

Post-operative wound infection by Nontuberculous Mycobacteria; case series in Dhaka Medical College Hospital of Bangladesh

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Title

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Introduction: Nontuberculous Mycobacteria (NTM) are ubiquitous group of bacteria that include mycobacterial species other than *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* (1). NTM are the diverse group of organisms that are isolated from environmental sources like soil, water, dust, lakes, rivers, streams and also from municipal water sources like water that people drink or shower (2). *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Mycobacterium fortuitum*, and *Mycobacterium smegmatis* are most often linked with NTM infections after surgical intervention worldwide (3). Usually, Ziehl-Neelsen (Z-N) staining and mycobacterial cultures are not routinely performed, that's why the detection of NTM is missed and the burden is increasing (4). Resistance to common antiseptics and disinfectant solutions used in hospital settings is also making these illnesses a growing threat. Inaccurate sterilization of instruments used in operation theater (OT) is usually responsible for such types of infections and makes it a great problem mainly affecting developing countries (5). Thus, proper sterilization of such instruments is essential to prevent the occurrence of post-laparoscopic wound infections with atypical Mycobacterium. NTM can produce biofilms which are collections of microorganisms that stick to each other, adhere to the surfaces of moist environments, become resistant to antibiotics, are difficult to eliminate, and ultimately increase the likelihood of chronic infection (3,6). Globally, in the last few years nosocomial post-operative wound infections by NTM have been increasing (7). NTM is becoming more common day by day yet it may be difficult to diagnose and long-term medication with lowered tolerance make it more challenging to treat effectively (8). Since treatment differs from species to species, species and sub-variants should be identified and even must be recognized from environmental NTM (9).

Materials methods: We collected data on such infections from February 2021 to July 2022 from the patients who presented with complaints of chronic serous discharge from the post-operative wound. The cases were examined, detailed history was taken, and wound discharge was collected for microbiological laboratory testing at the Microbiology department of Dhaka Medical College, Bangladesh. To detect NTM from these cases, Gram stain, ZN stain, fungal microscopy of the samples, culture, Gene-Xpert and, polymerase chain reaction (PCR) tests were done. We identified three patients with post-operative skin and soft tissue infections caused by NTM.

In the microbiology department, with proper aseptic technique discharge was collected by sterile swab sticks from all the cases. Wet-film preparation for fungal microscopy, Gram stain, Z-N stain, Gene-Xpert, culture,

and PCR were done from wound discharge. Discharge was inoculated and incubated on blood agar media and MacConkey agar media for 7 days, and Lowenstein Jensen (LJ) media for up to 6 weeks at 37°C aerobically. Written informed consent was taken from each patient.

Detailed information about the operation history, the treatment regimens of antibiotics, duration of therapy that is completed or running, follow-up, and the ultimate outcome were collected from patients. In other cases, where NTM were suspected but not identified from the samples, were treated with another treatment protocol advised by Microbiology department of Dhaka Medical College.

Results: On Gram staining, in some cases, few to moderate amounts of pus cells and sometimes gram-positive cocci were seen under the microscope. Z-N stained smear revealed acid-fast bacilli (AFB) in all the cases (Fig-1). No fungal element was found in any sample on microscopic examination of the discharge. No *Mycobacterium tuberculosis* was detected in GeneXpert for MTB from any case.

Culture on LJ media and MacConkey agar media did not show any growth but in on Blood agar media pale and opaque colored colonies were found in two samples after 4 days of incubation at 37°C (Fig-2) which became yellow-pink after 7 days of incubation. Again, Z-N stain was done from culture isolates and revealed AFB. PCR was done, and NTM was detected in two specimens. In one sample, culture yielded additional growth of Methicillin-resistant *Staphylococcus aureus* (MRSA) on blood agar media after 24 hours of incubation. The patients were treated initially for 6 weeks with 4 drugs regimen (clarithromycin 500 mg 12 hourly, ciprofloxacin 500 mg 12 hourly, linezolid 400 mg 12 hourly, and, amikacin 500 mg 12 hourly) for 6 weeks, followed by 5 months with 3 drugs regimen (clarithromycin 500 mg 12 hourly, ciprofloxacin 500 mg 12 hourly and linezolid 400 mg 12 hourly) as a maintenance dose. Follow-up was done in every case after the completion of the proposed drug regimens. Cessation of discharge occurred within 3-4 weeks after starting treatment and the wound was also healed or healing in most of the cases (fig-3, 4).

Case series: Two males and one female, between the ages of 30-36 years, who underwent various surgery presented with serous discharge at the site of incision mostly within a few months of the surgical procedures, which progressed to chronic discharging sinus with a small opening. Most of the patients had a history of apparently healthy post-operative wounds and stitches were removed within 7-10 days after surgery. The discharge from the wounds was thin, serous, and non-purulent. Most of them did not give any history of high fever, pain, or any constitutional symptoms associated with the wound discharge but some had low-grade fever and mild pain by giving pressure on the wound site.

One case presented with nodular swellings that progressed to chronic discharging sinus from a small site over the incision sites. On applying local pressure the discharge from the sinus of the wound site increased. Those patients gave a history of taking several antibiotics such as fluoroquinolones, colistin, cefixime, vancomycin, ceftriaxone, meropenem, and linezolid previously but none was cured. One patient took anti-MTB drug regimens for several months without any improvement and could not show any diagnostic evidence for MTB. Detailed clinical profiles of study cases are shown in Table-1, and investigation profiles are given in Table-2.

Discussion: In recent years, frequently encountered NTM species in post-surgical wound infections are *M. chelonae* and *M. fortuitum* (10). NTM are transmitted through aerosol, soil, dust, or contaminated tap water (11). In our study, all the post-operative wounds were healed initially within 7-10 days of surgery. Then within the next 1-2 months incision sites became erythematous, and indurated, small blisters formed, burst out, and started serous discharge in small quantities. Several antibiotics were recommended for these wound-infected cases but did not respond to any of them, discharge continued and persisted for a long time before they were referred to the Microbiology department. Wound infections due to NTM usually do not occur as an early postoperative complication. During operation, wounds are contaminated with NTM from environmental sources and take some time to make their clinical appearance. After infection with NTM, the operation scar breaks down and develops a non-healing superficial ulcer with the sinus tract from which non-purulent serous discharge comes out (12).

Bhalla et al reported 10.9% of post-operative wound infections occurred by NTM infections in South India (13). Development of mild fever, small indurations, with or without mild local pain, and serous discharge from

a tiny opening of post-operative healed wound scar indicates the initiation of the onset of NTM infection. Specimens from such cases usually show no pus cell or organism on Gram stain and cultures show no growth on routine culture media for aerobic and anaerobic organisms. Hence, all these specimens should be collected through aseptic precautions, must be stained by the Z-N staining method for acid-fast bacilli (AFB), and incubated on LJ media and blood agar media to isolate NTM (14). The aim of accurate and early diagnosis is to formulate an appropriate treatment regimen that is specific to NTM.

NTM have commonly identified pathogens from post-operative wound infection patients and require a high suspicion for correct and early diagnosis (13). Chronic discharge with a prolonged course of expensive antibiotics makes it a serious type of nosocomial infection. Skin or soft tissue infection is the most common manifestation seen in NTM-infected individuals whose wounds may be exposed directly or indirectly to the soil, colonized tap water, unsterilized operative instruments, or medical devices contaminated with environmental NTM after traumatic injury, during surgery, or cosmetic procedures. Surveillance study of environmental culture from tap water, operation instruments, wall, floor, basin and operation theatre (OT) may not yield growth of NTM (11). Strict sterilization of all OT equipment, proper hand washing, and prevention of wound contamination with dust, soil, and tap water are needed to prevent wound infections with NTM.

Because of the long duration of treatment, side effects and cost of the drugs accurate diagnosis is necessary. The most preferred choice is a varying combination of antibacterial drugs like imipenem, amikacin, fluorinated quinolones, doxycycline, linezolid, and clarithromycin (15). However, first-line antitubercular drugs like ethambutol and rifampicin have a bactericidal effect against NTM but they are not used commonly.

When there is a sudden increase in such cases, it is urgent to conduct epidemiological studies, collect specimens from the surrounding environment, and confirm NTM infections whether are identical through culture studies, PCR, and also need antimicrobial susceptibility testing. Therefore, although it is not possible to test for NTM in all patients routinely in developing countries like Bangladesh, it is advisable to refer cases where the patients had an operation history, were exposed to contaminated water, dust or soil, and suffered from discharging post-operative wounds with delayed recovery to detect whether it is NTM infection or not. Furthermore, in this background, there is poor communication between clinicians and the laboratory due to poor awareness, unknown prevalence patterns due to lack of study, and the absence of standard diagnostic facilities and regimens for treatment.

Until now, there has been a lack of analysis regarding NTM in Bangladesh. Therefore, such cases have been treated initially as general wound infections and sometimes by anti-MTB regimens given without any diagnosis. Recently NTM infections have attracted more attention from clinicians due to the increase in such cases but still, there is a lack of awareness. When chronic discharge from post-operative wound infections occur after operations that cannot be cured by usual antibiotics, NTM infections should be suspected and ZN stain, culture, Gene-Xpert, and PCR must be considered as diagnostic tools.

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Table 1 - Copy.docx available at <https://authorea.com/users/624076/articles/646535-post-operative-wound-infection-by-nontuberculous-mycobacteria-case-series-in-dhaka-medical-college-hospital-of-bangladesh>

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