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Osteomyelitis Associated with *Nocardiopsis composta* in a Dog

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Case Report Rapport de cas

Osteomyelitis associated with *Nocardiopsis composta* in a dog

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Abstract — We report the first detection of *Nocardiopsis composta* in association with osteomyelitis in a young male miniature Australian shepherd dog. Findings included suppurative osteomyelitis containing intralesional Fite's acid fast bacilli, aerobic culture of branching Gram-positive rods, and positive identification via phenotypic analysis and 16S rDNA sequencing.

Résumé — Ostéomyélite associée à *Nocardiopsis composta* chez un chien. Nous signalons la première détection de *Nocardiopsis composta* en association avec l'ostéomyélite chez un jeune chien berger Australien miniature mâle. Les résultats incluaient une ostéomyélite suppurative contenant des bacilles alcoolo-acido résistants à la coloration de Fite, une culture aérobie de bâtonnets à Gram positif embranchés et l'identification positive par une analyse phénotypique et le séquençage de l'ADNr 16S.

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Osteomyelitis can be caused by bacterial or fungal agents or may be idiopathic. Cocci, bacilli, and filamentous bacteria such as members of the *Actinomycetes* have all been determined to be causes of osteomyelitis. Differential diagnoses for Gram-positive filamentous rods in the family *Actinomycetales* causing osteomyelitis in the dog, include members of the more frequently encountered genera *Nocardia* and *Actinomyces* (1,2). Bacteria gain access to the bone via several routes, but are most often associated with direct inoculation (such as percutaneous injuries, compound fractures, or secondary to foreign bodies such as surgical or other material including dirt and wood) and fracture instability. Less frequently, the route is hematogenous, as has been found with *Propionibacterium acnes* (3). Agents isolated from osteolytic lesions in dogs and cats have included Gram-positive *Staphylococcus* spp., *Streptococcus* spp., Gram-negative *Escherichia coli, Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp., anaerobic *Clostridium* spp., *Peptostreptococcus* spp., *Actinomyces* spp., *Bacterioides* spp., *Fusobacterium* spp., and rarely *Brucella canis, Nocardia* spp., and *Mycobacterium avium* (3,4). Fungal causes include *Coccidioides immitis, Blastomyces dermatitidis, Histoplasma capsulatum, Cryptococcus neoformans,* and *Aspergillus* spp. (3). Osteosarcoma with associated cellulitis is a reported noninfectious cause of osteomyelitis in dogs (5). Our report details the diagnosis, treatment, and resolution of osteomyelitis in a dog caused by a unique agent, *Nocardiopsis composta.*

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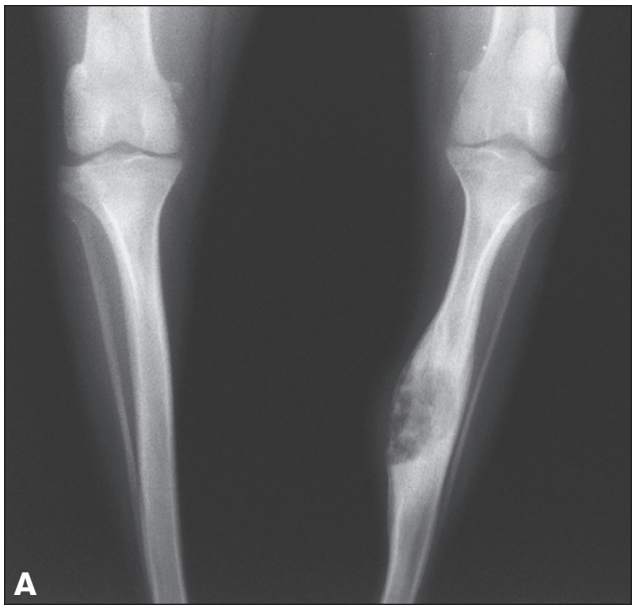
Case description

A 1-year-old neutered male miniature Australian shepherd dog was presented to a primary care veterinarian with a 6-week history of right hind limb weight-bearing lameness. Upon physical examination, a hard, 2-cm diameter mass was palpated at the medial right tibia. Radiographs revealed an osteolytic lesion in the mid-diaphysis of the limb (Figure 1). A 4-mm punch bone biopsy was taken 3 wk following initial examination and was submitted in 10% buffered formalin, decalcified in 12% hydrochloric acid for 4 h, processed, sectioned, and stained with hematoxylin and eosin (H & E). Histologic sections revealed moderate numbers of intact and degenerate neutrophils in the inter-trabecular spaces admixed with moderately abundant fibrin, mild amounts of karyorrhectic and cytolytic cellular debris, and few pyknotic nuclei. Osteolysis was characterized by disruption of boney trabeculae and replacement by fibrin and fibrosis. Few, multifocal, and prominent Howship's lacunae were present (osteoclastic resorption) (Figure 2A). Brown and Brenn Gram stain and Gomori's methanamine silver stain on histologic sections failed to reveal a bacterial or fungal etiology, respectively. However, due to the presence of neutrophils, radiographic findings of osteomyelitis, and age of the patient, an infectious, rather than neoplastic, etiology was suspected. Following culture results from a subsequent biopsy, a Fite's acid fast stain was conducted retrospectively on the initial fixed paraffin-embedded biopsy and revealed small numbers of intralesional, acid fast positive, branching bacilli approximately 3 μm × 1 μm in size (Figure 2B). Empirical antimicrobial treatment was initiated, as fresh tissues were not initially collected for culture. After 4 wk of therapy with amoxicillin and



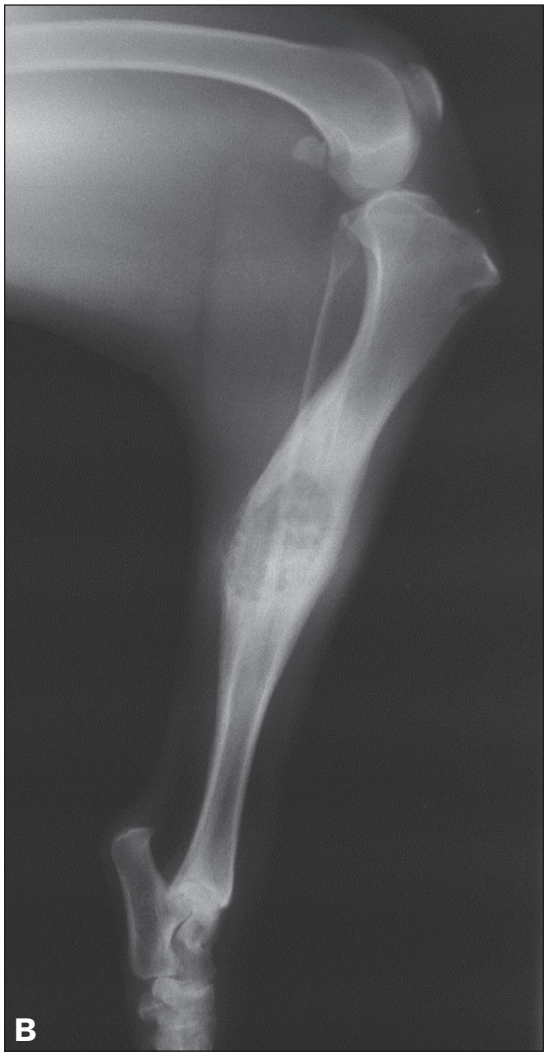
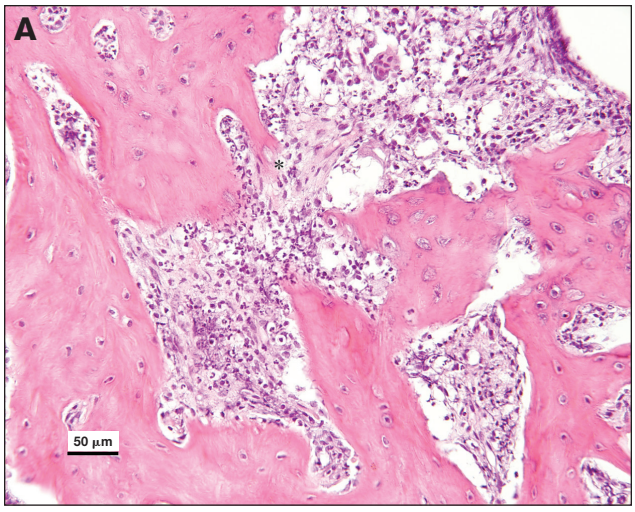


Figure 1. A – Dorsoventral radiograph of the rear limbs. A midshaft area of radiolucency consistent with boney lysis is seen in the right tibia. The left tibia is normal. B – Left to right lateral view of the left tibia. The area of lucency is oval and is bordered by mild boney proliferation.



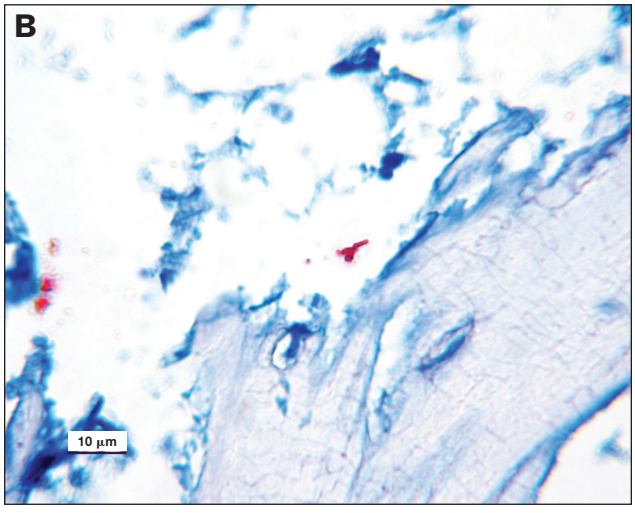


Figure 2. Punch biopsy of bone. A – Moderate numbers of neutrophils fill the intertrabecular spaces of lamellar bone. A small focus of osteolysis is present (asterisk) (Hematoxylin and eosin, Bar = 50 μm). B – Few, small, extracellular, 1 μm × 3 μm, Fite's acid-fast positive, branching bacilli are seen within intertrabecular spaces. (Fite's acid fast stain, Bar = 10 μm)

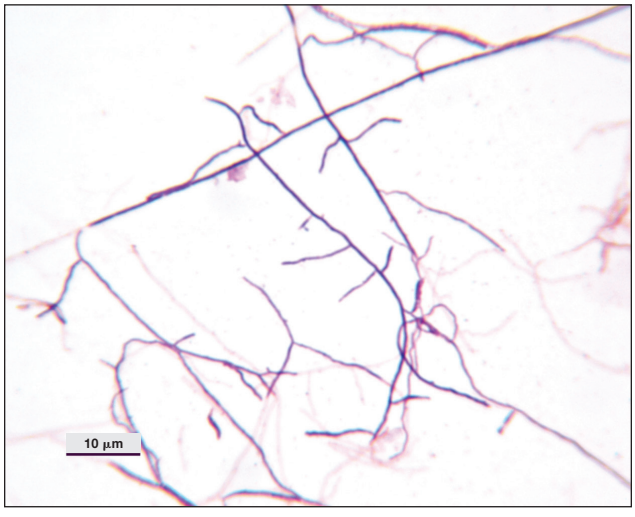


Figure 3. Gram-stained heat-fixed colony smear showing Gram-positive branching rods. (Bar = 10 μm)

Table 1. Kirby-Bauer antimicrobial susceptibility results for *Nocardiopsis composta* isolate.

Antimicrobial	IC	mm[a]	Antimicrobial	IC	mm
Ceftiofur	S	30	Tetracycline	I	15
Ceftriaxone	NI	26	Penicillin G	R	13
Clindamycin	R	6	Spectinomycin	R	6
Ciprofloxacin	NI	20	Tiamulin	R	6
Danofloxacin	I	21	Tilmicosin	R	6
Enrofloxacin	I	22	Tobramycin	NI	14
Erythromycin	R	6	Trimethoprim-sulfamethoxazole	S	41
Florfenicol	R	8	Tulathromycin	R	6
Orbifloxacin	R	13	Vancomycin	S	29

[a] Interpretive criteria (IC) of zones of inhibition (mm) using susceptible (S), intermediate (I), or resistant (R) were taken from gram positive rod category, where available (8). NI indicates no interpretive criteria exist.

clavulanate, radiolucency consistent with osteomyelitis of the right tibia was still apparent and clinical lameness persisted. We therefore initiated therapy with clindamycin.

Five months following cessation of antimicrobial treatment, lameness and radiographic osteolysis were still noted. A fresh, non-fixed, bone biopsy was taken and submitted for culture and identification of an etiologic agent. A branching Gram-positive bacillus was observed on Gram-stained colony smears (Figure 3) and was subsequently isolated in pure growth on tryptic soy agar with 5% sheep's blood following 72 h incubation at 37°C in an atmosphere of 5% CO_2. This organism was sent to a laboratory (Accugenix; Newark, Delaware, USA) where a 480 nucleotide amplic on of the 16S rDNA was amplified and sequenced. Analysis (NCBI BLASTn) of the DNA sequence showed 99.6% nucleotide identity to *Nocardiopsis composta* reference strain KS9 (NR_025152.1). This was sufficient to determine identification to the genus and species level according to Clinical Laboratory Standards Institute (CLSI) approved interpretive criteria for 16S sequencing of aerobic actinomycetes (6,7). Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion susceptibility test based on modification of methods from the CLSI (8). These included the use of Müeller-Hinton agar and an extended incubation time of 72 h. Zones of growth inhibition were measured using a BioMIC instrument (BioMIC Giles Scientific, Santa Barbara, California, USA). Recognizing that validated antimicrobial susceptibility testing methodology and specific interpretive criteria for this organism do not exist, susceptibility breakpoints for zones of inhibition in mm were based on CLSI guidelines for Gram-positive veterinary isolates where available (8). Zones of inhibition for specific antimicrobials are presented in Table 1. The isolate of *Nocardiopsis composta* appeared resistant to most drugs tested, including clindamycin and erythromycin, but was susceptible *in vitro* to ceftiofur, trimethoprim-sulfamethoxazole, and vancomycin.

A 60-day empirical therapy with clarithromycin had been initiated prior to identification of the etiologic agent. Clinical examination and radiographs taken at a 2-month follow-up examination indicated further progression of the osteolysis. The patient was then placed on high-dose trimethoprim/sulfamethoxazole for 21 d. Therapy was discontinued following elevation of blood urea nitrogen (17.5 mmol/L, reference range: 2.5 to 9.6 mmol/L) due to concern regarding potential antibiotic toxicosis. A follow-up examination 8 mo after cessation of antibiotics indicated a resolution of both the presenting clinical signs and radiographic boney lysis with probable clearance of the organism.

Discussion

The genus *Nocardiopsis* in the order *Actinomycetales* contains aerobic actinomycetes and was proposed by Meyer based on morphological and biochemical analyses (9). Although related, these organisms are a distinct genus from the more commonly identified genus *Nocardia.* The genus *Norcardiopsis* was separated from the genus *Actinomadura,* and originally represented actinomycetes with substrate mycelium that fragments into coccoid elements and has a specific cell wall composition of *meso*-2,6-diaminopimelic acid that contains no diagnostically useful sugars. The genus *Nocardiopsis* has since been refined, based on additional chemotaxonomic analysis, and was revised to include only organisms with a fatty acid composition of type 3d and DNA G + C content of 64 to 71 molar percent (10). *Nocardiopsis composta* was initially isolated from the atmosphere of a composting facility in Germany and is one of 41 validly named species or subspecies within the genus (11,12).

To our knowledge, this is the first report of isolation of *Nocardiopsis composta* in association with a pathologic process in any animal species. Another member of the genus, *Nocardiopsis alba,* was recently identified in association with a case of chronic laminitis in a horse, the only report found of a closely related species in animals (13). Other members of the genus *Nocardiopsis* have been rarely documented to cause infections in humans, and nearly all of these have been attributed to a single species, *Nocadiopsis dassonvelli. Nocardiopsis dassonvelli* has been isolated in association with human diseases that include cutaneous infection, cellulitis, actinomycetoma, respiratory infection, nasal vestibulitis, blood cultures of a child with respiratory distress and a man with cholangitis (14–22). Although the prevalence of these infections in human medicine is poorly understood, one report indicates that the Centers for Disease Control identified 21 isolates as *N. dassonvelli* between 1981 and 1986

(23). In many cases described in the literature, patients suffered from protracted disease. In 8 patients described in case reports, 1 had treatment with corticosteroids prior to presentation and another was concurrently diabetic (15,19). Two patients with cellulitis had reported previous trauma, presumably allowing for a decrease in localized cutaneous defenses (18,20). Thus, immunosuppressive conditions could play a role in pathogenesis of infections with organisms of this genus (18,20).

The human patients affected by cutaneous infection, subcutaneous infection, and nasal vestibulitis lacked osteomyeletis. When performed, histopathologic examination in human cases revealed a suppurative to pyogranulomatous infection with intralesional Gram-positive, periodic acid-Schiff negative, and modified acid-fast negative filamentous branching bacilli (15,18,20). In our case, initial histologic examination of H & E stained sections failed to reveal visible bacilli. However subsequent Fite's acid fast staining revealed branching acid fast bacilli. Peanut oil used in Fite's acid fast stain has been shown to enhance penetration of the stain in waxy tissue or bacterial cell walls (24). Thus, it is recommended using this stain over traditional modified acid fast stains if *Nocardiopsis* is suspected.

In human cases of *Nocardiopsis dassonvelli,* therapies that resulted in resolution of clinical signs included oral trimethoprim/sulfamethoxazole, combination oral therapy of trimethoprim and gentamicin, intravenous piperacillin and ciprofloxacin, trimethoprim/sulfamethoxazole in conjunction with clarithromycin and levofloxacin, and minomycin (14–16,18–20,25). Based on the antimicrobial susceptibility test results which showed resistance to clarithromycin, the patient was then treated with high dose trimethoprim/sulfamethoxazole with complete resolution of clinical signs after a 3-week course of treatment. This patient had no concurrent immunosuppressive conditions and it remains unclear how this organism was introduced into the bone. However, trimethoprim/sulfamethoxazole therapy may be a reasonable selection for treatment of osteomyelitis caused by *Nocardiopsis,* due to *in vitro* susceptibility of the isolate, efficacy against related organisms, the spectrum of activity, good absorption, and a high calculated volume of distribution throughout the body tissues (26,27). In the absence of official antimicrobial testing methodologies and interpretative data for this particular organism, the Kirby-Bauer disc diffusion susceptibility test results, although not validated, were utilized in a comparative manner to guide the successful antimicrobial therapy of this case of osteomyelitis caused by *N. composta.* Determination of the minimum inhibitory concentration would be more appropriate, but the methodology is not available in all diagnostic laboratories. This, combined with a paucity of published information regarding *in vitro* susceptibilities of these organisms and lack of specific interpretive criteria for *in vitro* antimicrobial susceptibility tests, makes comparison of therapy protocols challenging.

We conclude that nonresponsive cases of osteomyelitis warrant additional diagnostics and an index of suspicion for more fastidious organisms. Testing should include fresh and fixed biopsy, Gram stain, Fite's acid fast stain for optimal penetration into bacterial cell walls, aerobic culture for a minimum of 3 d, anaerobic culture, and 16S ribosomal DNA sequencing. Treatment should be guided by susceptibility testing using approved CLSI protocols when available (8). In light of our findings, *Nocardiopsis composta* should be considered along with other etiologies as a possible differential for osteomyelitis in animal patients.

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References

1. Bradney IW. Vertebral osteomyelitis due to *Nocardia* in a dog. Aust Vet J 1985;62:315–316.
2. Johnson KA, Lomas GR, Wood AKW. Osteomyelitis in dogs and cats caused by anaerobic bacteria. Aust Vet J 1984;61:57–61.
3. Johnson KA. Osteomyelitis in dogs and cats. J Am Vet Med Assoc 1994; 204:1882–1887.
4. Dernell WS. Treatment of severe orthopedic infections. Vet Clin North Am Small Anim Pract 1999;29:1261–1274.
5. Boston S, Singh A, Murphy K, Nykamp S. Osteosarcoma masked by osteomyelitis and celllulitis in a dog. Vet Comp Orthop Traumatol 2010;5:366–371.
6. Clinical and Laboratory Standards Institute. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline MM18-A.Vol. 28 No. 12. Wayne, Pennsylvania: CLSI, 2008.
7. Patel JB, Wallace RJ, Brown-Elliott BA, et al. Sequence-based identification of aerobic actinomycetes. J Clin Microbiol 2004;42:2530–2540.
8. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals; approved standard-third edition. VETO1-A4 and VET01 S-2. 4 ed. Wayne, Pennsylvania: CLSI, 2013.
9. Meyer J. *Nocardiopsis,* a new genus of the order *Actinomycetales.* Int J Syst Bacteriol 1976;26:487–493.
10. Grund E, Kroppenstedt RM. Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. Int J Syst Bacteriol 1990; 40:5–11.
11. Kampfer P, Busse HJ, Rainey FA. *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. Int J Syst Evol Microbiol 2002; 52:621–627.
12. Conville PS, Witebseky FG. *Nocardia, Rhodococcus, Gordonia, Actinomadura, Streptomyces,* and other aerobic actinomycetes. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. Manual of Clinical Microbiology. 10th ed. Washington, DC: ASM Press, 2011:443–472.
13. Onishi JC, Park JW, Haggblom MM, Fennell MJ, Fugaro MN. Chronic laminitis is associated with potential bacterial pathogens in the laminae. Vet Microbiol 2012;158:329–336.
14. Beau F, Bollet C, Coton T, Garnotel E, Drancourt M. Molecular identification of a *Nocardiopsis dassonvillei* blood isolate. J Clin Microbiol 1999;37:3366–3368.
15. González-López MA, González-Vela MC, Salas-Venero CA, Conde R, Val-Bernal JF. Cutaneous infection caused by *Nocardiopsis dassonvillei* presenting with sporotrichoid spread. J Am Acad Dermato 2011; 65:e90–e91.
16. Lejbkowicz F, Kudinsky R, Samet L, Belavsky L, Barzilai M, Predescu S. Identification of *Nocardiopsis dassonvillei* in a blood sample from a child. Am J Infect Dis 2005;1:1–4.
17. Mordarska H, Zakrzewska-Czerwińska J, Paściak M, Szponar B, Rowiński S. Rare, suppurative pulmonary infection caused by *Nocardiopsis dassonvillei* recognized by glycolipid markers. FEMS Immunol Med Microbiol 1998;21:47–55.
18. Philip A, Roberts GD. *Nocardiopsis dassonvillei* cellulitis of the arm. Clin Microbiol Newsl 1984;6:14–15.
19. Shivaprakash MR, Sumangala B, Prasanna H, et al. Nasal vestibulitis due to *Nocardiopsis dassonvillei* in a diabetic patient. J Med Micriobiol 2012;61:1168–1173.
20. Sindhuphak W, Macdonald E, Head E. Actinomycetoma caused by *Nocardiopsis dassonvillei.* Arch Dermatol 1985;121:1332–1334.
21. Singh SM, Naidu J, Mukerjee S, Malkani A. Cutaneous infections due to *Nocardiopsis dassonvillei* (Brocq-rousseau) Meyer 1976, endemic in members of a family up to fifth degree relatives. Program and Abstracts

of the XI Congress of the International Society for Human and Animal Mycology, Montreal, Canada. 1991:abstr no PS1.91.
22. Liegard H, Landrieu M. Un cas de mycose conjonctivale. Ann Ocul 1911;146:8.
23. Ajello L, Brown J, Macdonald E, Head E. Actinomycetoma caused by *Nocardiopsis dassonvillei*. Arch Dermatol 1987;123:426–426.
24. Fite GL, Cambre PJ, Turner MH. Procedure for demonstrating lepra bacilli in paraffin sections. Arch Pathol (Chic) 1947;43:624.
25. Bernatchez H, Lebreux E. *Nocardiopsis dassonvillei* recovered from a lung biopsy and a possible cause of extrinsic allergic alveolitis. Clin Microbiol Newsl 1991;13:174–175.
26. Kaplan SA, Weinfeld RE, Abruzzo CW, McFaden K, Jack ML, Weissman L. Pharmacokinetic profile of trimethoprim-sulfamethoxazole in man. J Infect Dis 1973;128:S547–S555.
27. Wallace RJ, Steel LC, Sumter G, Smith JM. Antimicrobial susceptibility patterns of *Nocardia asteroides*. Antimicrob Agents Chemother 1988;32:1776–1779.