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Pseudoepidemic of *Nocardia asteroides* Associated with a Mycobacterial Culture System

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***Nocardia* isolations increased from 0.7 to 11.7/1,000 acid-fast bacillus and mycological cultures ($P <$ 0.000001). Only three isolations from one patient represented infection. Pseudoepidemic strain identity was confirmed by DNA fingerprinting; the isolate causing infection was distinct. The end of the pseudoepidemic was associated with changing the needle sterilizer and prolonging needle sterilization time on the BACTEC 460 machine. To our knowledge, this is the first reported *Nocardia asteroides* pseudoepidemic.**

Nocardia asteroides is a soil-borne aerobic actinomycete which is usually introduced via the respiratory tract and causes disease in immunocompromised hosts. Occasional false-positive single isolations have been reported, and it can be a respiratory saprophyte (3, 9). To our knowledge, few outbreaks (6, 10) and no pseudoepidemics involving this organism have been previously reported.

The Yale-New Haven Hospital is an 875-bed tertiary care facility. The hospital's Clinical Microbiology Laboratory processes 300 mycobacterial (acid-fast bacillus [AFB]) and mycological cultures per month. In May 1989, the laboratory noted an increase in *N. asteroides* isolations with the BACTEC TB system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.). This system is a radiometric method for detecting AFB growth in media containing ^{14}C-labeled substrate (8). Because *N. asteroides* is an unusual epidemic organism and is an unlikely contaminant, an investigation was begun.

Laboratory culture records were reviewed for the period May 1988 to April 1991 to determine the number of AFB specimens processed and *N. asteroides* isolates recovered and to determine endemic and epidemic rates of *Nocardia* isolation. Laboratory procedures were reviewed, and laboratory personnel were observed. Environmental AFB cultures were obtained from the BACTEC 460 machine and from the AFB laboratory, including BACTEC tubing, needle heater, filters, needles, the exterior of the BACTEC machine, and the surrounding bench areas.

Uninoculated BACTEC TB bottles, as well as BACTEC TB bottles inoculated only with an antimicrobial solution used to suppress non-AFB organism growth, were cultured. This solution, BACTEC PANTA PLUS (Becton Dickinson Diagnostic Instrument Systems), is comprised of five antimicrobial agents (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin). Lot N8C1 was associated with a pseudoepidemic of *Mycobacterium gordonae* during May to June 1989 in which the source of contamination was attributed to ineffectively sterilized water (13).

An auramine stain was performed with specimens with a positive growth index from BACTEC bottles. A positive auramine smear resulted in inoculations of a 7H9 broth (BBL, Microbiology Systems, Cockeysville, Md.), a Lowenstein-Jensen slant (BBL), and a 7H11 agar plate (BBL). Initial indications that *N. asteroides* was present were a yellow pellicle at the surface of the 7H9 broth and/or dry white-orange filamentous colonies on the 7H11 plate and Lowenstein-Jensen slant. A modified Kinyoun stain was performed with colonies from the solid media to observe filamentous branching. *N. asteroides* was then identified by standard criteria (1).

Patient charts were reviewed for evidence of *Nocardia* infection. Case definitions for *Nocardia* infection associated with a positive culture for *N. asteroides* were established. A definite case required isolation of a *Nocardia* sp. from a sterile site associated with positive histopathology and compatible symptoms. A possible case was defined as a single *Nocardia* isolation from a sterile site or sputum, with negative or no histopathology in an immunocompromised patient. An unlikely case was defined as a single isolation from a sterile site or sputum with no compatible symptoms or another etiology definitely established.

The baseline period was defined as 1 year prior to the epidemic (May 1988 to April 1989) and the 17-month period after the epidemic (November 1989 to April 1991). Data were analyzed with EpiInfo 5.0 software (Centers for Disease Control, Atlanta, Ga.).

N. asteroides isolates were lysed by using a sodium dodecyl sulfate-lysozyme procedure previously published (7) with the following modifications. *N. asteroides* isolates were inoculated onto Sabouraud dextrose agar and incubated at 37°C for 5 to 7 days. One to three isolated colonies from each culture were selected for lysis and DNA isolation. Whole-cell DNA was digested with restriction endonuclease *Pvu*II according to the manufacturer's specifications. DNA was analyzed by 0.8% agarose gel electrophoresis in Tris-borate buffer at 30 V for 8 h.

Figure 1 shows *N. asteroides* isolations by week of specimen submission. Of note, the *M. gordonae* isolations began 1 week prior to the first *N. asteroides* isolation and continued through week 10. One patient early in the pseudo-epidemic (patient 3) had a culture positive for *N. asteroides* and *M. gordonae*. The *N. asteroides* isolations continued for 4 months after the *M. gordonae* isolations ended. Of nine bottles of BACTEC TB positive for *N. asteroides* for which the presence or absence of PANTA was noted, seven bottles

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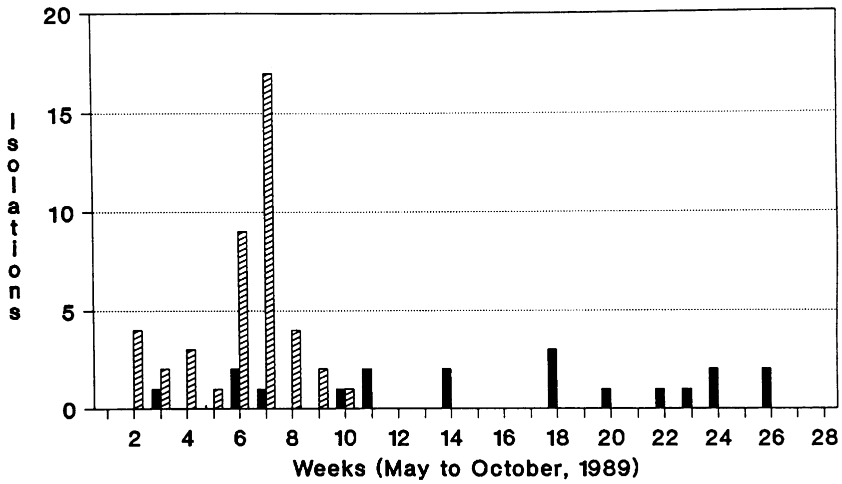

FIG. 1. *N. asteroides* isolations by week from May to October 1989 at the Yale-New Haven Hospital. ■, *N. asteroides*; ▨, *M. gordonae*.

contained PANTA solution; two did not. The extended time period of the outbreak suggested an ongoing point-source contamination.

Table 1 shows patients' characteristics and case classifications. Only 1 of the 19 patients with positive cultures (patient 14) had a definite *Nocardia* infection. Three different specimens from this patient were positive for *N. asteroides*. None of the other patients had repeated isolations. The three *N. asteroides* isolations from patient 14 were from pleural fluid, a surgical lung specimen, and a surgical deep-wound specimen.

Table 2 shows the *Nocardia* isolation rates during the baseline and epidemic periods. Six of six cultures positive for a *Nocardia* sp. during the baseline period before and after the epidemic were positive on other media. None of the *Nocardia* cultures positive from May to October 1989 were positive on media other than BACTEC TB, except for the culture from the definite case. Also, there were repeated isolations for all six cultures positive for a *Nocardia* sp. during the baseline period as well as the one definite case during the epidemic period. The rate of isolation of a *Nocardia* sp. increased from a baseline rate of 0.7 to 11.7/1,000 AFB and mycological cultures during the epidemic period ($P < 0.000001$).

No environmental cultures were positive for *N. asteroides*. Uninoculated BACTEC TB media and PANTA samples were also negative. *N. asteroides* isolations were not documented from consecutively sampled bottles. No breaks in aseptic technique by personnel were observed.

The *N. asteroides* isolates were compared by restriction

TABLE 1. Patients with *N. asteroides* isolations during the epidemic period from May to October 1989

Patient no.	Clinical history[a]	Culture source	Date of culture (mo/day/yr)	Case classification
1	AIDS, PCP	Blood	5/10/89	Possible
2	COPD	Sputum	5/30/89	Possible
3	AML, pneumonia	Sputum	6/3/89	Possible
4	MS, sepsis	CSF[b]	6/5/89	Unlikely
5	CHF	Pleural fluid	6/29/89	Unlikely
6	HTN, CVA	Pleural fluid	7/3/89	Unlikely
7	Pulmonary fibrosis	Bronchial washings	7/5/89	Possible
8	AIDS, cryptococcal meningitis	CSF	7/26/89	Unlikely
9	Tuberculosis	Pleural fluid	7/30/89	Unlikely
10	HIV positive, anemia	Blood	8/21/89	Unlikely
11	CHF, hematuria	Pleural fluid	8/22/89	Unlikely
12	*Staphylococcus aureus* sepsis	Sputum	8/22/89	Possible
13	HIV positive, syphilis	CSF	9/7/89	Unlikely
14	Breast cancer, steroids, pneumonia	Pleural fluid	9/21/89	Definite
		Lung	9/21/89	
		Deep wound	9/28/89	
15	Chronic renal failure	Bone marrow	9/28/89	Unlikely
16	AIDS, PCP	Blood	10/7/89	Possible
17	Rheumatoid lung	Pleural fluid	10/17/89	Possible
18	HIV dementia	CSF	10/4/89	Unlikely
19	Aseptic meningitis	CSF	10/16/89	Unlikely

[a] AML, acute myelogenous leukemia; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; HTN, hypertension; MS, multiple sclerosis; PCP, *Pneumocystis carinii* pneumonia.
[b] CSF, cerebrospinal fluid.

TABLE 2. *N. asteroides* isolation rates during baseline and epidemic periods

Medium	Baseline period[a] (n = 8,400)		Epidemic period[b] (n = 1,800)		P value
	No. of isolations	Rate[c]	No. of isolations	Rate[c]	
BACTEC TB only	0	0	18	10.0	<0.000001
Other	6	0.7	3	1.6	0.20
Total	6	0.7	21	11.7	<0.000001

[a] Baseline period, from May 1988 to April 1989 and from November 1989 to April 1991.
[b] Epidemic period, from May 1989 to October 1989.
[c] Rate per 1,000 AFB and mycological cultures.

endonuclease analysis of whole-cell DNA (Fig. 2). Single strain identity was confirmed in the 18 pseudoepidemic isolates. The distinct strain was from patient 14, the only patient with definite *Nocardia* infection.

Physicians of the affected patients were notified of suspected *Nocardia* contamination in the AFB cultures. None of the patients with suspected contaminated cultures were treated for *Nocardia* infection.

The needle heater on the BACTEC 460 machine was changed in June 1989 when the isolations began. Because ongoing point-source contamination was suspected, the needle heater was changed again in October 1989. In January 1990, the manufacturer prolonged the timing subcycle of the BACTEC 460 machine from 60 to 80 s for each bottle. The cycling time was increased to allow longer needle sterilization time, from approximately 16 to approximately 36 s in each timing subcycle.

A pseudoepidemic of *N. asteroides* in a clinical microbiology laboratory was suggested by a greatly increased rate of *Nocardia* isolations with no evidence of *Nocardia* infection in patients, with the lack of *Nocardia* isolation in media other than BACTEC TB, and with the lack of repeated isolations of *N. asteroides* in each case except one of a

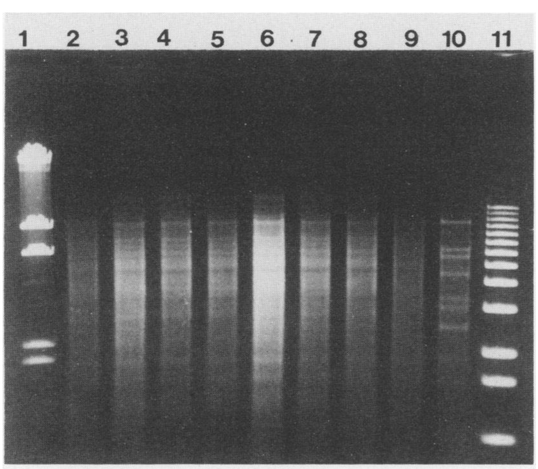

FIG. 2. Agarose gel electrophoresis of *N. asteroides* whole-cell DNA. Lanes: 1, molecular-size-standard bacteriophage lambda DNA *Hin*dIII fragments; 2 to 9, whole-cell DNA of pseudoepidemic *N. asteroides* isolates digested with the restriction endonuclease *Pvu*II; 10, whole-cell DNA of *N. asteroides* isolate from a patient with a definite case of *N. asteroides* pneumonia; 11, molecular-size-standard 1-kb ladder.

definite *N. asteroides* infection. Although the source of contamination was not found, analysis of whole-cell DNA documenting a single strain in 18 of the 19 isolates supported evidence that this was a pseudoepidemic. The distinct strain was from the definite case of *N. asteroides* pneumonia.

An ongoing point-source contamination was suggested by the small clusters of *N. asteroides* isolations during a 6-month period. Environmental cultures were negative, and the *N. asteroides* isolations did not end when *M. gordonae* isolations associated with contaminated PANTA solution ended. Since the *N. asteroides* isolations began at the same time as did the *M. gordonae* isolations and an AFB culture from one patient early in the pseudoepidemic was positive for both *M. gordonae* and *N. asteroides*, contaminated PANTA solution could have been the initial source and may have been retained on an environmental reservoir, such as the needle sterilizer, for some period of time.

Previous instances of pseudobacteremias involving the BACTEC 460 machine have been reported (2, 4, 5), and the pattern of cross-contamination may be low frequency, either clustered or sporadic (2). In our investigation, changing the needle heater a second time, after exposure to the contaminated PANTA lot, combined with the prolongation of the needle sterilization time for the BACTEC 460 machine was associated with the end of the pseudoepidemic. These interventions likely contributed to eliminating the *Nocardia* contamination.

N. asteroides is an unusual and, to our knowledge, previously unreported organism to be involved in a pseudoepidemic. It is a soil-borne aerobic actinomycete; because of its wide distribution, occasionally some isolates are deemed respiratory saprophytes or skin contaminants (3, 9, 12), and respiratory tract colonization may occur in patients with chronic pulmonary disease (9). In immunocompromised patients, however, its isolation is virtually always considered significant. A particular problem in our situation was that almost all the patients with specimens sent for AFB and mycological culture were to some degree immunocompromised. Indeed, 7 of 19 (37%) of the patients with *N. asteroides* isolations had possible infections. It was therefore necessary to review clinical information closely in correlation with the microbiology results which showed a pattern of isolations only from the BACTEC cultures and no repeated isolations, except for patient 14, who fulfilled the criteria for a definite case. The role of the laboratory in detecting the pseudoepidemic early was critical in avoiding unnecessary and potentially harmful therapy for affected patients.

Epidemiologic and clinical evidence suggested that the increased isolation rate of *N. asteroides* was a pseudoepidemic; however, the use of restriction endonuclease analysis of whole-cell DNA as a marker of strain identity was extremely useful confirmation. The single pseudoepidemic strain further implicated an ongoing point-source contamination. Molecular typing of *Nocardia* species done in another study suggests genetic heterogeneity between strains (11), as was suggested in our study by the distinct DNA fingerprint of the strain causing true infection. Thus, DNA typing of *Nocardia* species will likely be a useful tool for distinguishing strains in future studies.

Because clinical laboratory methods are increasing in sensitivity for detecting unusual pathogens from immunocompromised patients, the clinical laboratory should investigate sudden increased isolation rates of such pathogens. DNA typing methods should prove useful in such investigations.

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