

Nocardia caviae: a Report of 13 New Isolations with Clinical Correlation

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Thirteen isolates of *Nocardia caviae* from 12 different clinical sources were received and identified over a 5½-year period by the Mycology Division of the Center for Disease Control. The results of morphological, biochemical, and physiological studies on these isolates were compared with those obtained with four reference cultures of *N. caviae* received from the Institute of Microbiology, Rutgers University. Comparison showed that *N. caviae* isolates form a homogeneous group that is usually easily distinguished from *N. asteroides*, *N. brasiliensis*, and other pathogenic aerobic actinomycetes. The clinical sources included nine human and two animal infections and one human isolate apparently not associated with disease. Previous reports of *N. caviae* infections in man have been limited to rare cases of actinomycotic mycetoma. Among the human infections reported in this series are one case of mycetoma, one case of "mycotic" keratitis, one case of skin abscess, two cases of osteomyelitis, and four cases of serious pulmonary infection caused by *N. caviae*.

Aerobic actinomycetes can cause a variety of clinical diseases in man and animals. The most common of these are actinomycotic mycetomas, cutaneous and subcutaneous abscesses, and acute and chronic pulmonary infections which may later spread to other parts of the body via the bloodstream. In spite of the wide distribution of aerobic actinomycetes in nature, only a few species are known to be infectious for mammals: *Nocardia asteroides*, *N. brasiliensis*, *Actinomadura madurae*, *A. pelletieri*, and *Streptomyces somaliensis*. *N. caviae* has been known as the cause of occasional actinomycotic mycetomas in man and rare systemic infections in lower animals. Our recent identification of *N. caviae* as the cause of two fatal systemic infections in man (4) prompted this further investigation into the scope of disease caused by this agent. Thirteen isolates from a variety of human and animal infections are described in this report.

MATERIALS AND METHODS

Laboratory records were reviewed for all isolates identified as *N. caviae*. All such isolates that had been identified before the start of this study were retrieved from the stock culture collection, and during the course of the study other isolates identified as *N. caviae* were added to the series.

All isolates were studied by the methods of Gordon and Mihm (8) and Berd (2). Gross colonial morphology on modified Sabouraud dextrose agar slants

was described after 3 weeks of growth. Microscopic morphology was studied by using Gram-stained smears from solid media and slide culture preparations as described by Georg et al. (7). Microcolonies were examined directly on open agar plates at ×100 and ×400 magnification. Media used for slide cultures and agar plates for observing microcolonies included crude 2% tap water agar, cornmeal agar, and potato dextrose agar. Fourteen-day-old cultures grown on casein agar were tested for acid fastness by a modified cold Kinyoun technique for which 1% aqueous sulfuric acid was used as the decolorizing agent (7). Whole-cell hydrolysates were analyzed for the stereoisomer of diaminopimelic acid by the technique of Becker et al. (1). Monosaccharides in the hydrolysates were identified by Lechevalier's technique (14). All tests were done in duplicate or in triplicate.

Clinical information relating to the isolates was obtained from the referring physician, hospital, or laboratory. Pathologic materials demonstrating the microorganisms were requested, but were available in only three instances.

RESULTS

A review of laboratory records revealed that 10 isolates had been identified as *N. caviae* between June 1967 and June 1972. Among these 10 were 4 that had been obtained from the collection of the Institute of Microbiology, Rutgers University. These four were included in the current study as reference isolates for all tests. From July 1972 until January 1973, seven more cultures were identified as *N. caviae* and were

added to the series. Table 1 lists the cultures and gives their sources. In all, 13 new isolates from 12 different clinical sources were included in this series.

The results of the studies done on the current series demonstrate a much greater degree of uniformity among the strains of *N. caviae* than among those of the more common species, *N. asteroides* and *N. brasiliensis*. Gross colonial morphology on modified Sabouraud dextrose agar slants was similar among all isolates. They developed as slow-growing colonies that, after 3 weeks, were dry, raised, wrinkled or cerebriform, buff or peach colored, and somewhat waxy in consistency. Most produced a slight amount of powdery, white aerial mycelium.

The study of slide culture preparations showed that all possessed well-formed, branched substrate hyphae with some isolates showing a tendency toward fragmentation into coccobacillary elements. All produced aerial hyphae, but in only one instance were short chains of spherical spores noted. All isolates were gram-positive and 12 of 13 were partially acid-fast.

The isolates in this series could degrade xanthine, hypoxanthine, esculin, and urea, but not casein. Only one degraded tyrosine. In addition, all produced acid from fructose, glucose, glycerol, inositol, and mannitol, but not

galactose or rhamnose. Acid production from mannose and trehalose was variable.

Analysis of whole cell hydrolysates revealed the presence of the meso-stereoisomer of diaminopimelic acid and major amounts of galactose and arabinose.

Table 2 summarizes the results of the tests done on these isolates and compares our results with those reported by Gordon and Mihm (8). Table 3 emphasizes the most useful characteristics in distinguishing *N. caviae* from *N. asteroides* and *N. brasiliensis*.

Clinical correlation. All of the isolates received in the diagnostic laboratory were from suspected cases of nocardial infection—10 in humans and 2 in animals. Following are summaries of the clinical histories of the patients who were the sources of the isolates.

Case 1: (isolate N11). A 20-year-old man had been maintained on prednisone, azathioprine, and periodic doses of actinomycin D for 2 years after a kidney transplant from a living relative. He was admitted to the hospital because of fever, chest pain, and purulent sputum of recent onset. A large right upper lobe abscess was found on his chest roentgenogram. Smear of his sputum showed a variety of gram-positive cocci, gram-negative rods, a few yeast cells, and narrow, branched hyphal elements the same diameter as bacteria. Because the sputum cul-

TABLE 1. Isolates identified as *N. caviae*

CDC ^a no.	Referred no.	Place of origin	Source	Received as
Reference isolates				
N6	IMRU ^b 622	Not given	Soil	<i>Nocardia caviae</i>
N8	IMRU 736A	Not given	Not given	<i>N. caviae</i>
N12	IMRU 736B	Not given	Not given	<i>N. caviae</i>
N113	IMRU 616	Not given	Not given	<i>N. caviae</i>
Test isolates				
N11	Not given	Illinois	Lung abscess	<i>Streptomyces</i> sp.
N78B	Not given	Georgia	Osteomyelitis of foot	Unknown
N105	ISHD ^c 60	Illinois	Blood culture	Unknown
N112	ISHD C-51311	Illinois	Skin abscess	Unknown
N201	3637/70	Malawi	Goat lung	<i>Nocardia</i> sp.
N243	Not given	Georgia	Cow's milk	<i>Nocardia</i> sp.
N258	Not given	District of Columbia	Osteomyelitis of tibia	<i>Nocardia</i> sp.
N260	Not given	Arizona	Spinal fluid	<i>Nocardia</i> sp.
N264	CM-2255 ^d	Puerto Rico	Corneal ulcer	<i>Nocardia</i> sp.
N265	F73-5 ^e	Pennsylvania	Scalp hematoma	<i>N. caviae</i>
N279	M-42/72 ^f	India	Mycetoma	<i>Nocardia asteroides</i>
N280	M-45/72	India	Mycetoma	<i>N. asteroides</i>
N281	M-70/71	India	Thigh abscess	Unknown

^a CDC, Center for Disease Control.

^b IMRU, Institute of Microbiology, Rutgers University (Ruth E. Gordon).

^c ISHD, Illinois State Health Department Laboratory.

^d Clinical Laboratory, Medical Center of Puerto Rico (Lillian Vazquez).

^e PSHD, Pennsylvania State Health Department Laboratory.

^f Grace Koshi, Christian Medical College, Vellore, India.

TABLE 2. Results of comparative morphological, physiological, and biochemical tests on *N. caviae* isolates

Test	No. of positive results		Percent positive	
	Reference isolates (n = 4)	Test isolates (n = 13)	n = 17	n = 15 ^a
Morphology:				
Branched hyphae	4	13	100	100
Fragmentation	3	5	52	20
Aerial hyphae	4	13	100	100
"Spores"	0	1	6	7
Partially acid fast	4	12	94	67
Growth:				
10 C	1	0	6	7
25 C	4	13	100	100
35 C	4	13	100	100
45 C	3	10	76	60
50 C, survival, 8 h	4	12	94	93
Oxidizes glucose (Hugh-Leifson)	4	13	100	X ^b
Degradation of:				
Casein	0	0	0	0
Esculin	4	13	100	X
Hypoxanthine	4	13	100	100
Tyrosine	0	1	6	0
Urea	4	13	100	X
Xanthine	4	13	100	100
Acid from:				
Adonitol	0	0	0	0
Arabinose	0	0	0	0
Dulcitol	0	0	0	X
Fructose	4	12	94	X
Galactose	0	1	6	0
Glucose	4	13	100	100
Glycerol	4	13	100	100
Inositol	4	13	100	100
Lactose	0	0	0	0
Maltose	0	0	0	87
Mannitol	4	12	94	100
Mannose	1	3	23	47
Raffinose	0	0	0	0
Rhamnose	0	0	0	0
Salicin	0	0	0	X
Sorbitol	0	0	0	0
Starch	0	0	0	X
Sucrose	0	0	0	X
Trehalose	3	9	83	X
Xylose	0	0	0	0
Whole-cell hydroly-sates:				
L-Diaminopimelic acid	0	0	0	X
Meso-diamino-pimelic acid	4	13	100	X
Arabinose	4	13	100	X
Galactose	4	13	100	X
Madurose	0	0	0	X
Xylose	0	0	0	X

^a From reference 8, p. 634, Table 2 and text.
^b X, not reported.

ture grew out of a coagulase-positive *Staphylococcus aureus*, the patient was placed on high doses of penicillin and oxacillin. Neverthe-

less, the abscess enlarged and the patient deteriorated. A right upper lobectomy was done because of massive amounts of sputum that caused frequent bronchial obstruction, but the patient died several hours after the operation. A microorganism thought to be a *Streptomyces* sp. was grown from the resected lung abscess. The isolate was later identified as *N. caviae*.

Case 2: (isolate N78B). A 27-year-old woman was admitted to the hospital for reevaluation of a chronic draining abscess of the left foot that was associated with osteomyelitis of the foot and ankle bones. The illness had been going on for 9 years, and over the previous 6 years repeated cultures of the drainage and of the material that had been curetted from the abscess cavity had not revealed specific pathogens. However, microscopic examination of curetted material on several occasions had shown narrow mycelial elements resembling an actinomycete, although no "sulfur granules" were seen. Treatment with repeated courses of a variety of antibiotics (never sulfonamides) was ineffective, and the leg was amputated below the knee. Cultures obtained from the diseased foot yielded two actinomycetes, *N. caviae* and *Actinomadura (Nocardia) madurae*.

Case 3: (isolate N105). A 3-month-old infant was admitted to the hospital with a 1-day history of high fever and diarrhea with blood-

TABLE 3. Summary of the differential characteristics of *N. caviae*, *N. asteroides*, and *N. brasiliensis*^a

Characteristic	<i>N. caviae</i>	<i>N. asteroides</i>	<i>N. brasiliensis</i>
Decomposition of:			
Casein	-	-	+
Hypoxanthine	+	-	V
Tyrosine	-	-	+
Urea	+	V	+
Xanthine	+	-	-
Growth:			
45 C	V	V	-
0.4% gelatin	V	-	+
Survival, 50 C for 8 h	+	+	-
Acid from:			
Fructose	+	V	+
Galactose	-	-	+
Glucose	+	+	+
Inositol	+	-	+
Mannitol	+	-	+
Mannose	V	-	-
Rhamnose	-	V	-
Trehalose	V	V	+

^a Symbols: +, based on more than 90% of isolates showing positive reactions; -, based on fewer than 10% of isolates showing positive reactions; V, reactions that were positive in 10 to 90% of isolates.

streaked stools. She was born after a 7-month pregnancy complicated by hypertension, proteinuria, and edema. The baby weighed 3 pounds and 4 ounces at birth. After a 2-week stay in the newborn nursery she did very well, and was asymptomatic until the day before admission. Physical examination revealed the infant to be normal except for irritability, a pulse rate of 140 per minute, and a temperature of 39 C. Laboratory tests showed a hemoglobin of 9.9 g/100 ml, a packed cell volume of 36% and a white blood cell count of 13,800/mm³. Her urinalysis was normal, and three stool cultures were negative for enteric pathogens. A single blood culture grew out *N. caviae*. The patient was treated only with bowel rest and recovered uneventfully.

Case 4: (isolate N112). This isolate was recovered from a subcutaneous abscess in a woman who died of disseminated nocardial infection that involved the lungs, pericardium, kidneys, and skin. Details of this case were reported elsewhere (4).

Case 5: (isolate N201). This culture was obtained from autopsy material from a domestic goat that died of rapidly progressive granulomatous pneumonitis. Hyphal elements compatible with the isolate were demonstrated on a direct smear of the lung tissue. Details of this case were reported elsewhere (5).

Case 6: (isolate N243). A 5-year-old Jersey cow was seen because of a swollen, painful udder. She had a fever of 103.6 F, and a milk culture was taken. Penicillin, streptomycin, and nitrofurantoin were infused into the udder once daily for 3 days without response. The milk culture grew out two closely related actinomycetes, *N. caviae* and *N. autotrophica*. Although sulfonamides reduced the fever, the udder remained swollen until the animal was sold for slaughter 3 weeks later.

Case 7: (isolate N258). This isolate was recovered from a purulent osteomyelitis of the tibia in a child with chronic granulomatous disease. The organism was demonstrated in tissue sections of sequestrum removed at the time of diagnosis. Details of this case have been reported (3).

Case 8: (isolate N260). This culture was obtained from the spinal fluid of a man who died of nocardial meningitis and ventriculitis which developed after infection had spread from a nocardial lung abscess. Details of this case have been reported (4).

Case 9: (isolate N264). An adult male was seen by an ophthalmologist because of an eye irritation that had lasted 4 or 5 days. He was

thought to have a superficial corneal erosion and was given eyedrops containing polymyxin B, bacitracin, and neomycin. He was seen again the next day because his symptoms were markedly worse, and at that time he had a severe anterior chamber reaction with hypopyon and a central corneal ulcer. A smear of scrapings from the ulcer revealed many fine, gram-positive hyphae compatible with an actinomycete. He was treated with sulfacetamide drops and recovered completely in about a week. A culture of the scrapings revealed *N. caviae*.

Detailed case histories relating to isolates N265, N279 (and N280), and N281 are not available and a clinical judgment about their significance cannot be made with confidence. Table 4 illustrates the pertinent clinical features of the cases reported in this series.

DISCUSSION

N. caviae was first isolated from an infected ear of a Sumatran guinea pig by Snijders (18) in 1924, but it remained for Gordon and Mihm (8) to establish its taxonomic position and to set forth reliable criteria for its identification. Their study and subsequent reports have shown that this actinomycete is native to the soil (7, 10-12) and only very rarely inhabits man and animals.

N. caviae has been isolated from human mycetomas in Tunisia (9), Japan (6), India (20), and Mexico (19). In addition, at least two of the isolates reported in Gordon and Mihm's original study of the species were cultured from human mycetomas. These were the only *N. caviae* isolates from infected humans that I could find in the literature for which the criteria for identification of the isolate were given. Naturally acquired infections have also been reported in animals. Two of Gordon and Mihm's isolates were from cases of bovine mastitis. Systemic infections have been reported in a dog (10) and in a Pacific bottle-nosed dolphin (16).

Five of the infections in this series were probably pulmonary in origin (cases 1, 4, 5, 7, and 8). Dissemination of the infection outside the thorax was documented in cases 4 and 8, and probably occurred in case 7. In cases 1, 7, and 8, patients had serious underlying illnesses associated with impairment of immunity. Cases 2, 6, 9, 10, 11, and 12 resulted from traumatic implantation of the organisms into the skin, and none of these patients had any known impairment of their immunity. The isolate associated with case 3 was apparently not pathologically significant.

In two instances (cases 2 and 6), the *N. caviae* isolates were recovered from infected material

TABLE 4. Summary of the cases of *N. caviae* infection in humans and animals

Case no.	Isolate no.	Patient (age/sex)	Isolated from	Form of nocardiosis	Underlying condition	Treatment of nocardiosis	Outcome
1	N11	20/M	Sputum, lung	Pulmonary	Immune suppression	Penicillin, oxacillin	Death
2	N78B	27/F	Foot	Osteomyelitis	None known	Multiple antibiotics, amputation	Recovery
3	N105	3 mo/F	Blood	None	None	None	Recovery
4	N112	54/F	Skin abscess	Systemic	None known	Tetracycline, ampicillin, chloramphenicol	Death
5	N201	— ^a	Lung, goat	Pulmonary	None known	None	Death
6	N243	— ^a	Cow's milk	Bovine mastitis	None known	Sulfonamide infusion	Sold for slaughter
7	N258	9/F	Sequestrum	Osteomyelitis (? pulmonary)	Chronic granulomatous disease	Sulfonamides	Recovery
8	N260	54/M	Spinal fluid	Systemic	Postirradiation therapy, steroid therapy	Ampicillin, penicillin, sulfonamides	Death
9	N264	?/M	Corneal scrapings	"Mycotic" keratitis	None known	Topical sulfonamides	Recovery
10	N265	72/M	Scalp hematomata	Uncertain	Unknown	Unknown	Unknown
11	N279-280	25/M	Mycetoma of foot	Mycetoma	Unknown	Unknown	Unknown
12	N281	40/M	Abscess of thigh	Localized abscess	Unknown	Unknown	Unknown

^a —, Nonhuman cases; both were adult animals.

along with other closely related actinomycetes. *Actinomadura (Nocardia) madurae*, isolated along with *N. caviae* from case 6, is known to cause mycetomas in man, but its significance in other disease processes has not been established. *Nocardia autotrophica*, whose pathogenic potential is not known, was isolated along with *N. caviae* from case 2. The relative contribution of the two agents in each of these cases to the disease processes involved is uncertain.

The pathogenic potential of soil isolates of *N. caviae* for laboratory animals has been demonstrated (12). In a study of the comparative pathogenicity of *N. asteroides* and *N. caviae* in mice, Smith and Hayward (17) showed that the two were about equally virulent, although rather heavy inocula were required to produce disease or death in test animals. Mishra et al. (15) confirmed that the two species were of similar pathogenicity for mice, and that both *N. asteroides* and *N. caviae* were significantly more virulent than *N. brasiliensis*. They also showed that pretreatment of test animals with cortisone significantly reduced their resistance to infection when each of the three species was inoculated intravenously.

This report adds to the growing body of evidence indicating that all three of the recognized pathogenic nocardias can cause serious and even fatal disease in certain humans. Usually the patients susceptible to such severe

nocardial infections have immune defenses impaired by cancer chemotherapy, corticosteroids, primary immune deficiency diseases, or an accompanying malady such as diabetes mellitus. Nocardial infection of the skin and related tissues may not require the presence of these underlying conditions. Certainly when dealing with an immunologically compromised patient with an infection, physicians and laboratory personnel should suspect that unusual organisms might be involved. A relatively simple group of tests that should be within the capabilities of most laboratories handling specimens from this type of patient enables the laboratorian to identify the *Nocardia* species of medical importance.

APPENDIX

Since the conclusion of this study we have identified five additional isolates of *N. caviae*. The sources of two of these isolates are not known. They were sent out as evaluation cultures as *N. asteroides*. Two of the isolates were the cause of fatal pulmonary infections in nonhuman primates, and one isolate was recovered from the sputum of a man with chronic cavitary lung disease.

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