Isolation of *Nocardi a asteroides* from Respiratory Specimens by Using Selective Buffered Charcoal-Yeast Extract Agar

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Nocardiosis is difficult to diagnose clinically and by laboratory methods. A patient presented with disease compatible with pulmonary malignancy, but *Nocardi a asteroides* was isolated on buffered charcoal-yeast extract agar. Investigation revealed that this medium may be a suitable selective primary isolation medium for *Nocardi a* species from respiratory specimens.

Nocardiosis is a localized or disseminated infection which is caused by aerobic, partially acid-fast actinomycetes. These gram-positive bacilli exhibit rudimentary to extensively branched vegetative hyphae and are commonly found in decaying organic matter and soil (5). *Nocardi a asteroides* most commonly infects humans via the respiratory tract (8). Pulmonary involvement ranges from subclinical transient infection to prolonged severe illness mimicking lung abscess, tuberculosis, neoplasm, or fungal infection. The diagnosis may be overlooked because of the lack of pathognomonic features and difficulty in isolating the organism from contaminated specimens. In general, *Nocardi a* species are not fastidious organisms, and they grow well on a variety of media containing nitrogen and simple carbon sources (2, 3, 5). The suboptimal recovery rate is attributed to the slow growth of these organisms, which allows them to be overgrown by other microbes (7, 9, 10). We present a case in which the diagnosis was facilitated by isolating *N. asteroides* from bronchoalveolar lavage fluid by using buffered charcoal-yeast extract (BCYE) agar.

**Case report.** A slim, pale, 61-year-old Caucasian female was admitted on 4 March 1990 with a 3-month history of fatigue, decreased energy, anemia, and a left upper lobe density on chest X ray. There were no fevers, chills, or respiratory symptoms, nor was there a history of gastrointestinal bleeding. The admission physical examination disclosed no abnormalities.

The hemoglobin was decreased at 108 g/liter (normal range, 115 to 160), and the erythrocyte sedimentation rate was increased to 48 mm/h (normal range, 0 to 15). A computerized tomographic scan of the chest revealed a crescentic cavitated mass 7 by 3 cm in the superior left thorax near the axillary pleura. Adjacent pleural thickening was noted, as was a 1.5-cm parastrachial lymph node. A percutaneous aspiration biopsy of the thoracic lesion was performed, and studies for cytology, acid-fast bacilli, and fungi were all negative, although the specimen was noted to be of poor quality. Normal iron stores were noted on bone marrow examination. The patient was then monitored as an outpatient.

One month after the initial assessment, she was readmitted complaining of a constant burning pain in the anterior superior left chest, associated with coughing. On examination she was febrile, and a grade II of VI systolic ejection murmur was noted in the pulmonic area. Physical examination of the chest was still normal, and the chest X ray was unchanged. A second percutaneous aspiration biopsy was undertaken. A mixed inflammatory exudate which was negative for acid-fast bacilli and fungi was found. The bacterial cultures grew normal respiratory flora. The patient was discharged from the hospital with a prescription for a corticosteroid nasal inhaler for postnasal drip.

On 24 September 1990 she was readmitted to the hospital with progressive chest pain and a persistent nonproductive cough. Flexible fiberoptic bronchoscopy was undertaken, and endobronchial lesions were identified, but cytological examination of brushings revealed highly atypical cells suggestive of bronchoalveolar carcinoma. Ten days after admission her leukocyte count, which had been normal, rose to 14.5 × 10⁹/liter (82% granulocytes). The sputum was mucopurulent with moderate numbers of pus cells and mixed bacteria with a few gram-positive branching rods. Cultures revealed moderate growth of *Haemophilus parainfluenzae*, heavy growth of normal flora, and a few colonies of *N. asteroides* growing on the selective BCYE plate. No mycobacteria or fungi were isolated. She was treated with double-strength co-trimoxazole (1 tablet per os twice a day) and folic acid (5 mg per os once daily). Her leukocyte count rapidly returned to normal, and she was discharged from hospital. Follow-up chest X ray of 9 November revealed that the left upper lobe density had dramatically decreased, and the patient felt much better and was afebrile, reporting normal energy levels and freedom from chest pain. By 15 January 1991, the chest X ray showed minimal residual changes and the patient was gaining weight, with a normal leukocyte count and the erythrocyte sedimentation rate down to 20 mm/h. The initial concern about malignancy was discounted in favor of the final diagnosis, pulmonary nocardiosis.

**Microbiological methods and results.** A bronchoalveolar lavage specimen from the left upper lobe was spun down at 3,000 × g for 10 min. The sediment was used for microscopic examination and culture. Microscopic examination for acid-fast bacilli and fungi was negative, the Gram stain was unremarkable, and routine bacterial cultures (Columbia horse blood agar [Oxoid], MacConkey agar without crystal violet [Oxoid], and Columbia agar base with Fildes extract supplement) yielded normal respiratory tract flora at 48 h. The bronchoalveolar lavage was also inoculated on Lowenstein-Jensen medium (BBL 11359) after decontamination with a solution of 0.5% *N*-acetyl-1-cysteine–2% sodium...
hydroxide, 1.47% (wt/vol) trisodium citrate. Culture for fungi included inoculation on brain heart infusion agar (Difco) with 5% sheep blood (BHIB); BHIB with gentamicin, chloramphenicol, and cycloheximide (BHIB+); BHIB+ with albumin; Littman oxgall agar (Difco); and Mycelphil agar (BBL) with gentamicin. Culture for Legionella spp. included inoculation on selective and nonselective agar (Legionella CYE agar base [Oxoid no. CM655], Legionella BCYE supplement [Oxoid no. SR110], and Legionella BMPA supplement [Oxoid no. SR111]).

There was no growth on the Lowenstein-Jensen medium, but after incubation for 5 days at 35°C in 5% CO2 the BCYE agar yielded a few distinctive small white colonies mixed with normal respiratory tract flora. On the selective BCYE agar, only the white colonies had grown. Microscopic examination of the colonies revealed branching gram-positive rods which were partially acid fast by the modified Kinyoun procedure (4). Subculture yielded a pure growth which gave negative reactions on casein, xanthine, and tyrosine agar but grew well on Lowenstein-Jensen medium. Colonies were initially powdery and snow white but eventually produced a salmon-pink pigment on BCYE agar and a yellow-orange pigment on Lowenstein-Jensen medium. Our presumptive identification of N. asteroides was confirmed by the Mycology Department, Laboratory Services Branch, Ontario Ministry of Health.

A specimen of expectorated sputum was received 5 days after the bronchoalveolar lavage. The direct Gram smear from this specimen showed a moderate number of gram-positive branching rods. This specimen was plated on selective BCYE agar to compare its usefulness for the isolation of Nocardi sp. with that of Mycelphil agar (BBL 11445). The N. asteroides produced a few small colonies on blood agar and Mycelphil agar, but these were overgrown by the normal flora and were difficult to isolate in pure culture. On BCYE agar growth was more luxuriant, and on the selective BCYE medium a pure growth was obtained.

Subsequent subculturing of Nocardi spi. with the BCYE agar confirmed its usefulness as a growth medium. Twenty strains of actinomycetes were subcultured with a standard inoculum (150 CFU per plate or 10 μl of 1.5 x 10^7 CFU/liter) to BCYE agar and selective BCYE agar. The subculture plates were incubated for 6 days at 35°C. The results are given in Table 1.

To further support our hypothesis, culture studies were performed. Ten fresh sputum samples from randomly selected patients were pooled, cultured, and tested for the presence of antibiotics by growth inhibition. The mixture grew a heavy growth of normal respiratory tract flora, Pseudomonas aeruginosa, and yeast. No antibiotic substance was detected, and culture of the pooled specimen did not yield Nocardia species. The pooled sputum had a volume of 45 ml, to which an equal volume of dithiothreitol (Sputolysin; Behring Diagnostics catalog no. 869224) was added as a liquefying agent.

Twelve strains of N. asteroides were subcultured to fresh Columbia horse blood agar plates. Overnight cultures of these strains were used to prepare 12 standardized suspensions. Each isolate was made up to a 0.5 McFarland standard in antibiotic medium 3 (Difco) with 2% (wt/vol) Tween 80. One milliliter of a 1/1,000 dilution of each of these suspensions was added to 5-ml aliquots of the prepared sputum. Each of the 12 seeded samples was mixed well, and 20 μl of sample was inoculated on Columbia horse blood agar, Mycobal agar, BCYE agar, and selective BCYE agar. An acid wash pretreatment procedure using 0.2 M KCl-HCl buffer, pH 2.2, was performed on 0.5 ml of each of the 12 samples, and a second set of BCYE agar and selective BCYE agar was inoculated with 20 μl of the treated samples.

The remaining portion of each seeded sputum sample was decontaminated and inoculated on Lowenstein-Jensen medium. All media were incubated aerobically at 35°C and examined daily until N. asteroides was isolated or until the media became unreadable because of bacterial overgrowth (up to 10 days). Heavy growth of normal respiratory tract flora was observed on the blood agar, Mycelphil agar, and BCYE agar. A moderate reduction in growth occurred with the selective BCYE medium. There was a significant decrease in the flora after the acid wash pretreatment and after decontamination. Nocardia colonies were visible after 48 to 96 h of incubation on the BCYE agar, selective BCYE agar, and Lowenstein-Jensen medium (Table 2). The colonies were distinctive and easily picked for subculture. Two Nocardia isolates failed to grow on all media tested. Neither blood agar nor Mycelphil agar facilitated the isolation of the nocardioforms from the sputum specimens. Bacterial growth was heavy on these plates and obscured any Nocardia colonies which might have been present. Selective media and pretreatment of specimens improved Nocardia isolation. Without pretreatment, 8% of the Nocardia strains tested grew on BCYE agar and 33% grew on selective BCYE agar. Isolation improved to 58% after decontamination and to 67% when specimens were pretreated by acid wash.

Discussion. Although Nocardia species grow best on antibiotic-free media, the frequency of their isolation may be

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**TABLE 1.** Subculture results of 20 actinomycetes with BCYE agar and selective BCYE agar

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains subcultured</th>
<th>Source(s)</th>
<th>No. of strains growing on:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nocardia asteroides</td>
<td>12</td>
<td>8 clinical isolates, 1 ATCC 23824 isolate, 2 quality control strains, 1 Laboratory Proficiency Testing Program strain</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Nocardia brasiliensis</td>
<td>1</td>
<td>1 ATCC 19296 isolate</td>
<td>1</td>
</tr>
<tr>
<td>Nocardia species</td>
<td>1</td>
<td>1 clinical isolate</td>
<td>1</td>
</tr>
<tr>
<td>Rhodococcus equi</td>
<td>3</td>
<td>3 clinical isolates</td>
<td>3</td>
</tr>
<tr>
<td>Rhodococcus species</td>
<td>1</td>
<td>1 environmental isolate</td>
<td>0</td>
</tr>
<tr>
<td>Gordona (formerly Rhodococcus) bronchialis</td>
<td>1</td>
<td>1 clinical isolate</td>
<td>1</td>
</tr>
<tr>
<td>Streptomyces species</td>
<td>1</td>
<td>1 clinical isolate</td>
<td>0</td>
</tr>
</tbody>
</table>

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increased through the use of selective techniques to prevent overgrowth by more rapidly growing organisms (9). Nocardioforms grow on mycobacterial media such as Lowenstein-Jensen medium but are often killed by routine decontamination procedures (6). We suspect that this occurred during the primary isolation in the case we present, since the isolate grew well on Lowenstein-Jensen medium after subculture.

Sabouraud dextrose agar with chloramphenicol has been suggested as a selective medium for the recovery of Nocardia species from respiratory specimens (5), although chloramphenicol inhibits the growth of many Nocardia isolates (1). Paraffin baiting is also reported to be more efficient than conventional culture techniques for the isolation of N. asteroides from sputum (10). This technique is based on the ability of Nocardia species to utilize paraffin as a sole carbon source. A paraffin-coated glass rod is inserted into a carbon-free broth mixed with the sputum specimen. In positive cultures growth appears on the paraffin-coated rod just above the surface of the broth. Two selective agar media have also recently been described for the recovery of Nocardia species from respiratory specimens by using modified Thayer-Martin medium (7) and a synthetic paraffin agar medium (9).

This case is illustrative of the clinical and laboratory diagnostic problems faced when dealing with nocardiosis. Recognition of N. asteroides as the etiologic organism was accomplished by isolation of the organism on the standard Legionella medium, BCYE agar. This prompted us to investigate the usefulness of BCYE agar as a Nocardia medium. Microbiological evaluation of selective and nonselective BCYE agars demonstrated that these media support the growth of actinomyces and that these media are selective for Nocardia species in respiratory specimens. Isolation was not accomplished with blood agar or Mycobial agar, and Lowenstein-Jensen medium was inferior to selective BCYE agar. The highest isolation rate was obtained when the sputum specimens were pretreated by acid wash and inoculated on selective BCYE agar. This procedure may be suitable for primary isolation of Nocardia species from respiratory specimens.

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REFERENCES