

M72-5 Lung Biopsy: *Nocardia asteroides*

History: This isolate, recovered from a lung biopsy, was sent to "A" laboratories for identification. The culti-loop contained a pure growth of *Nocardia asteroides*. As 10 of 11 reference laboratories reported *Nocardia NOS* or *Nocardia asteroides*, M72-5 was considered suitable to grade.

Thirty-eight of 71 laboratories (53%) correctly identified this organism to genus level, and 16 identified to species level (23%); both identifications (76%) received excellent grades. Acceptable responses included: query *Nocardia*, referred (1), and gram-positive, branching bacilli, referred (6). The 5 laboratories that reported "gram-positive bacilli, referred" received unacceptable grades, because this response is too vague to provide useful clinical information. Other misidentifications of *Mycobacterium fortuitum-chelonae* (2), *Rhodococcus equi* (2), and coagulase-negative staphylococcus (1), also received unacceptable grades.

Nocardia is a common saprophyte in the soil and water. This organism does not commonly colonize the upper respiratory tract, therefore when the organism is isolated it should be considered a pathogen. *Nocardia asteroides* is the causative organism for 80% of nocardiosis in humans. *Nocardia brasiliensis* may occur in patients with normal immunity, but it is more often (up to 70%) recovered from patients immunosuppressed by cancer or transplant medication, or underlying systemic diseases such as AIDS. Normal hosts may present with mild chronic respiratory tract symptoms, however, frequently there is a cough, purulent sputum (sometimes blood streaked), pleural pain and night sweats. After the initial infection, the organism has a tendency to disseminate, especially in severely immunocompromised patients. The central nervous system is involved in 30% of cases while the subcutaneous may be involved in up to 15%. *Nocardia* should be considered in any pneumonia that is chronic and persists despite conventional antimicrobial therapy.¹

Nocardia, *Rhodococcus*, and rapidly growing mycobacteria can grow on many routine media, usually within 3 days, can survive mycobacterial concentration procedures, including sodium hydroxide methods, and grow on Lowenstein-Jensen media and 7H10 (oleic-acid-albumen agar). However, there are some *Nocardia* that may take one week or more to develop. The typical glabrous, pigmented orange colonies of *Nocardia* may resemble those of rapidly growing mycobacteria. *Rhodococcus* are usually cream coloured but some can be pink. In questionable cases, use of compound (plate microscope) to identify presence of aerial hyphae typical of the actinomycetes can assist to identify *Nocardia*.

In an ordinary Gram stain of the sputum, *Nocardia* appear as branched, beaded filaments and coccoid cells. A Gram stain from the primary plate is an important part of a work-up of any slow growing micro-organism. A Gram stain of a fresh culture of *Nocardia* shows Gram positive branching filaments (less than 1 m m. in diameter). Old colonies fragment into bacillary and coccoid forms. A Gram stain of an old culture has predominantly coccoid forms that could resemble staphylococci to the unwary. When you see branching gram- positive bacilli, it is important to transmit this information to the clinician as this is consistent with the actinomycetes group, and this information will aid in the focus of diagnosis and treatment. When gram-positive branching bacilli are identified in a Gram stain of clinical material like sputum or from a stain of primary culture the next test to be performed should be a modified acid fast.

Nocardia are weakly acid fast, and can be stained using a modified acid fast stain. Primarily acid fast stains are used in bacteriology for the detection of mycobacteria because of the high lipid content in their cell walls. Mycobacteria are resistant to most stains, but once stained with basic fushin, they resist decolourization with an acid alcohol mixture, hence the description "acid-fast". Acid fastness is variable in *Nocardia* species. In order to demonstrate acid-fastness of *Nocardia*, it is necessary to modify the decolourization step of Ziehl-Neelsen and Kinyoun staining methods to not exceed 5 – 10 seconds or to use Hank's Modified Acid Fast stain which uses a 5% H₂SO₄- methylene blue solution to counterstain and decolourize. The stain is controlled by the use of premade slides of *Nocardia* organisms that are grown on Middlebrook , Cohn 7H10, or casein agar. A facultative actinomycetes can be used as a negative control.²

If acid fast staining of the primary plate is equivocal, staining may be enhanced by passing the organisms through a high lipid medium such as Middlebrook 7H10, Lowenstein-Jensen or Casein agar. Of the remaining medically important actinomycetes (*Nocardiosis*, *Streptomyces* and *Actinomadura* species) none are acid fast and the latter two have the presence of spores in characteristic pattern.

Rhodococcus equi (formerly *Corynebacterium equi*) is a catalase producing aerobic actinomycetes, that can stain weak acid fast by modified Kinyoun, hence it can be confused with fragmented nocardial cells. When concerned about acid fast growers, differentiation can be done using tap water agar morphology medium (20 gm. plain agar in 100 ml. tap water). Inoculate the agar with a streak. *Nocardia* demonstrates fine-branched aerial hyphae, mycobacteria demonstrates frost-like to fir tree substrate hyphae, and *Rhodococcus* grow on the agar surface in classic diphtheroid fashion.^{3,4}

The following bio-chemical test can differentiate rhodococci from actinomycetes: Rhodococci species will degrade 1% ethylene glycol incorporated in solid 710 agar while other aerobic actinomycetes (including *Nocardia*) will generally fail. Additional presumptive tests are shown in table 1.

Table 1.	Casein	L-tyrosine	Xanthine
<i>N. asteroides</i>	-	-	-
<i>N. brasiliensis</i>	+	+	-
<i>N. caviae</i>	-	-	+

Some observations on therapy: Nocardiosis has been treated with sulphonamides since the 1940's. The present treatment of choice for localised pulmonary and cutaneous nocardiosis is trimethoprim/sulfamethoxazole. In severe illness in patients with disseminated disease, a combination of supplemental agents such as amikacin, ceftriaxone, cefotaxime or imipenen are added. Also with treatment failure (prolonged treatment) or severe illness with *Nocardia* sensitivity testing should be done on the isolate.⁵

REFERENCES:

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