

## Generalized Cutaneous Lesions Due to *Mycobacterium abscessus*: A Case Report

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### Abstract

**Introduction:** Generalized cutaneous lesions caused by *Mycobacterium abscessus* have not been reported in immuno competent individuals.

**Case presentation:** We describe here a case of 13-year old male child who developed a non-healing sternal wound after being operated upon for atrial septal defect surgery. Routine pyogenic and fungal cultures were negative. The wound did not clear with repeated courses of antibiotics. The continued serosanguinous discharge from the wound site ultimately progressed to generalized pustular non-healing lesions possibly through secondary bacteremia. Pustular pus showed acid-fast bacilli in Ziehl-Neelsen staining and grew Nontuberculous Mycobacteria on conventional LJ Medium and rapid MGIT medium. The NTM isolated was identified as *M. abscessus* through culture characters, biochemical testing, PCR and DNA fingerprinting technique. Treatment with multiple drugs helped in clearing of the skin pustular lesions.

**Conclusion:** This case of generalized lesions by *abscessus* infection appears to be the first ever reported finding.

**Keywords:** Acid-fast bacilli; Non tuberculous mycobacteria; Pustular lesions

### Introduction

Non Tuberculous Mycobacteria (NTM) is a diverse group of acid-fast bacilli in genus Mycobacteria that include more than 100 different species [1]. About one-third of NTM species have been associated with human disease and of all the species under this group, *Mycobacterium fortuitum*, *abscessus* and *chelonae* have been most frequently and are being increasingly associated with skin and soft tissue infections after skin injury following inoculation, minor trauma or surgery [2].

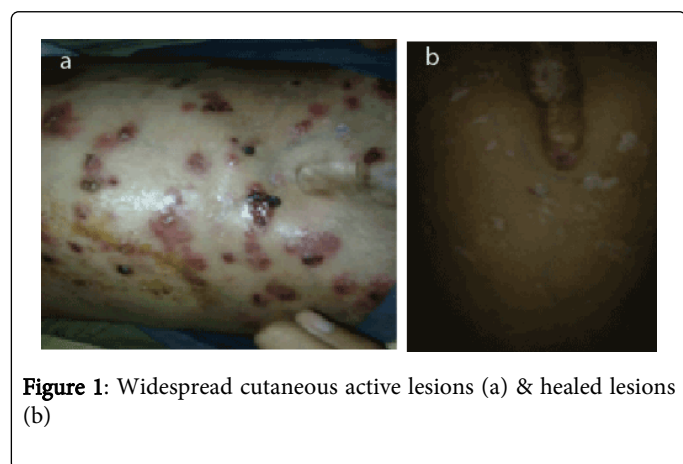
*Mycobacterium abscessus* was described first by Moore and Frerichs in 1953. In the beginning *M. abscessus* was classified as subspecies of *chelonae* but with the help of DNA homology studies, this has been classified as a separate species. *M. abscessus* has been further classified into three closely related species; *M. abscessus*, *M. massiliense* and *M. bolletii* on the basis of RNA Polymerase B sequencing pattern [3]. However little is known regarding the clinical impact of this species differentiation. *M. abscessus* is considered one of the most pathogenic and drug resistant NTM. We report a case of young child with generalized cutaneous lesions due to *M. abscessus* following cardiac surgery.

### Case Report

A 13-year old male child was admitted in the pediatric ward of our hospital with complaints of non-healing generalized pustular lesions and intermittent fever. Past history of the child was extremely significant. In 2004, at the age of 6 years, the child was operated for atrial septal defect correction surgery at All India Institute of Medical Sciences, New Delhi. The surgery was associated with sternal osteomyelitis which progressed to the development of a non-healing sternal wound for which the child was followed up with local wound care and different antibiotics but the wound did not clear. Failing to get any relief, the parents of the child started caring for the wound with betadine solution at home. During the next couple of years, there was no significant deterioration in the wound except that the child also started getting intermittent fever. The child also didn't gain weight and height proportionate to his age. In 2009, the child was put on four drug anti-tubercular treatment by one of the private doctors. This treatment modality was completely empirical and without any laboratory supported information. The child took the anti-tubercular treatment for two months only because the wound didn't respond much to the treatment and also the child could not tolerate the drugs and the parents reverted to the practice of local wound care.

In 2011, the child developed generalized pustular lesions (Figure 1) and was brought to the pediatric department. On admission, the child was started on empirical oral erythromycin and topical fusidic acid till the case was fully worked up. The child was 122 cm in height and

weighed 26 kg at first visit. Cardiovascular system was normal and chest was bilaterally clear. Liver and spleen were non palpable. Biochemical parameters for blood sugar, liver function and renal function were within biological reference ranges. Hb was 10.5 gm% and total leucocyte count 12300/ $\mu$ l (polymorphs 80, lymphocytes 18 and monocytes 2). Serology for HBsAg, HCV and HIV was non-reactive. Pyogenic cultures for blood, urine, stool and pus were sterile. Pus aspirated from the pustules was processed for fungal culture also but there was no growth after two weeks of incubation. Pus from the pustular lesions showed acid-fast bacilli in Ziehl-Neelsen stain. Histopathological examination of skin biopsy from the lesion site showed granulomatous reaction. Multiple retroperitoneal lymph nodes were enlarged in contrast enhanced computerized tomography.



**Figure 1:** Widespread cutaneous active lesions (a) & healed lesions (b)

Since the child had taken anti-tubercular treatment for two months in the past, it was considered scientifically more prudent to go in for culture and identification of the organism before starting the treatment afresh. Pus sample from the pustular lesions and 10 ml of blood sample was sent to LRS Institute of Tuberculosis and Chest Diseases for mycobacterial culture. Blood sample was investigated because mycobacteremia secondary to sternal wound appeared to be the most probable pathogenic mechanism involved in dissemination of infecting organism. LRS as a part of their protocol employ conventional LJ Medium and MGIT for culturing mycobacteria. Both pus and blood samples showed growth of cream-colored colonies within seven days of incubation on LJ Medium. MGIT also showed growth in same period. ZN stain of the cream colored colonies on LJ Medium and a drop of MGIT medium showed acid-fast bacilli. To identify the species biochemical reactions were put up and results of the reactions supported species *M. abscessus* (Table 1). The cultures were then sent to the Division of Biotechnology and Molecular Diagnostics, National Centre for Disease Control, Delhi for identification of mycobacterium species.

S.No.	Characteristic	Description
1.	Morphology	Colony was rough
2.	Growth Rate	Within 7 days
3.	Pigment Production	Cream colored
4.	Nitrate Reduction	Negative
5.	Niacin Production	Negative
6.	Semi Quantitative Catalase Test	Negative

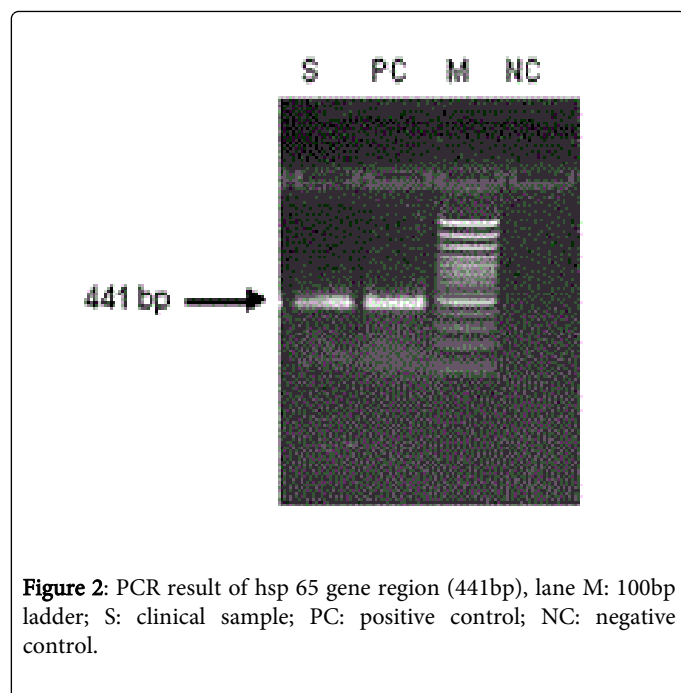
7.	Heat Resistant Catalase Test 68°C	Positive
8.	TCH	Positive
9.	PNB	Negative
10.	INH	Negative
11.	Urease	Positive
12.	Growth on Mc CONKEY	Negative
13.	Mc CONKEY with 5% NaCl	Positive
14.	Iron Uptake	Negative
15.	TWEEN Hydrolysis	Negative
16.	Tellurite Reduction	Negative
17.	Aryl Sulphatase 3 days 7 days	- Positive - Positive
18.	Pyrazinamidase 4 days 7days	- Negative - Positive

**Table 1:** Results of the biochemical reactions carried out for the identification of Mycobacterial species.

The DNA was extracted using the commercially available Qiagen kit (GmbH, Germany). Polymerase Chain Reaction (PCR) was carried out to amplify 441 bp region of hsp 65 gene of mycobacterium using the reported previously primers [4]. The hsp 65 gene is present in all mycobacteria and is more variable than the 16S rRNA gene sequence and therefore can be exploited to identify mycobacteria to the species level [4]. The amplified products were electrophoresed on 1.5% agarose gel and stained with Ethidium bromide to check for the presence of specified bands (Figure 2).

The PCR products were purified using Promega Wizard SV Gel and PCR Clean-up system. The purified PCR products were subjected to automated nucleotide sequencing using the same primers. Sequencing was carried-out using the Big dye-terminator cycle sequencing kit (ABI, USA). Approximately 25 ng of purified PCR product was mixed with 1 pM respective primer and a reaction mixture containing AmpliTaq DNA polymerase and four dye labeled di-deoxynucleotide terminators.

The reaction mixture was placed onto a preheated thermal cycler. Cycle sequencing parameters were: Denaturation at 96°C for 10 s, primer annealing at 50°C for 5 s, and an elongation step at 60°C for 4 min. The cycles were repeated 25 times. The reaction mixture was purified by precipitation with 3M sodium acetate (pH 4.6) and 75% isopropanol, and the extension product was vacuum dried. The DNA pellet was re-suspended in 10 ml of template suppression reagent (TSR), heated at 95°C for 2 min and immediately chilled on ice, mixed and after brief spin, finally loaded onto the ABI 3130xl genetic analyzer (ABI, USA) [6,8]. The sequence was identified using Blast search against a database of hsp 65 genes. On analysis, the sequence matched with the species *abscessus*. Thus the isolate was confirmed as *Mycobacterium abscessus*.



After getting the identification of the isolate, the child was put on amikacin (15 mg/KBW), doxycycline (4.4 mg/KBW), clarithromycin (250 mg BD), linezolid (10 mg/KBW 8 TDS), clofazimine (10 mg/KBW) and moxifloxacin (200 mg/KBW). This combination of drugs worked in this child and within two months of treatment the cutaneous lesions resolved. The parents of the child were advised to follow-up on monthly basis observe for any relapse phenomenon or adverse reactions of the drugs that were being given to the child.

## Discussion

Tracing the epidemiology of non-tuberculous mycobacterial infections is usually a difficult proposition because these are distributed ubiquitously in the environment and linking of these infections with any single particular source may not be possible. These have been isolated from soil, water, surgical solutions, equipment etc. and are known to cause disease in wide category of species [5,7]. Ubiquitous distribution of NTM coupled with their resistance to commonly used disinfectants makes them formidable hospital acquired pathogens. This child could probably have acquired the infection with *abscessus* at the time of cardiac surgery through the medium of any of the possible sources in the surgical theatre. In 2011, when the child developed generalized cutaneous pustular lesions, prior to that in the period between 2004 and 2011, there had been no history of major illness or infection except the fact that the sternal wound would not heal and the child didn't gain height and weight for his age. Serology for HIV was non-reactive, immunoglobulin levels and CD4 counts were within range but Mantoux intra dermal test was negative. Past history and laboratory parameters didn't signal any immune deterioration except negative Mantoux test which could be attributed to anergic phenomenon. Stunted growth and failure to thrive could thus reasonably be attributed to this chronic *abscessus* infection.

The mechanism of pathogenesis of nontuberculous mycobacteria is not very clear and has not been adequately investigated. Lipid-rich outer envelope may be acting as first line of defence but specific surface moieties may be important factors. Disseminated NTM

infections primarily occur under conditions of severe immune suppression and in people with normal immune function, these are usually associated with certain specific genetic syndromes [1]. Prima facie, there was no evidence of immune suppression and due to lack of facilities for evaluation of genetic syndromes, bacteremia secondary to sternal lesion appeared to be the only mechanism that could have been operative in dissemination of infection and or there could have been associated hypersensitivity element.

The diagnosis of cutaneous tuberculosis may be missed on account of false negative results of culture and direct microscopic examinations. In this particular case, it took almost 7 years to establish the cause of skin lesions because mycobacterial etiology was never anticipated and species status of *M. abscessus* could be established only after culture results were available and molecular techniques confirmed the isolate as *abscessus*. The major reasons for under diagnosis of NTM infections in tuberculosis endemic countries could be lack of awareness, inadequate laboratory facilities and overburden of other diseases. Treatment of infection with non-tuberculous mycobacteria depends upon the immune status of the patient, severity of disease and above all on the nature of infecting species. *M. abscessus* is usually resistant to conventional anti-tuberculous drugs and is otherwise also one of the most resistant NTM species [2,3]. This child was given two months of conventional ATT but the lesions did not respond at all and thus the treatment was withdrawn. The skin lesions resolved with two months of treatment with amikacin, doxycycline, clarithromycin, linezolid, clofazimine and moxifloxacin. After two months, clofazimine and doxycycline were withdrawn and the child was kept under observation. The child responded well to the drugs and his condition greatly improved during subsequent visits to the hospital. The decision to start this treatment protocol was purely consensually clinical because in India, diagnosis and management of tuberculosis is mainly administered through RNTCP (Revised National Tuberculosis Control Programme) and this programme doesn't define any regimens for treatment of MOTT group. Even the literature available on treatment of MOTT provides only fragmented information. The authors in no way advocate employing this treatment protocol rather underline the necessity and urgency of participation of all the stake holders to pool and share their scientific experience on clinical criterion to diagnose these infections and treatment of MOTT group for reference and consideration of medical fraternity.

This case signals lack of awareness among treating physicians and microbiologists about the real burden of problem of NTM infections. In the absence of any authentic data about their true incidence and prevalence, NTM infections are considered to be of least consequence because facilities for identification and anti-microbial sensitivity facilities for NTM are grossly inadequate in most of the microbiology labs.

Infections caused by NTM are a diagnostic as well as therapeutic dilemma. All the bacterial and fungal culture-negative skin lesions not responding to conventional interventional strategies need to be investigated for evidence of tubercular and non-tubercular mycobacterial causes. Role of microscopy in diagnosis and management of tuberculosis can't be under played but species identification appears to be absolutely essential component in the management of NTM infections since antimicrobial sensitivity and therapies differ significantly depending on the species isolated. Conventional culture and identification methods are time consuming and have inherent limitations in identification of NTM and

underscore the need for application of molecular techniques for rapid diagnosis and to account for other shortcoming of conventional methods.

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