucleic acids, and more recently proteomics for identification to the genus/species level, has provided a far more robust technique to understand the organisms’ relatedness, distribution, epidemiology, and pathogenicity in humans.

INTRODUCTION

The group of Gram-positive bacillary organisms broadly known as “aerobic actinomycetes” consists of heterogeneous and taxonomically divergent genera belonging to the phylum Actinobacteria, in the class Actinobacteria, subclass Actinobacteridae. The majority of human pathogens in this group are placed in the suborder Corynebacterineae, with some in the suborders Streptosporangineae, Streptomycineae, and Micrococcineae (1). Many of the clinically significant genera are characterized by the presence of at least rudimentary and sometimes longer branching filaments with the ability to produce spores or to fragment the filaments (1). Except for the Mycobacterium tuberculosis complex, Mycobacterium leprae, and the genus Dermatophilus (the only clinically significant genus in the suborder Micrococcineae, family Dermatophilaceae), most genera in this group are considered saprophytes and few are associated with human pathogenesis (2). An extensive number of aerobic actinomycete genera have been described, but only a minority of these have been associated with human or veterinary health.

The association with human disease is usually of an opportunistic nature, either through accidental means (i.e., traumatic percutaneous inoculation) or through involvement with immunocompromising conditions in the host (2). The number of infections directly related to the aerobic actinomycetes is increasing because of an...
increasing elderly population, together with increasing numbers of chronic debilitating conditions (e.g., chronic pulmonary diseases such as chronic obstructive pulmonary disease), and increasing number of immunosuppressive conditions within characteristic patient populations (e.g., organ/tissue transplantation, cancer, and HIV [human immunodeficiency virus] infections). For this reason, this chapter deals only with those genera having the most impact on human healthcare of patients with immunocompromising conditions. In some cases, microbes that have not caused human infection are discussed, due to the fact that immunocompromised patients are often the first to be identified with infections caused by what are thought to be saprophytes.

**TAXONOMY, EPIDEMIOLOGY, AND CLINICAL RELEVANCE**

The aerobic actinomycetes are found in a wide variety of natural and man-made environments but are rarely considered a part of normal human flora; infections generally originate exogenously. This microbial group causes a wide spectrum of diseases in humans. Symptoms may vary widely between the genera and even between species; however, depending on the species, a substantial amount of symptom overlap may also occur.

The prevalence of infections is probably greater than appreciated, since many may go unrecognized. Etiologic prevalence of specific genera and species varies geographically within the United States and worldwide. Strategies for traditional phenotypic identification methods that will separate the many genera and species of aerobic actinomycetes have met with great difficulties. Recent use of chemotaxonomic analyses and emerging technologies, such as molecular analysis of nucleic acids (including 16S rRNA genes, 16S-23S rRNA internal transcribed spacer, and 23S rRNA gene sequences), has been useful. More recently, proteomics for identification to genus and species level has provided laboratories with a far more robust technique, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF), to understand the organisms’ relatedness, distribution, epidemiology, and pathogenicity in humans.

The primary sources of human infection with aerobic actinomycetes are environmental. Even when categorized as nosocomial infections, they have been primarily associated with point sources in the environment. In such settings, dusty conditions during construction or aerosolization during manipulation of infected material have been implicated in some outbreaks. Because of their ubiquitous presence in the environment, aerobic actinomycetes have, on several occasions, been reported to cause pseudo-outbreaks through point-source contamination of specimens during processing or through cross-contamination of cultures being monitored using common needles.

Except for a few genera, the aerobic actinomycetes rarely invade immunocompetent hosts, except, perhaps, through traumatic inoculation. In humans, their primary pathogenic role is one of an opportunistic nature in an appropriate immunocompromised host, in the presence of a foreign body implant, or during chance percutaneous inoculation. In terms of overall numbers, infections are uncommon but can vary based on geographic location.

Species of *Nocardia* are the major group of aerobic actinomycetes that cause infections in humans, followed distantly by the *Rhodococcus* spp., *Gordonia* spp., and rarely *Tsukamurella* spp. Besides the mycetoma-causing species (*Actinomadura* and *Streptomyces*) in specific geographic areas, the other genera of aerobic actinomycetes also rarely cause disease, but the number of documented cases seems to be growing.

**Nocardia spp.**

The taxonomy of the genus *Nocardia* has undergone major changes with the advent of modern classification procedures. The uses of nucleic acid and proteomic characterization have expanded our knowledge of their phylogenetic relatedness and taxonomic status. Traditionally, *Nocardia* spp. were identified phenotypically using microscopy, growth characteristics (color, aerial filamentation), resistance to lysozyme (separating the *Nocardia* spp. from other genera in the aerobic actinomycetes group), and the hydrolysis of casein, tyrosine, xanthine, and hypoxanthine.

In 1988, Wallace and colleagues described six stable susceptibility patterns within the *Nocardia asteroides* group. They were initially able to define three separate species within this group, *Nocardia abscessus* (susceptibility pattern I), *Nocardia nova* (susceptibility pattern III), and *Nocardia farcinica* (susceptibility pattern V). As they studied these profiles, they found that a more distinguishable, stable species was discernible within the *N. asteroides* “group.” The susceptibility profiles within the traditional species of *N. asteroides* and other newer species, now assigned to those profiles, are depicted in Table 1.

As newer methods for characterization of species became available (originally high performance liquid chromatography, fatty acid analysis, PCR-restriction
endonuclease analysis of 16S RNA gene and of the heat shock protein gene, and most recently nucleic acid sequencing (e.g., 16S RNA or pyrosequencing) and proteomic characterization, it became clear that \textit{N. asteroides} species contained a multitude of species distinguishable in their own right (3).

Similarly, even the other previously individual species have yielded other separate species. The old \textit{Nocardia brasiliensis} genus is now known to be made up of two separate species, \textit{N. brasiliensis} and \textit{Nocardia pseudo-brasiliensis} (7, 8). These two species have significant genetic and clinical differences; the former causes primarily skin and lymphocutaneous infections, while the latter is associated with pulmonary and disseminated disease (8). At least 104 species have been presently recognized within the genus \textit{Nocardia}, which remains greatly heterogeneous and will continue to evolve (1, 3, 9).

The \textit{Nocardia} are found extensively worldwide and are saprophytic, making up an important component of normal soil microflora and often associated with fresh and salt water. In the U.S., the prevalence of nocardial infections seems to be greatest in the Southwest, where dispersal of infectious material may be facilitated by warm, dry, and windy (thus dusty) conditions (9). They may also be found in association with decomposing plant material and feces (1, 2).

At least 103 species of \textit{Nocardia} have been associated with human infections, but the geographic prevalence of each may be dramatically different throughout the world, with some being uncommon or rare. Seventeen species are more commonly implicated with human infection, while an additional 15 species have been associated with human infection but are either rarely isolated or of unknown prevalence; at least 21 other species have been described from the environment or from other animal species, but these have not yet been implicated in human infections (3).

\textit{Nocardiocyaageorgica} seems evenly distributed throughout the U.S., together with \textit{N. farcinica}, although the latter may be less prevalent. Distribution of other species may vary regionally. Although most often associated with tropical environments, \textit{N. brasiliensis} is relatively common in the U.S., with a higher prevalence in the southwestern U.S. (1, 10, 11). \textit{Nocardia otitidiscaviarum} has infrequently been recovered from soil throughout the world. The specific natural habitats of many nocardial species have not yet been ascertained (1, 2, 10).

In the U.S., the majority of nocardial infections are acquired through inhalation, followed in far smaller numbers by traumatic percutaneous inoculation (1, 3, 10). Infections with \textit{N. brasiliensis} and \textit{N. otitidiscaviarum} in the normal host are associated with percutaneous implantation via a foreign object. Little is known of the epidemiology of many of the newly recognized potentially pathogenic human species such as \textit{N. africana}, \textit{N. paucivorans}, and \textit{N. veterana} (12–14). Nocardiosis is not thought to be transmitted from person to person and is rarely acquired nosocomially (10). There have been reports of clusters of patients infected with identical strains of \textit{Nocardia} in which nosocomial acquisition was a probability (15–17).

The most common species that cause human disease include \textit{N. abscessus}, \textit{N. brasiliensis}, \textit{N. cyriacigeorgica}, \textit{N. farcinica}, \textit{N. nova}, \textit{N. otitidiscaviarum}, \textit{N. pseudo-brasiliensis}, \textit{N. veterana}, and \textit{N. wallacei}. Other species of \textit{Nocardia} are infrequently isolated or poorly documented as human pathogens (Table 2). The prevalence of various species appears to be related to geographic location.

### Table 1: Antimicrobial susceptibility profiles for the genus \textit{Nocardia}$^{a,b}$

<table>
<thead>
<tr>
<th>Organism</th>
<th>Amox/clavulanic</th>
<th>Ceftriaxone</th>
<th>Ciprofloxacin</th>
<th>Clarithromycin</th>
<th>Amikacin</th>
<th>Tobramycin</th>
<th>Gentamicin</th>
<th>Imipenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{N. abscessus}</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>V</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>\textit{N. brevicatena/paucivorans}</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>V</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>\textit{N. nova complex}</td>
<td>R</td>
<td>S</td>
<td>V</td>
<td>S</td>
<td>S</td>
<td>V</td>
<td>V</td>
<td>S</td>
</tr>
<tr>
<td>\textit{N. transvalensis complex}</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>V</td>
</tr>
<tr>
<td>\textit{N. farcinica}</td>
<td>S$^c$</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>V</td>
</tr>
<tr>
<td>\textit{N. cyriacigeorgica}</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>\textit{N. brasiliensis}</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>\textit{N. pseudobrasiliensis}</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>\textit{N. otitidiscaviarum}</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>V</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

$^a$Adapted from reference 138.

$^b$Abbreviations: Amox, amoxicillin; S, susceptible; R, resistant; V, variable.

$^c$About 80% of the \textit{N. farcinica} are susceptible to amoxicillin-clavulanic acid.
TABLE 2 Species of Nocardia more commonly associated with human infection

<table>
<thead>
<tr>
<th>Species and Isolation Frequency</th>
<th>Primary Skin</th>
<th>Pulmonary</th>
<th>Disseminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N. cyriacigeorgica</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N. farcinica</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>N. wallacei</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N. brasiliensis</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. nova</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>N. abscessus</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. pseudobrasiliensis</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>N. otitidiscaviarum</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>N. veterana</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Infrequently isolated or poorly documented

-Compiled from reference 139.

The Nocardia are primarily opportunistic pathogens with a predilection for immunocompromised patients. Cystic fibrosis may also be a condition predisposing patients to colonization with Nocardia, which can lead to infection (18). Infection is characterized by acute inflammation, necrosis, and formation of abscesses, while granulomas are not usually formed (10). The organisms may invade almost any organ system and present with a wide array of symptoms which are normally nonpathognomonic. Their primary clinical presentation is that of a respiratory infection, often of a chronic nature. Such pulmonary manifestations are usually associated with compromised patients, especially those on long-term corticosteroids for other respiratory conditions. Severe infections in the immunocompromised host are frequently fatal unless appropriate therapy is initiated.

In one evaluation of nocardiosis in patients over a one-year period, 75% were associated with underlying chronic lung conditions, followed by diabetes mellitus, hematologic and solid malignancies, transplantation, and AIDS (10). Fewer than 10% of patients had no definable immunocompromising conditions. Many of such seemingly healthy patients have infection caused by transcutaneous inoculation or infection secondary to abrasion (10). Additional manifestations of nocardiosis in the immunocompromised patient almost always occur via respiratory entry followed by dissemination and may include bacteremia, empyema, brain abscess, pericarditis, synovitis, peritonitis, and ocular infection (19).

In reality, almost any organ site may be a target of dissemination. The brain has been frequently reported as a site of metastasis, especially with N. farcinica, which, according to animal studies, seems to have greater invasive capabilities than other species (20, 21). Surprisingly, in at least one report from the arid Southwest U.S., the CNS was not a major site of Nocardia infections (10). In that study, less than two percent of 433 evaluable cases of nocardiosis over a five-year period had the CNS infections. In fact, the lower respiratory tract yielded 76% of isolates, while wounds (soft tissue, lymph nodes, and surgical wounds, including deep lines) yielded 16% and blood yielded 3%. The species N. cyriacigeorgica and N. transvalensis complex have mainly been associated with pulmonary disease and have infrequently caused infections of the brain, eye, and soft tissue (22–24). The N. nova complex is commonly related to disseminated disease, with involvement of multiple body sites. Common sites of infection include skin and/or soft tissue, pleural fluid, blood, joints, and cornea (25).

N. pseudobrasiliensis is associated with lower respiratory infection and dissemination (7, 26, 27). Infection may occur via percutaneous inoculation or entry via soft-tissue traumatic abrasions (thorns, cat scratch, or contamination of lesions by dirt). Such infections almost always occur in immunocompetent hosts and are associated specifically with N. brasiliensis, N. otitidiscaviarum, and N. transvalensis complex (28–31). Localized infections usually present as cellulitis, necrosis, and abscess formation or may spread lymphocutaneously in “sporotrichoid fashion,” similar to the fungus Sporothrix schenckii. Corneal ulceration after trauma can also occur. In the Western Hemisphere, N. brasiliensis is also one of the most common causes of actinomycotic mycosis, which is usually limited to the very southernmost areas of the U.S. and especially in Mexico. It is rarely found in the northern parts of the U.S.

Rhodococcus spp.

Once considered the rodocrous strains of the genus Mycobacterium, Rhodococcus gained generic status in 1977, based on the culmination of chemotaxonomic, serological and genetic studies (32). Redefining members within the genus Rhodococcus moved several species into a new genus now known as Gordonia and one species into the genus Tsukamurella (1, 2). The rhodococci contain 34- to 52-carbon mycolates, no mycobactins, and eight isoprene dehydrogenated menaquinones (33–35). Chemotaxonomic differences and
differences between molecular 16S rRNA sequences exist between Gordonia and Rhodococcus spp. The 53 species that have been separated within the genus Rhodococcus have been isolated from feces of animals (especially herbivores) and grow well in soil contaminated by the latter. Other sources that have been implicated include fresh water, marine habitat, fish, animal urine, and the gut of some arthropods (36).

Rhodococcus equi is the most common species associated with infections in humans as well as in a wide range of animal hosts. It is a major cause of suppurative bronchopneumonia or mesenteric lymphadenitis in young foals and is playing increasingly important roles in pulmonary and disseminated disease (often with presence of bacteremia) in immunocompromised humans. The organism has a predilection for causing pulmonary infection and bacteremia in patients with AIDS, those receiving organ transplants, or those undergoing chemotherapy (37–40). Patients receiving corticosteroids and those with long-term presence of a foreign body (such as a catheter) are also at increased risk for infection (41, 42). Other species of Rhodococcus, including R. aurantiacus, R. erythropolis, R. globerulus, R. gordoniae, R. corynebacteroides, and R. rhodochrous, have rarely been implicated in human disease (43–48).

In animals, virulence factors of R. equi include the presence of large plasmids which encode for the virulence-associated proteins A and B (VapA and VapB). The protein VapA has been shown to play a role in delayed phagosome maturation, intramacrophage survival, as well as cytotoxicity (49), whereas isolates that express the VapB protein alone are only intermediately virulent in nonhuman hosts. The presence or absence of vapA/vapB encoding plasmids in Rhodococcus equi shows a strong association between the animal host and specific plasmid types. Plasmid type vapA+B− correlates with equine isolates, vapA+B+ with porcine isolates, and vapA+B− with bovine isolates. Interestingly, all human strains analyzed were either vapA+ or plasmid-less (50). That being said, patients presenting with R. equi infection often have no history of direct exposure to animals. The presence of R. equi in sputum and central lines may indicate that inhalation or contamination of lines via air may be a primary source of infection (49).

Gordonia spp.
In 1988, the genus was initially introduced as Gordona to accommodate those members of Rhodococcus which were aberrant in their molecular structure and had differences in their mycolates (48- to 66-carbon mycolates) as well as in their dehydrogenated menaquinones (nine isoprene units) (51). The genus name was later changed to Gordonia. Presently, 38 species are listed in the genus.

Species in this genus are well distributed in nature and have been isolated from soil, sewage treatment plants, biofilters from waste gas treatment, and sputum (2). Historically, Gordonia infections have been overlooked as irrelevant coryneforms (52, 53) or misidentified as Nocardia or Rhodococcus (54, 55). Nevertheless, the Gordonia species G. aichiensis, G. bronchialis, G. effusa, G. oitidis, G. polyisoprenivorans, G. rubripertincta, G. spu, and G. terrae have been associated with central venous catheter-related bacteremia (53, 54, 56, 57) and peritonitis (52, 58) in patients with underlying malignancy or an immunocompromised state. There have also been rare incidents of an orthopedic device-associated infection caused by G. araii (59) and a cutaneous infection caused by G. amicalis (60).

Tsukamura spp.
Tsukamura was the first to study the organism isolated from sputum in 1971 and is the individual for whom the genus was named in 1988 (61). The primary species in this genus, Tsukamuraella paurometabola, was originally called Corynebacterium paurometabolum. The species in the genus Tsukamuraella contain 62 to 78 carbon atoms in their mycolates, and menaquinones are of the MK-9 type (62).

Tsukamuraella spp. have been retrieved from soil, sludge and arthropods. Most isolates seem to prefer cooler temperatures. Isolates in this genus are considered rare opportunistic pathogens; the species most often associated with infection are T. pulmonis, T. tyrosinosolvens, T. paurometabola, and T. spumae. Much like Gordonia, Tsukamuraella is frequently misidentified as Rhodococcus or Corynebacterium spp. and often requires molecular techniques to obtain a definitive identification. Most of the reported cases have been found in oncological patients, in patients undergoing dialysis and in those with indwelling catheters, or in patients with other immunocompromising conditions. Infections have mainly been associated with bacteremia (63–66), pulmonary disease (67–69), cutaneous infection (70), keratitis (63, 71), and knee prosthesis infection (72).

Other Aerobic Actinomycetes: the Genera Actinomadura, Amycolata, Amycolatopsis, Dermatophilus, Dietzia, Nocardiopsis, Pseudonocardia, Segniliparus, Streptomyces, and Williamsia
Several genera in this group (Actinomadura, Dermatophilus and Streptomyces) are primary pathogens in
several infectious clinical syndromes. In the U.S., they are rarely associated with diseases in humans and are rarely, if ever, involved specifically with immunocompromised patients. They are limited geographically to tropical or subtropical areas. Other species in this group are rarely described in human disease, and their in-depth characterization is not within the scope of this chapter. For additional insight on the subject, the reader is directed to the chapter on aerobic actinomycetes by Conville and Witebsky in the 10th edition of the Manual of Clinical Microbiology (1).

**Actinomadura spp.**

The organisms within the genus *Actinomadura* were originally classified within the genus *Nocardia* but were later assigned status in their own genus (73). They all share the chemotype III cell wall containing the sugar madurose (3-O-methyl-D-galactose), a characteristic shared only by *Dermatophilus* (74). The genus contains at least 75 species, of which two (*A. madurae* and *A. pelletieri*) are commonly associated with human infections.

*A. madurae* and *A. pelletieri* most commonly cause disease in humans through the evolution of a slow but progressive actinomycotic mycetoma (75). The infection is initiated by transcutaneous implantation of organisms from soil or debris, with the initial formation of a painless nodule. Thus, the usual site of infection is the foot and the reason for the name “Madura foot.” The nodule may become fluctuant and continue to evolve into a pus-draining sinus. Over time, additional nodules and sinus tracts form, and the tissues become fibrous and often deform the areas involved. Further progression may involve connective tissue, muscle, and bone. The cellular response in these actinomycotic mycetomas is pyogenic rather than granulomatous, thereby separating them from mycetomas caused by the fungi. Normally, these infections are seen in healthy immunocompetent patients and do not spread via lymphatics (2).

Mycetoma is common in tropical regions and is associated with walking shoeless; it is rarely found in the U.S., except perhaps in some of the southeastern states.

In rare instances, *Actinomadura* species have been associated with peritonitis, pulmonary infections, and bacteremia as well as dissemination to sites such as the spine and abdominal wall (76–79).

**Amycolata spp., Amycolatopsis spp., and Pseudonocardia spp.**

The members of *Amycolata* and *Amycolatopsis* were assigned their own genera and were moved from the *Nocardia* genus because they were Gram-positive but modified acid-fast stain negative (80). As for *Nocardia* spp, both genera have aerial mycelia. There are few isolates associated with human disease. The genus *Amycolata* has four recognized species, *Amycolatopsis* has 65 species, and *Pseudonocardia* has 54 species.

There have been no well-characterized or defined cases in humans associated with *Amycolata, Amycolatopsis*, and *Pseudonocardia* (1). That said, an isolate of *Amycolatopsis palatopharyngis* was found in association with an infected palatopharyngeal mucosa of an elderly patient (81).

**Dermatophilus spp.**

*Dermatophilus congolensis* is the sole member of this genus. *Dermatophilus* is separated from the others in this group by the presence of diaminopimelic acid and madurose (see *Actinomadura* also) in its cell wall (82). This genus also has a unique mechanism of division, which includes horizontal, longitudinal, and transverse septation of the mycelium, culminating in the production of motile zoospores under favorable climatic conditions. These vegetative mycelia are covered at points of such division by a gelatinous capsule forming a multicellular sporangium.

*D. congolensis* is an obligate animal pathogen which causes dermatitis in a wide variety of animals worldwide, including cattle, horses, goats, and sheep (83). *D. congolensis* infections in humans have been noted on only a few occasions and manifest exclusively in relation to the skin epidermis. Dermatophilosis in humans can present as pustular, exudative, and scaling lesions, recalcitrant verruca, hairy leukoplakia of the tongue, pitted keratolysis, and chronic nodular disease (84–88).

**Dietzia spp.**

The first of the species in the genus *Dietzia, Dietzia maris*, was moved from *Rhodococcus* to its own genus because of biological and molecular differences (89). There are 13 species recognized in the genus. Most identification schemes within the clinical laboratory incorrectly identify *Dietzia* species as *Rhodococcus equi*. The attainment of definitive genus-level identification normally requires 16S rRNA gene sequencing (90, 91). Only a few cases of *Dietzia* infection have been noted in humans. Cases are typically associated with *D. maris*, but in rare instances *D. cinnamea, D. pappillomatosis*, and *D. aurantiaca* have been isolated from clinical specimens (92, 93). *D. maris* has been associated with bacteremia, infectious aortitis, and a hip prosthesis infection (94, 95).
**Nocardiosis** spp.
Members of the species *Actinomadura dassonvillei* were given their own genus status (*Nocardiosis*) because they differed from other *Actinomadura* both morphologically and chemotaxonomically (96). The cell wall is made of mesodiaminopimelic acid and is considered a chemotype III but lacks mycolates and madurose. Chains of arthrospores fragmenting from aerial and substrate hyphae are characteristically produced. Only two of the 45 species in this genus have been shown to be pathogenic (2). Infection in humans is very rare and always caused by *N. dassonvillei* and *N. synnemataformans*. These opportunistic pathogens cause mycetoma, suppurative infections, and abscesses (97).

**Segniliparus** spp.
The *Segniliparus* genus contains rapidly growing, strongly acid-alcohol-fast-staining bacteria. The cell wall contains meso-diaminopimelic acid and mycolic acids. By 16S rRNA gene sequence, the genus is most closely related to genus *Rhodococcus*. Currently, there are two closely related species (*S. rotundus* and *S. rugosus*) in the genus (98). Both were recovered from respiratory specimens and were originally phenotypically identified as *Mycobacterium* species. *S. rugosus* has been reported in patients with cystic fibrosis and *S. rotundus* from a patient with non-cystic fibrosis bronchiectasis (99–101). By studying immune response and animal modeling, it was shown that *S. rugosus* is more virulent than *S. rotundus* (102).

**Streptomyces** spp.
The *Streptomyces* genus contains an extremely large assortment of poorly defined species. With almost 668 species, the nomenclature is in a state of flux and is tentative for many. The cell wall in this group is of the chemotype I (L-diaminopimelic acid and glycine but no sugar) (74). The streptomycetes primarily cause localized, suppurative, mostly chronic mycetomas (similar to *Actinomadura* above) (10). *Streptomyces somaliensis* is the most common species in this genus to cause mycetoma (103). It prefers arid regions with sandy soil; thus, it is normally found primarily, but not exclusively, in Africa, Mexico, and portions of South America. The lower extremity is the most common site of infection. Nonsubcutaneous *Streptomyces* infections are rare and are typically associated with preexisting conditions such as malignancy, HIV infection, AIDS, the presence of a central venous catheter, or prosthetic heart valve (104). Most cases consist of pulmonary infections or bloodstream infections (104–106). Nonetheless, microbiological and pathological results must correlate, as most isolates found in cultures from patients presenting with infections, other than mycetoma, are almost always either contaminants or colonizers.

**Williamsia** spp.
The recently established genus *Williamsia* includes environmental organisms similar to genera in family *Nocardiaceae*. It possesses mycolic acids with carbon chain lengths of 50 to 56. Based on its mycolic acids, it seems that *Williamsia* takes an intermediate position between *Rhodococcus* (mycolic acid chain lengths of 34 to 45) and *Gordonia* (mycolic acid chain lengths of 54 to 66) (107). Presently, nine species are recognized in this genus. *Williamsia*-related infections in humans are rare. *Williamsia* murals has been associated with pulmonary disease in an elderly patient and with endophthalmitis in an individual suffering from diabetic maculopathy (108, 109). There is one reported case of *Williamsia serinedens* causing perinatal sepsis (110).

The numbers of species for each of the genera listed in the above section were derived from the List of Prokaryotic Names with Standing in Nomenclature (LPSN, http://www.bacterio.net/index.html).

**GOALS OF LABORATORY TESTING**
In diagnosis of clinically significant infections caused by the aerobic actinomycetes, evaluation of appropriate specimens by direct microscopy and culture remains the primary goal of the laboratory. With appropriate attention, detection in smear and isolation on primary and/or selective media are not overly difficult (10). The laboratory should be able to differentiate quickly between probable colonizers or contaminants and true pathogens. They should be able to help the clinician interpret direct microscopic and culture results and to provide guidance in choosing both empiric as well as laboratory-guided therapeutic modalities. Laboratories should be able to recognize when an organism must be further identified (i.e., when it will be relevant in patient care decisions or important for epidemiologic purposes).

While it is usually fairly simple to identify the presence of a potential pathogen in an overtly morbid infectious process and to identify the microbe to a group or genus level, it is much more difficult to identify members of the aerobic actinomycetes to a species level due to the vast numbers of organisms that have recently been implicated in human infection. When necessary, laboratories must be ready to rapidly forward the isolate to an appropriately competent licensed reference laboratory in
full compliance with the regulatory measures for the safe transportation of potentially pathogenic isolates. In some instances, special susceptibility studies are also necessary to help guide therapy and should be performed or quickly referred to another appropriate laboratory.

Screening for, and prevention of, infections caused by the aerobic actinomycetes in the immunocompromised host play no real role. Surveillance cultures generally have no utility because most of the aerobic actinomycetes are ubiquitous in the environment and may easily contaminate specimens from nonsterile sites. Isolation from such sites does not implicate an organism in any specific infection, and isolation may confound or mislead the clinician.

Likewise, prophylactic or preemptive therapy of patients with prolonged immunocompromising states is not universally recommended. There are some authorities that support such suppressive therapy for nocardiosis, although there are no well-designed studies to show that such therapy has a positive effect on outcomes (111). Serologic screening or diagnosis is unreliable, and serologic tests are not commercially available for the aerobic actinomycetes. Skin tests may be of some value in diagnosis of actinomycotic mycetoma, but the utility of these assays is limited by their sensitivity and cross-reactivity with tuberculosis and leprosy (112, 113).

**Specimen Collection**

The collection and transportation of appropriate specimens is crucial to the diagnosis of infections with the aerobic actinomycetes. Respiratory specimens are most commonly submitted, except in more tropical regions of the world where actinomycotic mycetomas prevail. Bronchoscopically collected lower respiratory secretions result in a higher recovery rate of true etiologies of infections than do expectorated sputum secretions (3). Other specimens, such as tissue and normally sterile body fluids (including cerebrospinal fluid, synovial fluid, peritoneal fluid, etc.) make up a significant portion of material submitted for evaluation; such specimens are normally associated with extrapulmonary dissemination and in traumatically caused infection. Blood may also yield aerobic actinomycetes as etiologic agents, especially in immunocompromised patients and often in those with long-term catheters. Thus, each clinical setting must be evaluated on its own characteristics when deciding the most appropriate specimen choice.

Granules may be present in the drainage material from mycetomas and other chronic infections. These are usually associated with infections caused by *Actinomadura* and *Streptomyces* spp. but may also occasionally be found in chronic infections caused by *N. brasiliensis*. It is important to look for such granules in the appropriate specimens and, if found, to wash them in sterile saline and to crush them between glass slides prior to microscopic examination. Contaminated specimens are often pretreated in an attempt to decrease the contamination load prior to culture set-up. Unfortunately, decontamination methods used for the mycobacteria (e.g. with N-acetyl-l-cysteine) are too harsh for the other aerobic actinomycetes and lower their recovery rates (114). To diminish the effect of the pretreatment process, the specimens should be exposed to the decontaminating reagents for shorter periods of time (15 minutes). Alternatively, Convive describes a procedure in which the sample is diluted 1:10 in a preparation of 0.2 M HCL-0.2M KCL at a pH of 2.2 for 2 to 3 minutes before inoculation (115, 116). Paraffin baiting method was also shown to be promising for isolation of *Nocardiae* from contaminated specimens but is probably not clinically practical (117, 118).

**Microscopy and Direct Visualization**

Direct microscopic visualization can help provide early clues to the identity of the etiologic agent and to its actual role in the disease process. Early diagnosis and therapy of nocardiosis has been associated with better clinical outcomes. A number of wet-mount, Gram stain, or other special stains can be used to detect the aerobic actinomycetes either directly in specimens or after their decontamination and/or their concentration (e.g., decontamination of specimens from highly contaminated areas or concentration of normally sterile specimens such as synovial, peritoneal, or pleural fluids). Not only can stained or unstained smears and/or wet mounts of specimens show characteristic morphologies, they can also demonstrate the presence or absence of specific cellular material, such as polymorphonuclear cells, mononuclear cells, macrophages, and squamous epithelial cells (indicating presence of contaminated material). Evaluation of direct material can help interpret the cellular response, presence or absence of normal flora, the potential etiologic agent (in association with the appropriate cellular response), and the elucidation of their clinical significance (10).

Specialized stains such as the modified acid-fast stain (MAFS) and Kinyoun stain can further differentiate between some groups and genera of aerobic actinomycetes (10). Full-strength acid-fast stains should not be used on the nontuberculous aerobic actinomycetes, except to
separate the mycobacteria from the other genera in the group. The MAFS uses a 0.5 to 1.0% sulfuric acid de-colorizer rather than the full concentration used in the acid-fast stain for mycobacteria.

A nonspecific acridine-orange stain or a modified auramine-rhodamine acid stain may also be used to detect some organisms in specimens. Both methods, however, require use of a fluorescent microscope. Additionally, the Brown and Brenn Gram stain and the Gram-Weigert stain will usually stain the aerobic actinomycetes, but the hematoxylin and eosin stain does not stain adequately (2).

**Nocardia spp.**

In Gram-stained preparations, *Nocardia* are usually observed as Gram-positive and are beaded, filamentous, branching bacteria (Fig. 1A and 1B). They may, on occasion, fragment into coccobacillary forms. *Nocardia* spp. are considered MAFS-positive (Fig. 2A and 2B), but controversy surrounds the comparative efficacies of several staining techniques to demonstrate *Nocardia* in specimens. In some laboratories, the experience supports the Gram stain as the most sensitive microscopic method by which to visualize and recognize *Nocardia* in clinical specimens (10). In one study of 50 individual patients...

**FIGURE 1** Direct Gram stain of lower respiratory tract secretions showing polymorphonuclear infiltrates with associated filamentous, branching, beaded Gram-positive bacteria of the genus *Nocardia* (1000x magnification).

**FIGURE 2** Direct modified acid-fast stain (MAFS) of lower respiratory tract secretions showing filamentous, branching, beaded Gram-positive bacteria of the genus *Nocardia* (1000x magnification).
in whom *Nocardia* was directly visualized by Gram stain, only 51% had visually detectable MAFS-positive organisms; importantly, there were no Gram stain-negative but MAFS-positive specimens (10). The MAFS is not as reliable and should be used only to confirm the acid-fastness of organisms detected by Gram stain.

**Rhodococcus** spp.
Microscopically, rhodococci are usually diphtheroid-like, Gram-positive, pleomorphic coccobacilli without branching when viewed in stained specimens or on both solid and liquid media. They may also appear rod-like or filamentous as young cultures grown in liquid media. Occasionally, rudimentary branching may be seen on filaments when grown in liquid media. They are considered to be MAFS-positive, but only a very small portion of the cellular population may actually exhibit this characteristic; negative modified acid-fastness is especially prevalent in populations grown on media containing tryptic soy agar, such as with 5% sheep blood as well as chocolate agar (119).

The rhodococci do not have a characteristic morphology and may be readily confused with diphtheroids. However, microscopic examination of bronchial secretions often shows presence of short pleomorphic Gram-positive bacilli within polymorphonuclear cells. Histopathology may also show microabscesses, pseudotumors, and granulomatous inflammation (120, 121).

**Gordonia** spp.
*Gordonia* typically present as beaded Gram-positive diphtheroid-like, nonbranching coccobacilli and are occasionally arranged in a cuneiform “Chinese lettering” fashion. MAFS staining of *Gordonia* is typically weakly positive or negative.

**Tsukamurella** spp.
Microscopically, the organisms in this genus have been described as long, and usually straight, thin rods that may show a slight curvature and no branching. They are MAFS-positive but might show only slight staining.

**Other Aerobic Actinomycetes: the Genera Actinomadura, Amycolata, Amycolatopsis, Dermatophilus, Dietzia, Nocardiopsis, Pseudonocardia, Segniliparus, Streptomyces, and Williamsia**

**Actinomadura** spp.
The colonies and organisms in this genus resemble those in *Streptomyces*. They are short, thin, branching rods, which are MAFS-negative. *Actinomadura madurae* infection often results in draining granules, which are white to yellow in color, while *A. pelletieri* results in granules that are red to pink.

**Amycolata** spp., *Amycolatopsis* spp., and *Pseudonocardia* spp.
These organisms are Gram-positive bacilli but are MAFS-negative. Like the *Nocardia*, they can occasionally fragment into bacillary, coccoc or square forms.

**Dermatophilus** spp.
These organisms are Gram-positive bacilli but are MAFS-negative and may be coccobacillary. On culture, microscopic examination often reveals branching forms and the development of longitudinal as well as transverse septa along the filaments.

**Dietzia** spp.
These organisms almost never branch but show coccoc and bacillary Gram-positive cell forms and are MAFS-negative.

**Nocardiopsis** spp.
These organisms form long, Gram-positive, branched filaments that fragment into zigzag chains of arthroconidia-like spores.

**Streptomyces** spp.
These organisms are solidly Gram-positive filamentous bacillary forms but are MAFS-negative. Fragmentation of mycelia and beading may be found. Granules found with *S. somaliensis* are usually white to yellow in color.

**Segniliparus** spp.
These organisms are Gram-positive non-branching rods, occasionally in V forms. Both species are strongly acid-fast positive.

**Williamsia** spp.
Short Gram-positive rods or coccobacilli, not branching. These organisms are not acid-fast.

**Antigen Testing**
Antigen testing or *in vitro* test systems for antigens of aerobic actinomycetes are not commercially available. Such studies play no role at this time in the diagnosis of infections caused by this group of organisms.

**Culture**
Most clinically relevant aerobic actinomycetes are able to grow on routine bacteriology or mycology media (2).
Primary media such as 5% sheep blood agar, chocolate agar, buffered charcoal yeast extract agar, Mueller-Hinton agar, tryptic soy agar, brain-heart infusion agar, and various broths will readily support the growth of clinical isolates. Well-isolated colonies that are considered presumptive aerobic actinomycetes should be evaluated using the algorithm listed in Fig. 3.

However, because other organisms present in contaminated specimens may readily overgrow the slower-growing aerobic actinomycetes, it is imperative to add selective media to enhance the latter's recovery in culture. Thus, for potentially contaminated specimens, primary culture media should be supplemented with selective media such as selective buffered charcoal yeast extract agar (containing polymyxin B, anisomycin, and either vancomycin or cefamandole), modified Thayer-Martin agar, Lowenstein-Jensen agar, Colistin-Nalidixic acid agar, and Sabouraud dextrose agar. The Nocardia grow well on fungal media containing cycloheximide as well. Chloramphenicol should not be used as a selective agent as it can also inhibit the aerobic actinomycetes. In general, selective media may also have an inhibitory effect on some species and should never be used alone without the primary media (2, 122).

Various commercial blood culture systems can also support growth of the aerobic actinomycetes, with recovery usually occurring between 3 and 19 days (123, 124). Terminal subcultures are useful when these organisms are suspected. Use of fungal blood culture systems may also be helpful.

Some clinical scientists believe that media should be incubated at both 30°C and 35°C (1). If the strepto-
mycetes are suspected, then incubation at 25°C is also warranted. Cultures should be treated as fungal cultures with incubation extending for at least 2 weeks and perhaps longer, depending on the species that is suspected. Agar plates should be sealed to keep them from dehydrating. The cultures should be examined every 2 days for the first week and then at least twice a week for the remainder of the incubation period (2).

Once clinically significant isolates are recovered, they may be inoculated to slide cultures to help with the identification process. Agar blocks (using agar with minimal nutrients such as tap water agar) are cover-slipped, incubated at 25°C for up to 2 to 3 weeks in a moist environment, and periodically examined with a dissecting microscope for aerial and substrate filaments (mycelia) and branching (1).

**Nocardia spp.**
*Nocardia* will normally appear within 2 to 7 days on most routine bacteriologic media such as 5% sheep blood agar and chocolate agar. Although the majority of isolates will be detected within the first several days of incubation, media should be examined for up to 2 or 3 weeks. Use of a dissecting microscope may help in the recognition of the filamentous, white to yellow to orange colonies with aerial mycelia and delicate, dichotomously branched substrate mycelia typical of the genus *Nocardia* (2). Organisms can occasionally fragment into bacillary or coccal forms. Of the MAFS-positive species, *Nocardia* are the only ones that produce aerial mycelia.

**Rhodococcus spp.**
Rhodococci are frequently recovered from respiratory secretions, in material on infected catheter tips or lines, and from blood. As mentioned earlier, on culture, the rhodococci are usually seen as pleomorphic, short cocccoid forms but may show filaments in younger cultures in liquid media. Colonies have variable morphologies and may be rough to smooth and mucoid. Some, but not all colonies, may show red to yellow pigmentation with age. Except for colonies of a few nonpathogenic species, most species' colonies show no macroscopically visible aerial mycelia. Growth optimally occurs at 28 to 35°C, but not at 45°C. The organisms are aerobic, catalase-positive and nonmotile.

**Gordonia spp.**
Colonies are commonly wrinkled and salmon to orange in color after several days of incubation. Normally, these organisms show either rudimentary or no branching structures.

**Tsukamurella spp.**
Colonies do not produce aerial mycelia and are usually small, smooth to rough, and with pigmentation varying from whitish to orange. The organisms are catalase-positive.

**Other Aerobic Actinomycetes: the Genera Actinomadura, Amycolata, Amycolatopsis, Dermatophilus, Dietzia, Nocardiopsis, Pseudonocardia, Segniliparus, Streptomyces, and Williamsia**

**Actinomadura spp.**
Colonies may vary between isolates, are frequently wrinkled, and have evidence of aerial mycelia.

**Amycolata spp., Amycolatopsis spp., and Pseudonocardia spp.**
As in *Nocardia*, colonies in these genera have aerial mycelia.

**Dermatophilus spp.**
These organisms are facultative anaerobes. The colonies produce aerial mycelia under increased CO2 conditions, are heaped, opaque, and beta-hemolytic on sheep blood agar. The colonies may turn yellow to orange in color as they grow older. They have characteristic vegetative mycelia with long filaments that branch laterally as well as transversally and longitudinally.

**Nocardiopsis spp.**
The colonies are often wrinkled or folded. Aerial mycelia are produced but may be sparse in some isolates. The filaments associated with the agar fragment into coccal forms, while the aerial filaments break up into spore-like forms of various sizes.

**Streptomyces spp.**
Colonies between isolates may vary, are frequently wrinkled and have evidence of aerial mycelia.

**Segniliparus spp.**
The genus presents as smooth and rough nonpigmented colonies, which may produce a soluble pigment. Growth on the Middlebrook media resembles rapidly growing mycobacteria (98).

**Williamsia spp.**
Smooth, yellow to orange, or red colonies.
**Identification**

Initial visualization of phenotypic colony coloration and morphology, together with presence of aerial hyphae using a dissecting microscope, often provides initial clues to the genus of the isolate. Presumptive identification can be achieved if a filamentous, branched isolate stains with the MAFS but not with the traditional Kinyoun acid-fast stain (10). Resistance to 0.005% lysozyme differentiates *Nocardiopsis* spp. from *Streptomyces* spp. *Tsukamurella* spp. are also MAFS-positive and are resistant to lysozyme but do not produce aerial filaments. *Gordonia* spp. have variable resistance to lysozyme but have no aerial filaments.

**Phenotypic identification**

Identification to species level using phenotypic characteristics may be more tenuous and problematic, and in some species it may be nearly impossible. Originally, identification of the *Nocardiopsis* spp. was based on hydrolysis of casein, tyrosine, xanthine, and hypoxanthine. However, differing stable susceptibility profiles within *N. asteroides* complex showed that at least six unique species were identifiable (3). Molecular as well as further phenotypic studies of the species confirmed their disparity and uniqueness.

Presently, phenotypic characteristics, and in some cases susceptibility profiles, may be useful in separating the major genera and some species of aerobic actinomycetes, but molecular means are required for some of the newly described species. Some phenotypic characteristics that are useful in differentiating the more common clinically significant aerobic actinomycetes include the microscopic and colony morphologies and a number of biochemical or susceptibility profiles. Phenotypic characteristics that are useful include use of acetamide, aroylsulfatase, acid production, utilization of carbohydrates as sole carbon sources, esculin hydrolysis, Middlebrook opaciﬁcation, substrate decomposition, temperature studies, and urease studies (1). Schematic ﬂow charts, use of commercial test systems, and review of phenotypic characteristics have been described by others (1, 2, 10, 125).

However, these algorithms do not address the large number of newer species that have recently been reported; thus, they cannot differentiate between many of the newer species recovered. To identify these would require a huge number of phenotypic studies with a large enough database from which to be able to conﬁrm identity.

**Genotypic identiﬁcation**

Evaluation of whole-cell hydrolysates, chromatographic procedures such as paper, gas, thin-layer and high-performance liquid chromatography, and mass spectrometry for fatty acids, as well as cell wall and mycolate analysis, have been used to differentiate between some of the genera and species (126). These methods are not adequate for overall identiﬁcations, nor are they amenable for use in the clinical laboratory.

Presently, moderately successful molecular methods used to identify isolates to the species level include restriction endonuclease analysis of an ampliﬁed portion of the 16S rRNA gene, ribotyping, PCR using randomly ampliﬁed polymorphic DNA, and DNA probes. However, these techniques have a number of shortcomings, and thus, they cannot be used to identify most of the aerobic actinomycetes species and are not frequently used (1). Gene sequencing methodologies, such as that of the 16S rRNA or DNA have recently become popular for identiﬁcation of most aerobic actinomycetes (2, 18, 127). Gene sequencing of a 500-bp region of 16S rRNA has gained momentum for identiﬁcation of many such isolates; but a longer bp region may be needed for better discrimination (128). Sequencing of other gene targets like HSP, the secA1, and gyrB gene allow for greater discrimination among species than does 16S rRNA (129–131).

**Proteomic identiﬁcation**

MALDI-TOF MS has become a widely used technology in the clinical laboratory to quickly and easily identify commonly encountered bacteria and yeast. Thus, from a practical perspective, MALDI-TOF MS has the potential to eliminate conventional phenotypical methods, which are time consuming and difﬁcult to interpret even by experienced staff. Unfortunately, the use of MALDI-TOF MS remains limited as aerobic actinomycetes are not currently part of the reference databases found in commercially available MALDI-TOF MS systems. That being said, several groups have shown the utility of MALDI-TOF to identify aerobic actinomycetes either through the use of research-based reference databases or through the development of laboratory-developed data sets (132–134).

**Interpretation of Data**

The presence of aerobic actinomycetes in the environment can lead to contamination and/or colonization of clinical specimens and can confuse clinical relevance. That being said, the aerobic actinomycetes are rarely seen as contaminants in the laboratory, and each isolate has to be carefully evaluated to discern clinical signiﬁcance (127). The presence of organisms in normally sterile sites or on direct microscopic examination of potentially contaminated specimens (especially in associa-
tion with a pyogenic cellular response) such as sputum greatly increases the likelihood of an organism’s role being one of an etiologic agent (3, 10). Immunocompromising conditions or corticosteroid use in patients also significantly increases the clinical relevance of isolates. Semiquantitation of isolates in specimens can further elucidate their significance. A colony or two on primary media or isolated from a single medium or from broth may not be considered as significant as the presence of large quantities of growth from multiple media and plates or tubes, but low colony counts cannot be universally dismissed in immunocompromised individuals. It is extremely important that isolates be evaluated in conjunction with the patient’s clinical presentation as well as any underlying conditions. Finally, the organisms’ identity is also very important and is associated with clinical significance. N. farcinica is far less likely to be a contaminant or colonizer than a member of the streptomycetes; thus, it is important to include all facets of the case in evaluating the significance of a specific isolate in each setting.

**SUSCEPTIBILITY TESTING AND THERAPY**

The majority of aerobic actinomycetes have variable susceptibilities to a wide variety of agents and require in vitro susceptibility testing to help guide therapy. A standard for susceptibility testing of the aerobic actinomycetes using broth microdilution and cation-supplemented Mueller-Hinton broth has been published by the CLSI (Clinical Laboratory Standards Institute) (11). The organisms included in the standard include Nocardia, Actinomadura, Rhodococcus, Gordonia, Tsukamurella, and Streptomyces spp. The primary recommended antimicrobial agents for testing are amikacin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline, moxifloxacin, sulfamethoxazole or trimethoprim-sulfamethoxazole, and tobramycin. The secondary set of recommended agents include cefepime, cefotaxime, and doxycycline. Interpretative guidelines and reporting formats are provided in that publication. In general, therapeutic efficacy in patients is dependent on the presenting infection, susceptibilities of the recovered isolates and on the extent of the immunocompromising conditions in patients. In vitro susceptibilities of various isolates, can vary dramatically and susceptibility studies are indicated.

**Nocardia spp.**

Nocardia species can vary in their antimicrobial susceptibility patterns. Therapeutic efficacy in individual patients may depend on species identity and on in vitro susceptibility studies (1, 3, 10). Susceptibility testing should be especially considered in refractory cases. Besides the standard microdilution method published by CLSI, disk agar diffusion and gradient strip agar dilution (E test) methods have all also been used for susceptibility testing of the Nocardia (1, 20, 135). Studies have shown inter- and intralaboratory agreement and reproducibility of above 90% between these methods (1, 10, 135). Multisite reproducibility of the microdilution method has shown more than 90% agreement for amikacin, ciprofloxacin, clarithromycin, moxifloxacin, amoxicillin-clavulanic acid, linezolid, minocycline, and tobramycin. However, the reproducibility of ceftriaxone with N. cyriacigeorgica and N. wallacei was less than 90% even with mode +/- 2 dilutions. There was also lack of reproducibility testing with sulfonamides for N. farcinica and N. wallacei (136). Prospective clinical studies attempting to correlate results of susceptibility testing to patient therapy and outcomes have not, however, been systematically performed. Most of the available data are accumulated from anecdotal and individual cases.

Sulfa-containing antimicrobials remain the drugs of choice for nocardiosis and improve survival when used alone for respiratory tract infections, or in combination with other antimicrobials when dealing with severe or disseminated disease in the immunocompromised patient (3, 127, 137). However, other antimicrobial agents are needed for patients intolerant to sulfonamides or in whom infection is refractory to sulfonamide therapy. Primary agents that have been used successfully, alone or in combination, include minocycline for less-severe infections such as skin and soft tissue or respiratory, amikacin, imipenem, linezolid and ceftriaxone (normally in combinations) for more serious infections. Linezolid should not be used for more than 4 weeks because of its high association with hematologic toxicity. Empiric therapy using combinations of a sulfa-containing agent with one or more of the latter has been recommended for serious, systemic disease (3, 127). Amikacin in combination with imipenem or ceftriaxone has also been suggested for serious infection. Imipenem seems more active than meropenem against the Nocardia (3). Other potentially efficacious choices include the third-generation cephalosporins, amoxicillin-clavulanate, ampicillin/sulbactam, newer macrolides, other aminoglycosides and the fluoroquinolones (1, 3, 10). The latter agents should be avoided unless susceptibility data are available; susceptibility data should be sought with all significant isolates of Nocardia. Duration of therapy is
uncertain but should be protracted because of considerable relapse after shorter courses (127). Cutaneous disease requires at least 3 to 6 months of therapy, serious infections at least 6 weeks with maximal dosing, followed by reduced dosing for between 6 and 12 months. Immunocompromised patients and those with CNS manifestations require at least a year of therapy.

**Rhodococcus spp.**
Isolates of *R. equi* are most frequently susceptible *in vitro* to a number of agents, including erythromycin and other extended spectrum macrolides, rifampin, fluoroquinolones, aminoglycosides, glycopeptides, and imipenem. Antimicrobial agents with some activity include TMP-SMX, clindamycin, and chloramphenicol, but these should not be used until susceptibility studies demonstrate them to be active. Most isolates are resistant to the penicillins and cephalosporins, and resistance to beta-lactams has been noted to arise during therapy for initially susceptible strains. Resistance has also been noted to develop during therapy with TMP-SMX, doxycycline, and rifampin.

Strains of *R. equi* may be resistant to intracellular killing, and thus an agent that penetrates into macrophages (e.g., azithromycin, fluoroquinolones) should be chosen in combination with other agents. In immunocompetent patients, single-agent therapy with a macrolide or fluoroquinolone is adequate, while in the immunocompromised patient combination therapy with two or more agents is required. Depending on patient immune status, the clinical presentation, and the susceptibility profile of the isolate recovered, one may choose amongst the macrolides and the fluoroquinolones in combinations with rifampin, vancomycin, and/or gentamicin. Rifabutin may be substituted for rifampin in patients with HIV disease.

**Tsukamurella spp.**
*Tsukamurella* spp. are susceptible to amikacin, sulfa drugs, quinolones, and clarithromycin. They are generally resistant to ampicillin, cephalosporins, and amoxicillin-clavulanic acid. *Tsukamurella paurometabola* is resistant to imipenem; other species are susceptible. There is no standardized regimen for treatment.

**Gordonia spp.**
*Gordonia* spp. are usually susceptible to ampicillin, macrolides, quinolones, and cephalosporins, but the main course of treatment is removal of the foreign body and surgical drainage. There is no standardized regimen for treatment.

**Segniliparus spp.**
Known clinical isolates of *S. rugosus* tested using *Nocardia* CLSI guidelines were susceptible to sulfadiazine, imipenem, and rifabutin and were resistant or intermediate to amikacin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, clarithromycin, linezolid, minocycline, and tobramycin.

**SUMMARY**
The aerobic actinomycetes have gained increased prominence as etiologic agents of significant disease, primarily in immunocompromised patients. The combination of increasing conditions impairing host resistance to invasion by environmental pathogens, and the rapidly progressing technological capability to identify isolates recovered from such patients, has allowed the widespread documentation of species now known to be capable of causing disease in humans (albeit under special circumstances). A better understanding of the epidemiology, clinical course, and antimicrobial susceptibilities of aerobic actinomycetes is paramount to their rapid diagnosis and to early selection of therapeutic modalities so that better outcomes for patients can be achieved. Molecular identification and typing techniques will bring a better understanding of the interrelatedness of the various genera and species. Clinical laboratories must become aware of the new role played by the aerobic actinomycetes in disease and must recognize when identification, susceptibility testing, and therapeutic and surgical interventions are necessary. Laboratories will be required to better interact with clinicians and to better interpret and understand the clinical significance of each isolate based on the parameters described in this chapter.

**REFERENCES**


Aerobic Actinomycetes of Clinical Significance


