Isolation of *Mycobacterium Fortuitum* from BACTEC 9240 Blood Culture System: A Case Report

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We describe an 89-year-old male patient who admitted due to pneumonia documented bacteremia by isolating *Mycobacterium fortuitum* (*M. fortuitum*) from BACTEC 9240 blood culture system in April 2009. During his admission, two sets of blood culture were reported as positive. Microscopic examination revealed atypical gram-positive bacilli and acid-fast organism. This organism was subsequently identified as *M. fortuitum*. Concerning that *M. fortuitum* could be cultured and isolated in this case, not only owing to it is a rapidly growing mycobacteria, but also the professionalism that the experienced technicians have. Laboratory staff should have awareness of performing mycobacteria culture when encountering an atypical gram-positive pathogen accompanying with delayed aerobic culture result.

Key words: Rapidly growing mycobacteria, Mycobacterium fortuitum, BACTEC 9240 Blood Culture System

**Introduction**

Runyon described the four groups of non-tuberculous mycobacteria (NTM), a grouping that encompasses all mycobacteria outside of the *Mycobacterium tuberculosis* complex, according to pigment production and rate of growth in 1959 [1]. These are Runyon Group I: the photochromogens, Runyon Group II: the scotochromogens, Runyon Group III: the nonchromogens, which are classified as slowly growing mycobacteria, and Runyon Group IV: the rapid growers, which may be photochromogenic, scotochromogenic, or more usually nonchromogenic, are defined as visible growth on Löwenstein-Jensen slant medium (Becton Dickinson) within seven days on subculture [2]. *Mycobacterium fortuitum* (*M. fortuitum*) is a member of the rapidly growing Runyon Group IV NTM. Besides *M. fortuitum*, the common organisms of Runyon group IV are *Mycobacterium peregrinum*, *Mycobacterium senegalense*, *Mycobacterium abscessus* and *Mycobacterium chelonae* [1].

*M. fortuitum* is a gram-positive and acid-fast bacilli. It is also a saprophyte whose natural habitat includes soil, water and dust. Nowadays *M. fortuitum* is increasingly recognized as an opportunistic pathogen causing disseminated infection [3-5]. Clinical presentation of *M. fortuitum* includes mainly cutaneous and soft tissue infections, localized posttraumatic wound infections, surgical wound infections and keratitis [6-8]. In general, immunocompetent patients tend to experience limited infections associated with low mortality.

Bacteremia is a serious clinical condition which can lead to death, and consequently a rapid and accurate detection and identification of the pathogen plays the crucial role in effective treatment. More than one blood sample are collected and examined to detect the microorganism exists and, to identify its species, and to determine its drug susceptibility.

It goes without saying that shortening the turn-around time of microbiological analyses is fairly significant and is closely related to declining patients’ morbidity and mortality [9-10]. In clinical laboratories, the BACTEC 9240 blood culture system (Becton Dickinson...
Diagnostic Instrument Systems, Sparks, Md.) is one of the automated, continuous-monitoring and widely exploited systems [10]. It uses noninvasive fluorescent patented technology to detect increases in CO₂ produced by microbial growth. Each bottle contains a fluorescent CO₂ sensor which is monitored every ten minutes for increases in fluorescent appearance. Computer algorithms determine whether sustained linear increases or increasing rates of change in fluorescent indicate microbial growth. Commercial bottles are available for every variety of clinical use. For instance, BD BACTEC Plus Aerobic/F and Plus Anaerobic/F media are used in a qualitative procedure for the aerobic and anaerobic culture respectively. Generally speaking, when blood culture bottles were positive, removed them from the BACTEC instrument, the contents were gently mixed, direct Gram staining of the blood culture fluid, and some of the fluid was inoculated onto a combination of agar plates, suited for culturing aerobic, anaerobic, and fastidious microorganisms. In this case report, subsequently mycobacteria culture should be considered whenever suspicion of mycobacterial infection. Finally, identification of the microorganism and determination of its susceptibility pattern.

**Case Report**

We describe a case of an 89-year-old male patient with poor-controlled diabetes who was bedridden and underwent a long-term antibiotics and steroid therapy documented *M. fortuitum* bacteremia in April 2009. This patient had a prolonged hospitalization for two years because of repeated episodes of pulmonary edema and nosocomial pneumonia. Two sets of blood culture were collected appropriately, incubated in BACTEC 9240 blood culture system and were positive after 48 hours of incubation. Gram stain of these positive blood culture specimens revealed Gram-positive rods (Figure 1), which were preferred spore forming bacilli than cocci. Due to no microbial growth on aerobic agar plates in the first two days, we tried to perform Ziehl-Neelsen stain and unexpectedly found the acid-fast bacilli (Figure 2). For further identification, mycobacteria culture was followed by submitting the positive culture broth to Löwenstein-Jensen slant medium. The individual colonies displayed smooth and buff-color appearance both on aerobic agar plates (Figure 3) and Löwenstein-Jensen slant medium (Figure 4) in the following two and three days. The microorganism was subsequently identified as *M. fortuitum* by traditional biochemical method [11]. The following microbiological and biochemical reactions were applied. There was growth at 3 days on Löwenstein-Jensen slant medium, individual colonies were smooth and buff colored in the dark and after exposure to light. The isolate was positive for arylsulfatase, nitrate, 5% NaCl and urease and negative for niacin and Tween 80 hydrolysis.

![Fig. 1. *Mycobacterium fortuitum* (Gram stain 1000x) revealed gram-positive rods](image1)

![Fig. 2. *Mycobacterium fortuitum* (Ziehl-Neelsen stain 1000x) showed acid-fast bacilli](image2)

![Fig. 3. Smooth and buff-color appearance of *Mycobacterium fortuitum* colonies grew on aerobic sheet blood agar plates](image3)
Isolation of Mycobacterium fortuitum from BACTEC 9240 Blood Culture System

Discussion

This is the first case of *M. fortuitum* isolation from positive blood culture bottle in the laboratory of Taipei Medical University- Wan Fang Hospital. According to the manufacturer’s instructions, samples of blood culture bottle are going to be asserted negative if BACTEC 9240 does not detect the appearance of fluorescence in culture bottles within six days. *M. fortuitum*, a member of rapidly growing Runyon IV NTM, has the property to grow on standard mycobacterial media within seven days and this property may contribute to the primary positive culture in blood sample culture system in this case.

This finding has highlighted that *M. fortuitum* is not only a well-known acid-fast bacillus, but can also forms diphtheroids, Gram positive rods appearance, under Gram stain microscopy [1]. Consequently, clinical technicians should show considerable regard for Gram positive diphtheroids, beads or rods from the blood culture sample and should proceed with mycobacteria identification rather than ignore it as contaminants.

*M. fortuitum* is ubiquitous in nature and is considered as a non-pathogen to normal people, while it may cause opportunistic infection for immunocompromised patients. In fact, cases of cervical lymphadenitis and meningitis arisen from *M. fortuitum* infection in patients with immunosuppression, the acquired immunodeficiency syndrome patients for example, have also been reported [3]. Nevertheless, even though bacteremia resulting from *M. fortuitum* is relatively rare, the incidence of such mycobacterium infections is gently growing, and probably becoming common in the future.

In view of the predilection groups of mycobacteria infection, it is essential to administer appropriate medical treatment, and therefore the susceptibility testing for mycobacteria is extremely recommended.

References

案例報告

自 BACTEC 9240 血液培養系統分離出 Mycobacterium Fortuitum－案例報告

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我們描述一位89歲的男性病人因肺炎住院治療，病歷記錄由Mycobacterium fortuitum (M. fortuitum)引起的菌血症。於住院期間做了兩套血液培養且呈現陽性結果。革蘭氏染色見到不典型陽性桿菌，推測可能是分枝桿菌，操作抗酸菌染色並呈現陽性結果。經過分枝桿菌培養鑑定出M. fortuitum。此案例的血液培養分離出M. fortuitum，不僅是因爲此菌屬於快速生長分枝桿菌，也由於經驗醫檢師的判斷及專業。醫檢師如遇到不典型革蘭氏陰性病原菌且培養需時較長的個案，應考慮操作分枝桿菌培養。

關鍵詞：快速生長分枝桿菌、Mycobacterium fortuitum、BACTEC 9240 血液培養系統

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