JOURNAL OF CLINICAL MICROBIOLOGY, June 1997, p. 1582–1584 Vol. 35, No. 6
0095-1137/97/$04.00+0

Investigation of a Pseudo-Outbreak of *Nocardia asteroides* Infection by Pulsed-Field Gel Electrophoresis and Randomly Amplified Polymorphic DNA PCR

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Received 31 October 1996/Returned for modification 4 December 1996/Accepted 9 January 1997

Molecular strain typing by pulsed-field gel electrophoresis and by randomly amplified polymorphic DNA analysis was used to investigate a cluster of four *Nocardia asteroides* isolates associated with the BACTEC 460 TB system. An instrument motor drive misalignment resulted in inadequate needle sterilization and cross-contamination of BACTEC vials. This pseudo-outbreak illustrates the importance of proper BACTEC 460 needle sterilization and maintenance and confirms the usefulness of molecular typing methods for epidemiologic investigations.

Nocardia asteroides, a ubiquitous soil saprophyte, is a known opportunistic pathogen in immunocompromised hosts (8, 16, 18). The organism has been reported to cause a variety of infections, but primary pulmonary infection occurs most often (2, 8). Disseminated infection, especially with central nervous system involvement, and other localized extrapulmonary infections may also occur (2, 5). Outbreaks of infection due to *Nocardia* species have been documented (4, 12), but there has been only one previous report of a pseudoepidemic due to *N. asteroides* (7). We report here a *N. asteroides* pseudo-outbreak related to specimen processing with the BACTEC 460 TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). We also describe the application of molecular typing techniques by pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) PCR analysis in the investigation of the pseudo-outbreak.

The Microbiology Laboratory at Sunnybrook Health Science Centre processes approximately 120 specimens submitted for mycobacterial culture each month with the BACTEC TB system, a radiometric method for detection of mycobacterial growth in media containing a ^{14}C-labelled substrate. During 1 month, *N. asteroides* was isolated from specimens processed by the BACTEC TB system from four patients, whereas *Nocardia* organisms had been isolated only three times in the entire previous year.

On 30 October 1995, a leg abscess aspirate from a patient (patient 1) was processed through the BACTEC 460 instrument for isolation of mycobacteria. An aliquot of the specimen was also planted on standard agar and broth media for bacterial culture. The patient was receiving chemotherapy for lymphoma and had a febrile illness with soft tissue, pulmonary, and cerebral abscesses. The specimen was decontaminated and treated with an antimicrobial solution, PANTA PLUS (Becton Dickinson Diagnostic Instrument Systems), before inoculation of a BACTEC 12B vial. On 7 November 1995, *N. asteroides* was isolated from the BACTEC 12B vial of patient 1. The sample was removed, decontaminated, and inoculated into a new BACTEC vial with PANTA PLUS. No mycobacteria were subsequently isolated, but conventional bacterial cultures of the leg aspirate and of bronchial washings also grew *N. asteroides* on blood agar plates. On 12 and 13 December, BACTEC bottles inoculated with specimens from three other patients yielded *N. asteroides*. Two of these specimens were blood cultures from patients with AIDS that were obtained in October 1995 for detection of *Mycobacterium avium* complex and had been inoculated into BACTEC 13A vials. The third specimen was a skin biopsy that was also obtained in October and had been inoculated into a BACTEC 12B vial. These three specimens were processed on separate racks at positions far removed from one another and from the vial of the index patient. None of these specimens had been treated with PANTA PLUS, and the organism was not recovered from conventional culture media. These three patients had no clinical or radiographic features of *Nocardia* infection.

A review of the BACTEC 460 instrument maintenance log indicated that the needle heater was overdue for replacement, as recommended by the manufacturer. Moreover, it was noted that there had been an instrument needle jam in September 1995. Although the damaged needles had been replaced, a motor drive realignment was thought to be unnecessary. However, a second needle jam occurred on 5 December 1995, 1 week prior to the cluster of three *Nocardia* isolates. A review of laboratory records indicated that there had been no cross-contamination with mycobacterial species during this time.

Environmental cultures were collected by using swabs moistened with sterile saline from the BACTEC 460 instrument's needles, needle heater, and tubing; *N. asteroides* was isolated from each of these sites. The BACTEC 460 instrument's motor drive was realigned, and the needle heater, tubing, and needles were replaced. Following these interventions, no further *Nocardia* isolates were recovered from BACTEC specimen vials or from the instrument.

Each strain of *N. asteroides* was identified by standard techniques (1). Molecular typing by PFGE and RAPD analysis was undertaken to determine whether or not the *N. asteroides* isolates were related. Three other, unrelated clinical isolates of *N. asteroides* recovered in previous years were also typed by these methods. For PFGE analysis, DNA extraction was performed as described by Wallace et al. (15), with the following modifications. Bacterial growth from a blood agar plate was inoculated into 1.5 ml of TE buffer (10 mM Tris-HCl [pH 7.6], 0.1

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mM EDTA [pH 9.0]) to a density of a no. 5 McFarland standard. Total genomic DNA was prepared in agarose plugs and lysed in 3 ml of lysis buffer (2 mg of lysozyme per ml in TE buffer) for 18 h at 37°C and then exposed to 3 ml of proteinase K solution (0.5 M EDTA [pH 9], 1% sodium dodecyl sulfate, 1 mg of proteinase K per ml) at 55°C for 48 h. The plugs were then digested with *Xba*I (Boehringer-Mannheim, Laval, Quebec, Canada) according to the manufacturer's instructions. DNA fragments were separated on a 1% gel in a CHEF Mapper system (Bio-Rad, Mississauga, Ontario, Canada) with linearly ramped pulse times of 5 to 20 s over 20 h, 6 V/cm at 14°C (11). PFGE profiles were visualized after ethidium bromide staining under UV illumination.

For RAPD PCR, DNA extraction was performed by the method of Sritharan and Barker (10). Briefly, several colonies of *N. asteroides* were resuspended in 100 μl of TE-Triton buffer (100 mM NaCl, 10 mM Tris-HCl [pH 7.6], 0.1 mM EDTA [pH 9], 1% Triton X-100) and boiled for 30 min. Samples were centrifuged for 1 min, and 5 μl of the lysate was used in a 25-μl reaction mixture containing approximately 25 ng of DNA; 1 μM primer; 0.5 U of *Taq* DNA polymerase (Life Technologies, Burlington, Ontario, Canada) per ml; 200 μM each dCTP, dGTP, dATP, and dTTP (Perkin-Elmer Cetus, Norwalk, Conn.); 4 mM $MgCl_2$, 20 mM Tris-HCl (pH 8.4); and 50 mM KCl. The mixture was subjected to 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 35°C for 30 s, and 72°C for 30 s. The 10-mer primer used was UBC157 (5′-CGTGGGCAGG-3′) (Nucleic Acid-Protein Service Unit, University of British Columbia, Vancouver, British Columbia, Canada). PCR products were separated by using a 1% agarose gel and visualized under UV illumination after staining with ethidium bromide. To assess the reproducibility of the procedure, the RAPD PCR was repeated with a new preparation of the organisms.

Figure 1A shows the *Xba*I-digested DNA PFGE profiles of the four *Nocardia* strains recovered in November and December 1995, the environmental isolates from the BACTEC instrument, and the three unrelated clinical isolates. The four recent isolates from the BACTEC TB system and the environmental isolates all had the same DNA profile, which was distinct from those of the three epidemiologically unrelated strains. Figure 1B shows the RAPD profiles of the same isolates; these results were reproducible (data not shown). The two molecular typing methods produced concordant DNA profiles that were able to clearly distinguish the pseudo-outbreak strains from epidemiologically unrelated isolates.

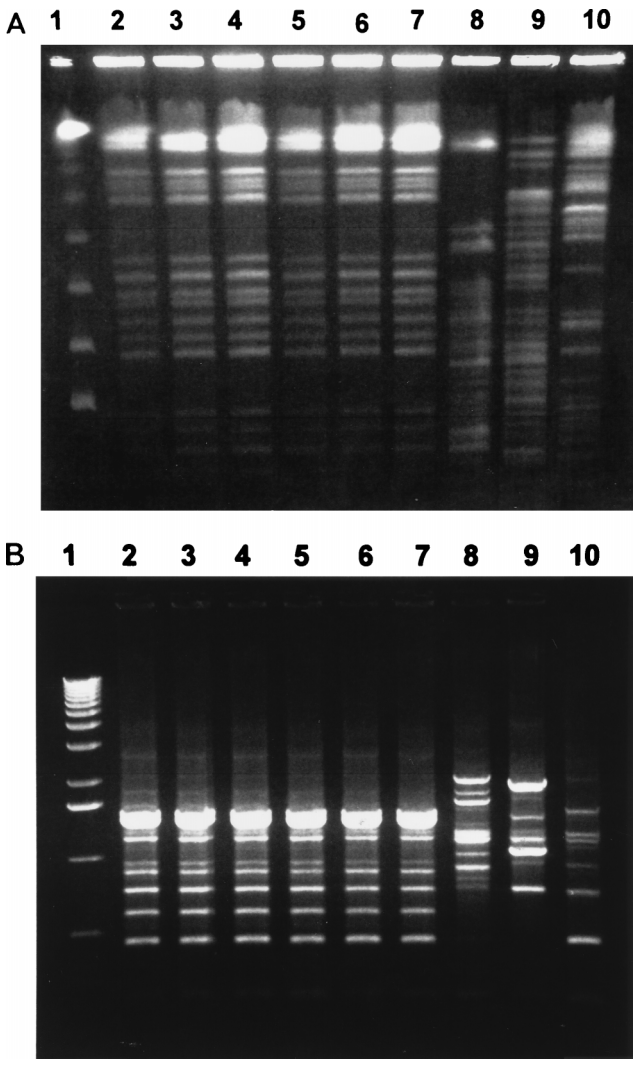

FIG. 1. *Xba*I-digested PFGE and RAPD PCR DNA profiles of *N. asteroides*. (A) PFGE profiles. Lanes: 1, lambda DNA ladder; 2, isolate from the index case, patient 1; 3 to 5, pseudo-outbreak isolates; 6, BACTEC needle heater isolate; 7, BACTEC needle isolate; 8 to 10, unrelated clinical isolates of *N. asteroides*. (B) Corresponding RAPD PCR profiles. Lanes: 1, 1-kb DNA ladder; 2, isolate from the index case, patient 1; 3 to 5, pseudo-outbreak isolates; 6, BACTEC needle heater isolate; 7, BACTEC needle isolate; 8 to 10, unrelated clinical isolates of *N. asteroides.*

The BACTEC 460 TB system has proven to be an extremely valuable adjunct for the laboratory diagnosis of tuberculosis. However, cross-contamination of specimens processed through the instrument has been described. Most of the reported instances of cross-contamination have involved mycobacterial species (3, 6, 9, 14, 17) and have been related to inadequate needle sterilization, although contamination of commercially distributed PANTA PLUS solution has also been described (13). We now report only the second pseudo-outbreak of *N. asteroides* associated with processing of specimens with the BACTEC 460 TB system. In the previous report by Patterson et al. (7) describing a *Nocardia* pseudoepidemic, no source of contamination was identified; the outbreak was interrupted only when the BACTEC needle sterilizer was changed and the sterilization time was prolonged. The source of contamination in the current report appeared to be related to a specimen from a patient (patient 1) that was inoculated into a BACTEC 12B vial and processed through the BACTEC 460 instrument. Due to an oversight in the routine maintenance schedule, a misalignment of the motor drive and overused needle sterilizer were undetected for at least 4 to 6 weeks. These problems likely contributed to contamination of the needle heater, needles, and tubing, leading to contamination of BACTEC vials containing samples from patients. We believe that only three vials became contaminated with *Nocardia* organisms because most of the specimens processed through the BACTEC instrument were respiratory samples that were pretreated with PANTA PLUS, containing antimicrobial agents that could have suppressed the growth of *Nocardia* organisms. Our experience with a *Nocardia* pseudo-outbreak associated with processing through the BACTEC 460 TB system emphasizes the importance of rigorous and timely instrument maintenance to ensure adequate needle sterilization.

This report also confirms the value of molecular typing methods in the investigation of possible laboratory-based contamination. Both PFGE and RAPD PCR were able to clearly differentiate the pseudo-outbreak strain from epidemiologically unrelated clinical isolates. The RAPD PCR analysis was

technically easier to perform and provided results within a day, whereas PFGE took several days to perform. These preliminary results, obtained by molecular typing of a small number of *N. asteroides* strains, should be validated with a larger number of isolates.

We thank E. Mandarino for technical assistance and L. Cook for secretarial services.

REFERENCES

1. **Beaman, B. L., M. A. Saubolle, and R. J. Wallace.** 1995. *Nocardia*, *Rhodococcus*, *Streptomyces*, *Oerskovia*, and other aerobic actinomycetes of medical importance, p. 379–399. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
2. **Boiron, P., F. Provost, G. Chevrier, and B. Dupont.** 1992. Review of nocardial infections in France 1987 to 1990. Eur. J. Clin. Microbiol. Infect. Dis. **11:**709–714.
3. **Conville, P. S., and F. G. Witebsky.** 1989. Inter-bottle transfer of mycobacteria by the BACTEC 460. Diagn. Microbiol. Infect. Dis. **12:**401–405.
4. **Exmelin, L., B. Malbruny, M. Vergnaud, F. Prosvost, P. Boiron, and C. Morel.** 1996. Molecular study of nosocomial nocardiosis outbreak involving heart transplant recipients. J. Clin. Microbiol. **34:**1014–1016.
5. **McNeil, M. M., and J. M. Brown.** 1994. The medically important aerobic actinomycetes: epidemiology and microbiology. Clin. Microbiol. Rev. **7:**357–417.
6. **Murray, P. R.** 1991. Mycobacterial cross-contamination with the modified BACTEC 460 TB system. Diagn. Microbiol. Infect. Dis. **14:**33–35.
7. **Patterson, J. E., K. Chapin-Robertson, S. Waycott, P. Farrel, A. McGeer, M. M. McNeil, and S. C. Edberg.** 1992. Pseudoepidemic of *Nocardia asteroides* associated with a mycobacterial culture system. J. Clin. Microbiol. **30:**1357–1360.
8. **Simpson, G. L., E. B. Stinson, M. J. Egger, and J. S. Remington.** 1981. Nocardial infections in the immunocompromised host: a detailed study in a defined population. Rev. Infect. Dis. **3:**492–506.
9. **Small, P. M., N. B. McClenny, S. P. Singh, G. K. Schoolnik, L. S. Tompkins, and P. A. Mickelsen.** 1993. Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. J. Clin. Microbiol. **31:**1677–1682.
10. **Sritharan, V., and R. H. Barker, Jr.** 1991. A simple method for diagnosing *M. tuberculosis* infection in clinical samples using PCR. Mol. Cell. Probes **5:**385–395.
11. **Steingrube, V. A., R. J. Wallace, Jr., B. A. Brown, Y. Pang, B. Zeluff, L. C. Steele, and Y. Zhang.** 1991. Acquired resistance of *Nocardia brasiliensis* to clavulanic acid related to a change in β-lactamase following therapy with amoxicillin-clavulanic acid. Antimicrob. Agents Chemother. **35:**524–528.
12. **Stevens, D. A., A. C. Pier, B. L. Beaman, P. A. Morozumi, I. S. Lovett, and E. T. Houang.** 1981. Laboratory evaluation of an outbreak of nocardiosis in immunocompromised hosts. Am. J. Med. **71:**928–934.
13. **Tokars, J. I., M. M. McNeil, O. C. Tablan, K. Chapin-Robertson, J. E. Patterson, S. C. Edberg, and W. R. Jarvis.** 1990. *Mycobacterium gordonae* pseudoinfection associated with a contaminated antimicrobial solution. J. Clin. Microbiol. **28:**2765–2769.
14. **Vannier, A. M., J. J. Tarrand, and P. R. Murray.** 1988. Mycobacterial cross contamination during radiometric culturing. J. Clin. Microbiol. **26:**1867–1868.
15. **Wallace, R. J., Y. Zhang, B. A. Brown, V. Fraser, G. H. Mazurek, and S. Maloney.** 1993. DNA large restriction fragment patterns of sporadic and epidemic nosocomial strains of *Mycobacterium chelonae* and *Mycobacterium abscessus*. J. Clin. Microbiol. **31:**2697–2701.
16. **Wilson, J. P., H. R. Turner, K. A. Kirchner, and S. W. Chapman.** 1989. Nocardial infections in renal transplant recipients. Medicine **68:**38–53.
17. **Witebsky, F. G., J. F. Keiser, P. S. Conville, R. Bryan, C. H. Park, R. Walker, and S. H. Siddiqi.** 1988. Comparison of BACTEC 13A medium and Du Pont Isolator for detection of mycobacteremia. J. Clin. Microbiol. **26:**1501–1505.
18. **Young, L. S., D. Armstrong, A. Blevins, and P. Lieberman.** 1971. *Nocardia asteroides* infection complicating neoplastic disease. Am. J. Med. **50:**356–366.