ANNALS OF CLINICAL AND LABORATORY SCIENCE, Vol. 11, No. 6

Rapid Detection of *Mycobacterium chelonei* ss *abscessus* by a Radiometric Technique

EDWARD R. BANNISTER, PH.D.,* ELAINE CONDON,*
ROCHELLE COHEN, M.D.,† and JOHN P. MANOS, M.D.*

Department of Laboratory Medicine, and †Department of Family Medicine, Medical University of South Carolina, Charleston, SC 29425

ABSTRACT

Mycobacterium chelonei ss *abscessus* was isolated from a subcutaneous abscess that had been submitted in Bactec blood culture bottles. Detection occurred in three days as compared to seven days in classical mycobacterial media.

Introduction

Mycobacterium chelonei ss *abscessus* infections have been well documented and are usually the result of direct trauma or needle inoculation.[2,4,6,9,11] This rapidly growing organism, included as a member of the *M. fortuitum* complex, may be distinguished from *M. fortuitum* on the basis of morphologic, biochemical, and serologic characteristics.

Therapy has consisted of surgical hot soaks followed by drainage or excision because these mycobacteria are usually resistant to most antimycobacterial agents.[3] This paper reports on the radiometric detection, isolation, and identification of a rapidly growing Mycobacterium from an aspirate of a subcutaneous abscess which was submitted in blood culture bottles (Bactec).*

Case Report

K.F., a 63 year old black female with adult onset diabetes mellitus, was on 22 units NPH insulin daily. Because of poor compliance, her diabetes was difficult to manage. Her past medical history was remarkable only for adult onset diabetes mellitus and that she had been insulin dependent since 1972 but with poor regulation of medication and diet. She also had a positive PPD† by history prior to 1974.

She presented to Family Medicine with a one and a half week history of a tender, superficial erythematous swelling (2.5 × 4 cm) in the subcutaneous fat of the abdominal wall. Her temperature was 100.8°F. There was no history of previous trauma, and she was instructed to use frequent warm soaks to the area at home. Missing her follow-up appointment, she returned six weeks later with 3+ glycosuria (for which she was given 5 units regular insulin s.c.) afebrile, and increased warmth and

* Bactec is a semiautomated instrument manufactured by Johnston Laboratories, Cockeysville, MD. It was designed for the rapid detection of microorganisms in blood and other sterile body fluids. The detection is performed by a radiometric system which measures the amount of $^{14}CO_2$ produced by a microorganism from the metabolism of 14C substrates (glucose, amino acids in blood culture medium). The amount of $^{14}CO_2$ produced is measured in an ionization chamber, and an electrometer in the machine measures the change in current produced. The current is amplified and displayed as a growth index on the front panel and is also printed with the vial identification number. A growth index of ≥35 for aerobes and ≥20 for anaerobes would be an indication that a microorganism might be present.

† Purified protein derivative (skin test antigen for *Mycobacterium tuberculosis*).

0091-7370/81/1100-0538 $00.60

tenderness of the abdominal swelling which now measured 6 cm. The patient had not applied the warm soaks as prescribed and, after further inquiry, remembered injecting her insulin s.c. into the painful site several weeks prior to her original visit. Five ml of pus were aspirated, inoculated into Bactec blood culture media (6B, 7C) and sent for fungal and routine bacterial cultures. Request for acid-fast studies was not made at this time. She was discharged on erythromycin therapy and warm soaks. The cultures submitted in blood culture bottles produced a rapidly growing Mycobacterium within three days. On return visit ten days later, there was increased pain and warmth with fluctuance, again following a non-compliant course. The mass was incised, drained, and packed with iodoform gauze; erythromycin was discontinued at this time. A culture submitted for acid-fast bacilli at this time was also positive for a rapidly growing Mycobacterium. When seen again a week later, there was decreased pain, erythema, and swelling with a serosanguinous discharge present. The gauze was removed at this time. Five days later, there was a minimal area of tenderness and induration over the now closed wound and one ml of serosanguinous fluid was aspirated but was not submitted for culture. She returned two weeks later with some increased tenderness; however, over the next two months, there was complete resolution of the abscess.

Comment

The specimen for routine culture was submitted in aerobic and anaerobic blood culture bottles to the bacteriology laboratory. Examination of the bottles by Bactec on the day of receipt in the laboratory and 24 hrs later did not give growth index readings high enough (≥35 for aerobic bottles and ≥20 for anaerobic bottles) to be considered positive. A methylene blue stain and blind subcultures to a chocolate agar plate and an anaerobic plate within 24 hrs after receipt did not reveal any organisms. Bottles were examined daily by Bactec and three days after receipt, the aerobic bottle had a growth index of 40. Examination of the bottle by Gram stain revealed Gram positive beaded bacilli. This was suggestive that mycobacteria might be present. The organism was referred to the mycobacteriology laboratory where examination by fluorochrome revealed acid-fast bacilli. Subcultures were made from the bottle to Lowenstein-Jensen slants for identification and susceptibility testing by the mycobacteriology laboratory. This organism was subsequently identified as *M. chelonei* ss *abscessus* (table I).

The second specimen, received 10 days after the initial culture was submitted to the mycobacteriology laboratory, grew an acid-fast bacillus after seven days that was identified as *M. chelonei* ss *abscessus*. Susceptibility tests were performed on both isolates by using standard techniques.[15] The results are shown in table I and are compared to the biochemical patterns of the other organisms in the *M. fortuitum* complex. Characterization of the organism was also performed by the South Carolina Department of Health and Environmental Control and the Mycobacteriology Laboratory, National Jewish Hospital, Denver, CO.

Infections with rapidly growing mycobacteria have been a problem to diagnose. *M. fortuitum, M. chelonei* ss *chelonei,* and *M. chelonei* ss *abscessus* apparently are the only pathogens for man found in this group. Suspicion that one may be dealing with a rapidly growing Mycobacterium is almost of paramount importance before diagnosis is achieved. In most cases, infections have resulted from direct trauma or needle inocula-

TABLE I
Identification and Susceptibility Results of the MUSC Isolate Compared with the *M. fortuitum* Complex

	M. fortuitum Complex			*MUSC Isolate*
Identification Tests	*fortuitum*	*chelonei - ss chelonei*	*chelonei - ss abscessus*	*chelonei - ss abscessus*
Growth				
Colony	smooth	smooth	smooth	smooth
Optimal temperature	35 - 37C	35 - 37C	35 -37 C	35 - 37C
Time	< 7 days	< 7 days	< 7 days	< 7 days
Pigment	-	-	-	-
Biochemical tests				
Niacin	-	-	-	-
NO_3 reduction	+	-	-	-
Fe uptake	+	-	-	-
Urease	+	+	+	+
NaCl tolerance	+	-	+	+
Indirect drug susceptibility on 7H 10 agar				
Rifampin				
Isoniazid				
Streptomycin	Usually	resistant		†
PAS	Usually	resistant		†
Ethambutol				
Ethionamide				
Cycloserine				
Kanamycin	Usually	resistant		‡

MUSC = Medical University of South Carolina
PAS = Para-aminosalicylic acid
†Resistant
‡Sensitive

tion.[2,4,6,9,11] Disseminated infections with *M. chelonei* ss *abscessus* have occurred in patients who have received renal homografts, but usually these are well localized.[10] Bacteremia has also been reported in patients with adenocarcinoma or leukemia.[13] This group of organisms has also caused endocarditis, pulmonary, inguinal and bone and joint infections and have been isolated from individuals who have had venous stripping performed.[1,7,8,12,18] Thus, cultures from these areas might include examination for acid-fact bacilli when routine and fungal studies are negative.

Diagnosis of infections owing to rapidly growing mycobacteria are made basically by culture and definitive biochemical testing of the isolate. The use of skin tests to aid in diagnosis has not proved to be very successful.[9]

Treatment of localized abscesses as seen in our patient, usually consists of hot soaks followed by surgical drainage.[3] Studies of the susceptibilities of rapidly growing mycobacteria to the various antimycobacterial agents have appeared in the literature.[8,9,10,18] Generally, these organisms have shown resistance to these agents. Recent *in vitro* studies[5,14,16,17] have shown that some antibacterial agents, such as amikacin, gentamicin, tobramycin, kanamycin, and tetracyclines, might be useful in treating infections due to the *M. fortuitum* complex. It does appear from these studies, however, that *M. chelonei* demonstrated greater resistance to amikacin than *M. fortuitum*. The remaining antibiotics also were less consistent than amikacin in their *in vitro* activity against these organisms. Our isolate was resistant to all antimycobacterial drugs tested except kanamycin (table I). There have been some deaths in disseminated disease,[10] thus, the decision to treat a patient with antimicrobials must be individualized.

This is the second report of the radiometric detection of a *M. chelonei* ss *abscessus* using Bactec blood culture media.[13] The specimen inoculated into the bottles was an aspirate from a subcutaneous abscess rather than blood. Detection of organisms in routine blood cultures are usually performed by examining the bottles macroscopically for turbidity, hemolysis, and gas production, or microscopically by Gram stain or other suitable stain, and by blind subculture to an enriched medium which is incubated at 35 C in 5 to 10 percent CO_2 for a maximum of 48 hours. Any or all of these techniques might miss a mycobacterial organism if it were growing in a blood culture medium.

With our patient, examination of the bottle for the production of $^{14}CO_2$ by Bactec was sensitive enough after three days incubation to give a growth index high enough (40) to be considered positive and to proceed to staining and culture techniques for isolation and identification of the suspect organism. The growth index values were as follows: 24 hrs: 21 and 15; 48 hrs: 29 and 28. A growth index value of 35 for aerobes has been set for 4.5 years. After reviewing positive blood cultures for 1979 and 1980, it was found that our false positive blood cultures were reduced by 50 percent by having a growth index value of 35 instead of 30 and, at the same time, the detection time was not reduced. Very few, if any, of the positives have a value between 30 to 35. A subsequent specimen received 10 days after the initial culture took seven days to grow on routine mycobacterial culture media. Thus more rapid detection was obtained (three days after receipt) because the specimen had been submitted in blood culture bottles. The specimen as submitted in a blood culture bottle did not indicate that it might be an acid-fast organism. As soon as it was determined that an acid-fast organism was present, the bottle was no longer run on the Bactec. It does seem apparent from these results and one other[13] that a routine blood culture medium (Bactec) is capable of supporting

the rapid growth of the *M. fortuitum* group of mycobacteria. Perhaps this medium or the 7H12 medium* for acid-fast bacilli should be used for patients who may have a mycobacterial bacteremia or patients who may have an abscess or other suppurative infection which may be due to a *Mycobacterium*, in particular, a rapid grower.

In summary, as with our patient, a localized abscess that fails to heal and that has occurred in an area of direct needle inoculation should be suspect for a rapid growing Mycobacterium. If routine and fungal cultures are negative, then other procedures should be instituted to establish the cause of the abscess. Cultures for acid-fast bacilli must be requested, as most microbiology laboratories do not incubate plates long enough (> four days) to allow a rapid growing Mycobacterium to be detected. If blood culture bottles are inoculated with blood or other material (such as aspirated pus) and Gram stains reveal thin Gram positive beaded bacilli and aerobic and anaerobic cultures are negative, then an acid-fast stain should be performed.

References

1. ALTMAN, G. A., HOROWITZ, A., KAPLINSKY, N., and FRANKL, O.: Prosthetic valve endocarditis due to *Mycobacterium chelonei*. J. Clin. Microbiol. *1*:531–533, 1975.
2. BORGHANS, J. G. A. and STANFORD, J. L.: *Mycobacterium chelonei* in abscesses after injection of diptheria-pertussis-tetanus-polio vaccine. Amer. Rev. Resp. Dis. *107*:1–8, 1973.
3. CHAPMAN, J. S.: *Mycobacterium fortuitum* (with *M. chelonei*, *M. borstelense* and *M.* abscessus). The Atypical Mycobacteria and Human Mycobacteriosis. Topics in Infectious Disease, 1st ed. New York, Plenum Medical Book Company, 1977, pp. 137–144.
4. CROWLEY, J., LIU, P. I., and GLASSMAN, A. B.: Primary isolation of *Mycobacterium chelonei* subspecies *abscessus* from pus inoculated into peptone broth. Appl. Microbiol. *28*:943–945, 1974.
5. DALOVISIO, J. R. and PANKEY, G. A.: *In vitro* susceptibility of *Mycobacterium fortuitum* and *Mycobacterium chelonei* to amikacin. J. Infect. Dis. *137*:318–321, 1978.
6. DAMSKER, B. and BOTTONE, E. J.: Nontuberculous mycobacteria as unsuspected agents of dermatological infections: diagnosis through microbiological parameters. J. Clin. Microbiol. *11*:569–571, 1980.
7. DREISEN, R. B., SCOGGIN, C., and DAVIDSON, P. T.: The pathogenicity of *Mycobacterium fortuitum* and *Mycobacterium chelonei* in man: A report of seven cases. Tubercle *57*:49–57, 1976.
8. FOZ, A., ROY, C., JURADO, J., ARTEGA, E., RUIZ, J. M., and MORAGAS, A.: *Mycobacterium chelonei* iatrogenic infections. J. Clin. Microbiol. 7:319–321, 1978.
9. GANGADHARAM, P. K. and HSU, K. H. K.: *Mycobacterium abscessus* infection in a puncture wound. Amer. Rev. Resp. Dis. *106*:275–277, 1972.
10. GRAYBILL, J. R., SILVA, J., FRASER, D. W., LORDON, R., and ROGERS, E.: Disseminated mycobacteriosis due to *Mycobacterium abscessus* in two recipients of renal homografts. Amer. Rev. Resp. Dis. *109*:4–10, 1974.
11. INMAN, P. M., BECK, A., BROUIN, A. E., and STANFORD, J. L.: Outbreak of injection abscesses due to *Mycobacterium abscessus*. Arch. Dermatol. *100*:141–147, 1969.
12. KHERMOSH, O., WEINTRAUB, S., TOPILSKY, M., and BARATZ, M.: *Mycobacterium abscessus (M. chelonei)* infection of the knee joint. Clinical Orthopedics *140*:162–168, 1979.
13. LANDAU, W., FRECZKO, J., and KAPLAN, R. L.: Radiometric detection of mycobacteria in routine blood cultures. J. Clin. Microbiol. *12*:477–478, 1980.
14. SANDERS, W. E., JR., HEARTWIG, E. C., SCHNEIDER, N. J., CACCIATORE, R., and VALDEZ, H.: Susceptibility of organisms in the *Mycobacterium fortuitum* complex to antituberculous and other antimicrobial agents. Antimicrob. Agents Chemother. *12*:295–297, 1977.
15. VESTAL, A. L.: Procedures for the isolation and identification of mycobacteria. U.S. Department of Health, Education and Welfare, Washington, D. C. Publication No. (CDC) 75-8230, 1975.
16. WALLACE, R. J., JR., DALOVISIO, J. R., and PANKEY, G. A.: Disk diffusion testing of susceptibility of *Mycobacterium fortuitum* and *Mycobacterium chelonei* to antibacterial agents. Antimicrob. Agents Chemother. *16*:611–614, 1979.
17. WELCH, D. F. and KELLY, M. T.: Antimicrobial susceptibility testing of *Mycobacterium fortuitum* complex. Antimicrob. Agents Chemother. *15*:754–757, 1979.
18. ZINA, A. M., DEPOOLI, M., and BOSSANO, A. F.: Cutaneous mucous infection with lymphadenopathy. Dermatologica *160*:376–379, 1980.

* Available from Johnson Laboratory.

Mycobacterial species gave a variety of beta-lactamase patterns. Identity was established between some strains of M. chelonei. IEF distinguished between enzymes within both the chelonei and abscessus sub-species that could not be differentiated by other methods. This technique could provide a means of identifying the source of a M. chelonei infection. Authors: J Sparks; G W Ross. Related Documents : 9990135 - Partial purification and characterization of a novel endo-beta-mannosidase acting on n- 3873255 - Mechanism of inhibition of the pc1 beta-lactamase of staphylococcus aureus by cephalos Contact Technical Service. Medicina cutanea ibero-latino-americana 1988-1-1. [Cutaneous mycobacteriosis caused by Mycobacterium chelonei var. abscessus]. [G Martinez, J Bizcarguenaga, J M Agurruza, A Rezusta, F J Carapeto]. PMID 3059086. Abstract. We are commenting on a case of cutaneous mycobacteriosis (M. chelonei) in an asthmatic 60-year-old patient under continuous steroid treatment, who months after an accidental trauma on his hand, developed painful papular lesions, located over the trauma scar, and on the dorsal lateral side of the forearm. The lesions on the forearm were accompanied by