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CASE REPORT

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Mycobacterium chelonae bacteraemia in a patient with myasthenia gravis receiving long-term steroid therapy

Arghadip Samaddar[1], Saumya Srivastava[1], Salman Khan[1], Vibhor Tak[1,*], Anuradha Sharma[1], Vijaya Lakshmi Nag[1] and Gopal Krishna Bohra[2]

Abstract

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms found in soil and water. Infections caused by NTM are increasing with conditions ranging from harmless colonization to invasive infections, the latter being more common in immunocompromised hosts. In this report, we present a case of bacteraemia caused by *Mycobacterium chelonae*, a rapidly growing NTM belonging to Class IV in the Runyon classification, in a 71-year-old male with ocular myasthenia gravis undergoing treatment with oral prednisolone. Gram staining of these organisms from blood culture can be easily overlooked or confused with diptheroids. Detection of Gram-positive bacilli should prompt Ziehl–Neelsen staining to distinguish diphtheroids from rapidly growing mycobacteria in immunosuppressed patients. In addition, speciation and antimicrobial susceptibility testing are of paramount importance in such cases as there is considerable variation in the resistance patterns between different species of NTM. Line probe assay provides a rapid and reliable method for identification of NTM to the species level, which can guide treatment with appropriate antibiotics. This case report highlights the importance of early detection of such cases so as to optimize management and improve patient outcomes.

INTRODUCTION

Mycobacterium chelonae is a rapidly growing, non-tuberculous mycobacterium (NTM) belonging to Class IV in the Runyon classification. *M. chelonae* and *Mycobacterium abscessus* were considered identical until 1992 when they were reclassified as separate species [1, 2]. The incidence of infections caused by NTM has increased significantly, probably due to increased use of immunosuppressive drugs and advances in identification techniques [3]. *M. chelonae* is ubiquitous and has been isolated from soil, water and environmental sources [4]. Infections occur in both immunocompetent and immunocompromised hosts. In immunocompetent individuals, the most common clinical presentation is localized skin and soft tissue infections. Disseminated and invasive infections are seen mainly in immunocompromised patients [5]. *M. chelonae* has no pathognomonic characteristics, which makes diagnosis challenging. The treatment of infections caused by *M. chelonae* is difficult as it is frequently resistant to most antimicrobial agents. The present case report highlights the importance of early detection of such cases so as to plan appropriate management and improve patient outcomes.

CASE REPORT

A 71-year-old male was admitted to the medical ward of the All India Institute of Medical Sciences, Jodhpur, Rajasthan, India, on 20 October 2018, with complaints of high-grade fever associated with chills, generalized body aches, weakness, headache and anorexia for the last 15 days. He was diagnosed with ocular myasthenia gravis 25 years previously for which he was treated with pyridostigmine (a cholinesterase inhibitor) and azathioprine (an immunosuppressive agent) and had been receiving oral prednisolone for the past 10 years. He was also a known case of chronic kidney disease, coronary artery disease, hypertension, dyslipidaemia and hyperuricaemia for which he was receiving appropriate medications. Clinical examination revealed reduced chest expansion, proximal muscle weakness and proptosis. On auscultation, a systolic murmur was heard in the precordium radiating to the axilla and basal crepitations were heard in the left lung field. Chest radiography revealed an opacity on the lower lobe of the left lung. Examination of other systems revealed no significant abnormality. His complete haemogram revealed microcytic hypochromic anaemia with anisopoikilocytosis,

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Author affiliations: [1]Department of Microbiology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India; [2]Department of General Medicine, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India.

***Correspondence:** Vibhor Tak, drvibhortakaiims@gmail.com

Keywords: bacteraemia; non-tuberculous mycobacteria; *Mycobacterium chelonae*; line probe assay; bloodstream infection.

Abbreviations: LPA, line probe assay; NTM, non-tuberculous mycobacteria.

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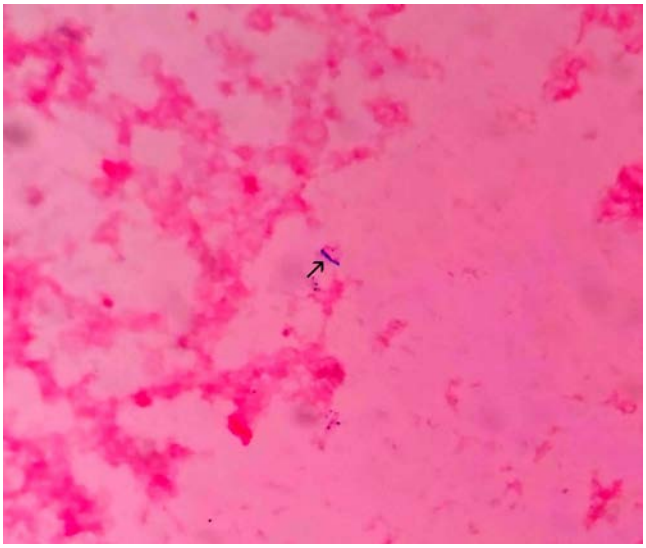

Fig. 1. Beaded Gram-positive bacilli (arrow) in Gram-stained smear from positive blood culture bottle (magnification: x1000).

Fig. 2. Colonies of *Mycobacterium chelonae* on MacConkey agar after 48 h of aerobic incubation at 28 °C.

elliptocytosis, mild thrombocytopenia and elevated erythrocyte sedimentation rate (ESR) (69 mm in the first hour). High-sensitivity C-reactive protein (hsCRP) was 89 mg l^{-1} (normal range 0.5–10 mg l^{-1}). A presumptive diagnosis of lower respiratory tract infection (LRTI) was made and the patient was started on intravenous amoxicillin plus clavulanic acid (1000/200 mg) three times daily and oral azithromycin 500 mg once daily.

His urine sample was sterile on culture. A blood culture bottle (BACTEC 9120; BD) inoculated with venous blood collected with aseptic techniques was positive after 13 h of aerobic incubation, and a Gram-stained smear from the bottle showed Gram-positive bacilli (Fig. 1). Ziehl-Neelsen (ZN) staining was performed, which was positive for acid-fast bacilli. On subcultures, white to pale, smooth colonies, 2–3 mm in diameter on blood agar (HiMedia Laboratories) and small, pink colonies on MacConkey agar (HiMedia Laboratories) were obtained after 72 h of aerobic incubation (Fig. 2). Gram staining and ZN staining of smears prepared from colonies on MacConkey agar showed beaded Gram-positive and acid-fast bacilli, respectively (Figs 3 and 4). The isolate was negative for MPT64 antigen (BIO-LINE SD Ag MPT64 TB test). A presumptive identification of *M. chelonae* was made based on the following tests: growth on para-aminobenzoic acid-containing Lowenstein–Jensen (LJ) medium, growth on MacConkey agar at 28 °C, negative nitrate reduction test, negative 5% NaCl tolerance test and lack of pigment production. A repeat blood culture also showed Gram-positive and acid-fast bacilli having similar morphologies on blood agar and MacConkey agar and with an identical biochemical profile. The identity of isolates obtained on both the occasions was further confirmed by line probe assay (LPA) using GenoType Mycobacterium CM VER 2.0 (Hain Lifescience) according to the manufacturer's instructions. The process involved PCR amplification, hybridization of amplicons to probes bound to the test strips and detection of bound products. Antimicrobial susceptibility testing of the isolates revealed sensitivity to ciprofloxacin, azithromycin and imipenem but resistance to gentamicin and tetracycline.

The antibiotic treatment was revised and the patient was started on ciprofloxacin 500 mg orally once daily and azithromycin 500 mg orally once daily. The patient defervesced within 48 h of initiation of the revised regimen and his clinical condition improved. He was discharged from the hospital with oral antibiotics after 7 days of treatment. The patient

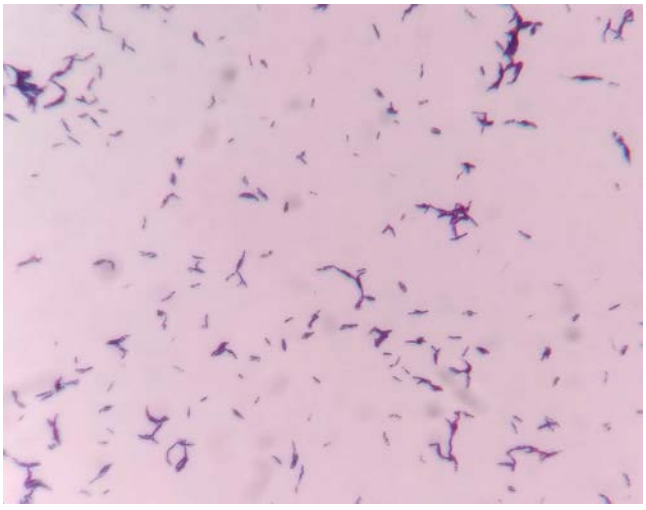

Fig. 3. Gram-stained smear prepared from colonies on MacConkey agar showing beaded Gram-positive bacilli (magnification: x1000).

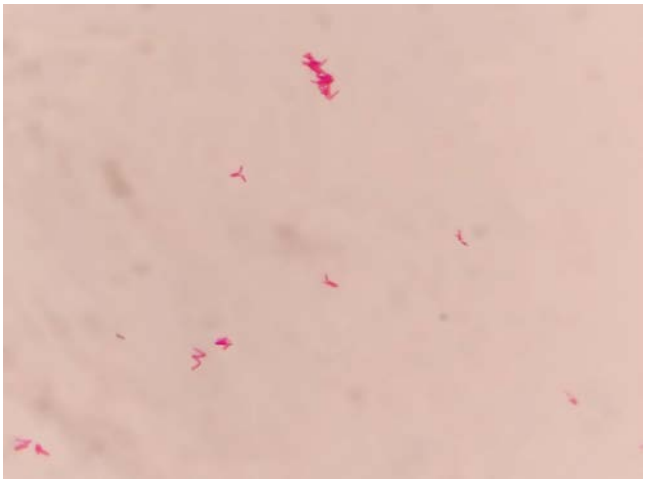

Fig. 4. Ziehl-Neelsen staining of colonies on MacConkey agar showing beaded acid-fast bacilli (magnification: x1000).

remained afebrile with sterile blood cultures on follow-up visits for 4 months, with no treatment-related adverse effects.

DISCUSSION

NTM numbering over 95 species are mostly environmental saprophytes but are known to cause four different categories of human infections: (i) pulmonary infections resembling tuberculosis; (ii) extra-pulmonary infections affecting lymph nodes, skin and soft tissue; (iii) multifocal disseminated infections; and (iv) infections in immunocompromised hosts [6]. The incidence and prevalence of NTM infections are increasing, largely due to therapeutic intervention-induced immunocompromised status [3]. *M. chelonae* is a rapidly growing mycobacterium that causes disease in both healthy and immunocompromised hosts [7]. Although most infections involving *M. chelonae* are limited to cutaneous lesions, invasive infections such as bacteraemia, endocarditis, osteomyelitis, peritonitis, keratitis and disseminated infections are common in immunocompromised patients, particularly those on steroids, immunosuppressive drugs and post-transplant immunosuppression [8]. Our patient was a known case of ocular myasthenia gravis for the last 25 years who had undergone treatment with the immunosuppressive drug azathioprine and had been receiving oral prednisolone for the last 10 years. Chronic kidney disease patients are also susceptible to disseminated and invasive infections due to *M. chelonae* [9]. Therefore, therapeutic immunosuppression coupled with underlying chronic kidney disease were likely to be the main reasons for our patient developing *M. chelonae* bacteraemia.

Despite their low pathogenicity, NTM can result in serious infection when introduced to sterile body sites. Because NTM are ubiquitous and common laboratory contaminants, isolation of these organisms from clinical specimens should meet certain criteria to confirm their aetiological significance, such as (i) repeated isolation of the same organism from a patient; (ii) supportive clinical and radiological evidence:; (ii) isolation from sterile body fluids such as blood, cerebrospinal fluid and pleural fluid; (iv) presence of predisposing factors/underlying diseases; and (v) the immune status of the patient [10, 11]. A single isolate of NTM from a sterile body site or multiple isolates from a non-sterile site are considered significant, especially in immunocompromised hosts [12]. In our case, the same isolate was obtained twice from blood cultures from the same patient on two separate occasions. The appearance of beaded Gram-positive rods in blood culture and other sterile sites should prompt ZN staining to distinguish diphtheroids from NTM. Molecular methods play an important role in the definitive identification of these organisms, which is crucial because antibiotic susceptibility patterns vary among different species of NTM [9]. Our isolate was identified as *M. chelonae* by LPA using GenoType Mycobacterium CM VER 2.0 (Hain Lifescience), which involves DNA amplification targeting the 23S rRNA gene region, followed by reverse hybridization to specific oligonucleotide probes immobilized on membrane strips. Singh *et al.* [13] reported that the GenoType Mycobacterium CM assay was able to identify 96.7% of NTM to the species level correctly. Similar findings were reported by other studies [14]. Therefore, the GenoType Mycobacterium CM assay can provide rapid speciation of NTM, and can be useful in targeted therapy and management of infections caused by these organisms, thus reducing antibiotic resistance.

Antibiotic susceptibility testing of clinically significant isolates of NTM is recommended because they differ in their susceptibility to the commonly used antimicrobial agents; for example, *M. abscessus* is considered to be more drug-resistant than *M. chelonae* and is extremely difficult to treat [15]. Moreover, NTM are resistant to commonly used anti-tubercular drugs. As reported by several studies, the use of combination antimicrobial agents is superior to monotherapy in the treatment of NTM bacteraemia and is associated with lower relapse rate [5, 7]. Disseminated infection caused by *M. chelonae* requires treatment with at least two drugs that include a macrolide and an aminoglycoside [16]. In our case, resistance of the isolate to gentamicin and a history of chronic kidney disease warranted the use of aminoglycoside antibiotics. Therefore, a combination regimen consisting of azithromycin (a macrolide) and ciprofloxacin (a fluoroquinolone) was initiated based on the susceptibility report. Although studies have demonstrated reduced susceptibility of *M. chelonae* to quinolones [17, 18], good therapeutic response was observed in our patient. He was discharged from the hospital after 7 days of treatment with the revised antibiotic regimen and his subsequent blood cultures were sterile during follow-up.

Although the isolation rate of NTM from patients in India has been reported to range from 0.5 to 8.6% [19], the exact burden of such infections remains unclear. A study from western India reported an increasing incidence of NTM infections from 1 % in 2005 to 3.5 % in 2008 with 88.6 % of the isolates being clinically relevant [19]. However, there are limited data on the incidence and prevalence of clinically significant NTM infections from India as these are frequently

under-diagnosed in countries endemic for tuberculosis due to non-specific clinical symptoms, unfamiliarity and confusion among clinicians, and inadequacy of laboratory services to diagnose such infections. Furthermore, accurate identification of acid-fast bacilli and a clear distinction between *Mycobacterium tuberculosis* and NTM is important to avoid therapy with anti-tubercular drugs, particularly in India where tuberculosis is endemic.

CONCLUSION

Clinicians need to be aware of the significance of NTM infections in immunocompromised patients, and microbiology laboratories should rule out NTM before dismissing Gram-positive bacilli as diphtheroids. LPA using GenoType Mycobacterium CM VER 2.0 is a rapid and reliable method for speciation of NTM, and can help clinicians to understand the spectrum of associated diseases and plan appropriate management. Antibiotic susceptibility testing should be performed for all isolates of NTM as the resistance patterns vary among different mycobacterial species.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Written informed consent for publication of the clinical details and/or images was obtained from the patient. A copy of the consent form is available for review from the Editor of this Journal.

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