Delayed post-operative wound infections due to non-tuberculous *Mycobacterium*

Juri B. Kalita, H. Rahman* & K.C. Baruah

Department of Microbiology, Down Town Hospital, Guwahati, India

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*Background & objectives:* The non-tuberculous mycobacteria (NTM) have emerged as important opportunistic pathogens of human beings in the recent years. The NTM are rapid growing mycobacteria (RGM), which include *Mycobacterium fortuitum* and *M. chelonae* and grouped as *M. fortuitum-chelonae* complex. Though there are reports on isolation of NTM from various parts of India, information on its occurrence in northeastern India is lacking. We therefore undertook this preliminary investigation to report on the occurrence of NTM-associated with non-healing post-operative wound infections that did not respond to antibiotics used for pyogenic infections and having sterile routine aerobic cultures in patients from northeastern part of India.

*Methods:* Pus/discharge from 25 patients with delayed onset of post-operative wound infections not responding to antibiotics used for pyogenic infections were collected and examined for isolation and identification of the causative agents.

*Results:* Of the 25 pus/discharge specimens examined, 20 revealed growth of non-tuberculous *Mycobacterium* spp. All the isolates were identified as *M. fortuitum-chelonae* complex. Of these only 10 samples revealed acid-fast bacilli (AFB) on direct examination of Ziehl-Neelsen stained smears from the specimens. All cases where direct smear was positive for AFB were also positive for *Mycobacterium* culture.

*Interpretation & conclusion:* The results of the present study indicated that non-tuberculous mycobacterial post-operative wound infection was fairly common in northeastern India. Thus, mycobacterial infections should be considered in wounds that show delayed healing and do not respond to antibiotics used for acute pyogenic infections. Further, 80 per cent of the specimens yielded the growth of AFB in cultures as against only 40 per cent positive in the ZN stained direct smears. This indicates the possibility of missing a mycobacterial wound infection if only direct smears are taken for diagnosis.

**Key words** Delayed post-operative wound infections - *Mycobacterium fortuitum-chelonae* complex - non-tuberculous mycobacteria - rapidly growing mycobacteria

*Present address:* Principal Scientist & Head, Division of Animal Health, ICAR Research Complex for NEH Region Umroi Road, Umiam 793103, Meghalaya, India
Members of the genus *Mycobacterium* are ever expanding and presently stands at 95 species\(^1\). While tuberculosis, leprosy and paratuberculosis (Johne’s disease) are specific diseases caused by *Mycobacterium tuberculosis*, *M. leprae* and *M. paratuberculosis*, respectively, other members are usually saprophytes but can be opportunistic and at times deadly pathogens\(^1-5\), and are referred to as atypical mycobacteria or non-tuberculous mycobacteria (NTM). NTM are rapid growing mycobacteria (RGM), widely distributed in nature and have been isolated from natural water, tap water, soil, etc.\(^4\). NTM have been isolated from various sites and procedures *viz.*, cutaneous and soft tissue infections after skin injury following inoculation\(^6-7\), minor trauma\(^8\) and surgery including plastic surgery procedures\(^8\), median sternotomy\(^10\) and rhinoplasty\(^11\). They are known to cause systemic infection in patients suffering from acquired immune deficiency syndromes (AIDS) and in other immunocompromized individuals while in immunocompetent hosts the infections are of localized nature such as cutaneous and soft tissue infections\(^12,13\). The association of NTM with pulmonary disease in man has also been reported\(^14\). Though infections due to NTM like *M. fortuitum*, *M. chelonae*, *M. avium*, *M. scrofulaceum*, etc., have been reported from different parts of India\(^15-19\), information on the occurrence of NTM in northeastern part of India is lacking. This preliminary investigation was undertaken to report on the occurrence of non-tuberculous mycobacteria, associated with non-healing post-operative wound infections that did not respond to antibiotics used for pyogenic infections and having sterile routine aerobic cultures.

**Material & Methods**

*Patients and collection of samples:* The present study included 25 patients (11 males and 14 females, aged 25-55 yr with an average of 41 yr ± 0.012 SE) who had undergone surgery for various ailments between September 2000 to March 2002 in different parts of northeast India including Assam, Manipur and Meghalaya. The patients developed delayed post-operative wound infections (10 days to 3 wk after surgery) that were not responding to antibiotics used for pyogenic infections and were presented for microbiological investigation in the Microbiology Department, Down Town Hospital, Guwahati, Assam. The patients had undergone laparoscopic cholecystectomy (n=8), diagnostic laparoscopy (n=7), caesarian section (n=5), reduction mammoplasty (n=1), pacemaker implantation (n=1) and diagnostic laparotomy (n=3). One patient had undergone caesarian section in Mumbai and presented for treatment also included in this study. The clinical history was recorded in all cases at the time of specimen collection.

Pus/discharge from the post-operative wounds was collected with the help of sterile cotton swabs and/or sterile syringes and processed for isolation and identification of the causative agents immediately.

**Isolation and identification of the causative organisms:** Thin and uniform smears were prepared from each specimen and stained with Gram and Ziehl-Neelsen (ZN) stains (Hi-Media, Mumbai, India). For isolation of aerobic bacteria, the specimens were streaked on blood agar (BA, containing 5% sheep blood) and McConkey’s lactose agar (MLA) and incubated at 37°C aerobically up to 48 h. For isolation of anaerobic bacteria, the specimens were cultured anaerobically by the Gaspak method on anaerobic agar (Hi-Media, Mumbai) and blood agar at 37°C up to 72 h\(^20\). For isolation of anaerobic bacteria, the specimens were cultured anaerobically by the Gaspak method on anaerobic agar (Hi-Media, Mumbai) and blood agar at 37°C up to 72 h\(^20\). For isolation of fungi, the specimens were cultured on Sabouraud’s dextrose agar (SDA, containing chloramphenicol 50 µg/ml) in duplicate. One plate was incubated at 37°C and other plate at 22°C and observed for growth if any up to 6 wk. For isolation of mycobacteria, the specimens were cultured on Lowenstein-Jensen (LJ) medium in duplicate and observed daily for the growth and discarded after 8 wk.

The isolated organisms were identified as per the standard bacteriological techniques\(^21\). The growth on LJ medium was further processed and tested for the following additional characteristics: growth within 3-8 days on LJ medium, acid fastness of the isolates, pigment production, growth on McConkey’s medium without crystal violet, aryl sulphatase activity at 3 and 7 days, nitrate reduction, tellurite reduction, etc.\(^21\).
A total of 25 patients with delayed post-operative wound infections were included in this study. All patients had more or less similar clinical features: delayed onset of wound infection (10 days to 3 wk after surgery), appearance of erythema and oedema followed by breakdown of wound and suppuration and/or discharging sinuses, absence of systemic symptoms/illness and lack of response to antibiotics used for pyogenic infections.

Of the 25 pus/discharge samples examined microbiologically, 20 revealed growth of rapidly growing *Mycobacterium* spp. within 5-8 days of incubation. The remaining five negative cases received prior treatment with one or other fluoroquinolones which are known drugs for treatment of mycobacterial infections. Three of the 25 specimens revealed growth of coagulase-negative staphylococci. No anaerobic bacteria and fungi could be isolated from any of the samples examined. Ten samples revealed the presence of acid-fast bacilli (AFB) on examination of smear prepared directly from specimens. Of the 15 AFB-negative samples, 10 also yielded the growth of *Mycobacterium* on LJ medium. All cases where direct smear was positive for the presence of AFB were also positive for mycobacterium culture. Five samples which were negative for AFB on direct smears were also culturally negative (Table I).

All these isolates were rapidly growing, non-pigmented, and thus belonged to Runyon group IV. Further, they were positive to nitrate reduction, tolerant to NaCl, grew on MacConkey’s agar and three and seven day aryl sulphatase test positive (Table II). Based on the results of these key tests, all isolates were identified as *M. fortuitum-chelonae* complex.

As the cases were referred to our laboratory only for microbiological examination of wound infection, all could not be followed up. Only seven of the 25 cases were followed up, and all seven were cured with clarithromycin and amikacin for 2-3 months along with vigorous surgical debridement.
It was observed that 80 per cent of the specimens yielded the growth of AFB in cultures although only 40 per cent were positive in ZN stained direct smears. This indicates the possibility of missing a mycobacterial infection if only direct smears are taken for diagnosis. In contrast, LJ medium culture method for rapidly growing mycobacterium (RGM) was found to be a much more sensitive system as all direct smear positive specimens were also found to be positive in LJ medium.

Rapidly growing mycobacteria are important human pathogens and have been reported from post-surgical wound infections in many countries including India. Many such epidemic and sporadic cases in India probably remain unreported for a variety of reasons. Water, soil, animals and marine life have been mentioned as sources of *M. fortuitum-chelonae* complex, but no attempt was made to study the source of the organisms in this investigation due to technical difficulty of collecting suitable samples because of the diversity of the places of surgery. As the culture with strict criteria is still not routinely done in most parts of India and there is a tendency to ignore such isolates as contaminants, it would be difficult to comment on the exact magnitude of the problem.

Many of our cases with post-operative wound infections demonstrated sterile aerobic and anaerobic cultures in spite of having stopped taking antibiotics for a week or so prior to collection of the specimen. Although the post-operative wounds had initially healed after surgery, but it became erythematous and gradually broke down to make discharging wound later. The wounds were painless and patients were afebrile with no other systemic illness. A chronic non-healing wound may therefore present a confusing picture and in such cases mycobacterial infection should always be ruled out by proper investigations specially AFB culture. Typically, wound infections due to atypical mycobacteria do not occur as an immediate post-operative complication. There is apparent immediate post-operative healing and gradually over a variable period of time, the scar breaks down to a persistent non-healing superficial wound with discharging sinuses. Such wounds do not respond to antibiotics used for acute infection and persist for a prolonged period of time. In our study most patients had similar non-responsiveness to antibiotics and had delayed wound healing.

Mycobacterial infections must be considered in wounds that show delayed healing and do not respond to antibiotics used for acute pyogenic infections. As 80 per cent of the specimens yielded AFB in culture as against 40 per cent positive in the ZN stained direct smears, this indicates a possibility of missing a mycobacterial infection if only direct smears are taken for diagnosis. Further studies to reveal the sources of infection will also be helpful in the epidemiologic control of such infections.

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**References**


