Abstract
A 23-year old HIV-negative male, receiving immunosuppressive therapy for polymyositis, presented with a cutaneous abscess in the neck and regional lymphadenopathy caused by a drug susceptible strain of *Mycobacterium tuberculosis*. He was started on a 4-drug antituberculosis regimen to which he supposedly was adherent. He was readmitted 6 months later with miliary tuberculosis and multiple cutaneous abscesses. The *M. tuberculosis* strain isolated from all abscesses and sputum culture was confirmed to be identical to the initial strain with restriction fragment length polymorphism (RFLP) and phage assay. The strain was now resistant to 9 antituberculosis drugs according to susceptibility testing by BACTEC 460TB system and Loewenstein Jensen media (with antitubercular drugs in different concentrations). The patient was cured after immunosuppressive therapy was withdrawn, all cutaneous abscesses were surgically drained and the patient was treated for 1.5 years on a 6-drug antituberculosis regimen.
Background
Global Tuberculosis Control Report 2003 issued by World Health Organization on World Tuberculosis (TB) day ranks India as number one in the world in estimated number of tuberculosis cases. The incidence of smear positive cases of pulmonary TB in India is about 0.9 million every year whereas the incidence of extrapulmonary TB cases is estimated at about 0.2 million. Cutaneous TB was rare (0.1%) and of these lupus vulgaris was the most frequent manifestation (55%) among dermatology patients in a tertiary care hospital of northern India (1975-95). Similar low incidences (0.07% and 0.14%) of cutaneous TB were reported from Hong Kong (1983-1992) and Madrid (1980-1993) respectively. TB itself has reemerged as a serious problem since 1985 with the advent of HIV/AIDS. However, atypical mycobacteria such as M. kansasii, M. scrofulaceum, rather than M. tuberculosis are the most common etiological agents for cutaneous TB in HIV/AIDS and other immunocompromised patients.

The pathogenesis of cutaneous involvement is either due to true bacterial invasion of the skin or tuberculid (hypersensitivity reaction) associated with primary focus elsewhere. Erythema induratum (Bazin disease) and lichen scrofulosorum are the two forms of tuberculid lesions whereas true cutaneous TB in immunosuppressed patients manifests as primary chancre, lupus vulgaris, tuberculid verrucosa cutis, scrofuloderma, tuberculid cutis orificialis or tuberculid cutis miliaris disseminates. Poor management, including the prescription of incorrect regimens and non-compliance with treatment, can lead to the selection of M. tuberculosis with mutations conferring resistance to antitubercular drugs. WHO criteria define acquired drug resistance as the isolation of drug-resistant M. tuberculosis from a patient who has been treated for TB for 1 month or longer, and primary drug resistance as the isolation of drug-resistant strain from a patient without a history of previous treatment.

We report a rare case of multiple cutaneous TB abscesses and miliary TB caused by multidrug resistant (MDR) M. tuberculosis in a young man with polymyositis on immunosuppressive therapy.
Case Report
A 23-year-old male was diagnosed as a case of polymyositis on the basis of clinical symptoms, muscle biopsy, enzyme assay, needle electromyography (EMG) and nerve conduction velocity test (NCV). He was treated with immunosuppressive drugs including corticosteroids at 1 mg/kg per day for 3 to 4 weeks, tapered gradually over a period of 10 weeks to 1 mg/kg every alternate days and methotrexate at 7.5 mg weekly with gradual increase to 25 mg weekly for a period of 1 year. During this period, his muscle power improved slightly. He was HIV negative with normal chest radiograph findings and sputum microscopy was negative for acid-fast bacilli (AFB).

He was readmitted 6 months later with a single cutaneous abscess on the right side of the neck with no sinus formation along with cervical and axillary lymphadenopathy. Chest x-ray was again normal. Three early-morning sputum samples (induced in two occasions) were collected on three consecutive days, all of which were negative for AFB. The aspirated pus sample from cutaneous abscess showed plenty of AFB. Biopsy of the axillary lymph node showed nonspecific necrosis with disintegrating polymorphs, very few granulomatous cells, no epithelioid cells and plenty of AFB. *M. tuberculosis* was cultured and confirmed by niacin and nitrate reduction tests and rapid bacteriophage assay (FAST Plaque Tuberculosis kit, BIOTEC Laboratories Ltd, UK) was performed.

The strain was further confirmed by Restriction-fragment length polymorphisms (RFLP) analysis (figure 1) using species-specific probes for *devR* (Differentially expressed virulent gene). The *devR* gene (Gene bank nucleotide sequence accession no. U22037) encodes a response regulator that is part of a two-component signal transduction system of *M. tuberculosis*. The authenticity of the amplified product was established by hybridization of immobilized PCR products to an internal oligonucleotide, *devR*1, mapping within the *devR* gene. The chromosomal DNA was prepared by chloroform-isoamyl alcohol DNA extraction and 4.5 µg of DNA from the isolate was restricted with *Pvu*II. Separation of *Pvu*II-restricted DNA by electrophoresis, Southern blot hybridization with a 513-bp PCR probes for *devR* (*devRf*, 5΄ GGTTAGGC CGGGTGTCGTCGC 3΄; *devRr*, 5΄ CGCGGCTTTCGTCGAGCT CGTCGTTCACGT TC 3΄) and chemi-luminescence detection were done according to the standard method recommended for the DNA fingerprinting of *M. tuberculosis*.6
Ultrasonography (USG) of kidney, liver or spleen revealed no abscess. The patient was treated with a 4-drug regimen [isoniazide (H) 300mg/d, rifampicin (R) 450mg/d, ethambutol (E) 800mg/d and pyrazinamide (Z) 1.5 g/d] for 8 months (2EHRZ/6HR), first 2 months of which was supervised. The *M. tuberculosis* strain was susceptible to all the 4 drugs by drug susceptibility testing in BACTEC 460TB system. The patient improved clinically.

The patient returned 6 months later with multiple cutaneous abscesses, mainly on his back, left thigh and left arm. The CXR showed miliary mottling. Although treatment was not supervised after first 2 months, the patient denied irregular intake of antitubercular drugs. The patient was readmitted and all immuno-suppressive drugs (corticosteroids and methotrexate) were discontinued.

Two of 3 consecutive early-morning sputum samples and 5 aspirated pus samples from all 5-abscess sites were positive for AFB. All the abscess sites were completely drained. The isolate from the sputum as well as from aspirated pus was reconfirmed as the identical *M. tuberculosis* strain by niacin and nitrate reduction tests, rapid bacteriophage assay and RFLP analysis with 513-bp PCR probes for devR (figure 1). The isolate was resistant to 4 first-line drugs by BACTEC 460TB system and to nine drugs, tested into Loewenstein Jensen media [Hi-media, Mumbai, India]: H (1µg/ml), H (10 µg/ml), R (20µg/ml), R (50µg/ml), E (2µg/ml), E (10µg/ml), Z (50µg/ml, at pH 5.5), streptomycin (5µg/ml), streptomycin (50µg/ml), paraaminosalicylate (2.5µg/ml), cycloserine (30µg/ml), amikacin (700µg/ml) and ciprofloxacin (12.5µg/ml). The patient started treatment with H (5mg/kg/d) and R (10mg/kg/d), pyrazinamide (30mg/kg/d), ethambutol (15mg/kg/d), kanamycin (15mg/kg IM 5 times weekly) and sparfloxacine (500mg/d). Treatment was supervised for 2 months and thereafter given unsupervised to a total of 18 months. The patient responded to the therapy slowly and by the end, he was clinically, microbiologically and radiologically cured. He was followed up for another 16 months.
Discussion

India is an endemic country for TB where there is an estimated 20,000 infectious cases of MDR TB every year and 1-3.3% of new patients have MDR TB. Infections are common with more than one strain of *M. tuberculosis* during the same episode (multiple infection), in different lesions (multiple infection) or during successive episodes (reinfection) in HIV-positive as well as –negative individuals. In this study identical strains *M. tuberculosis* were isolated from the patient at 6 months interval, but contrary to the first, the strain in the second episode was resistant to all the first line and most of the second line antitubercular drugs like aminoglycosides, paraaminosalicylate, cycloserine, and ciprofloxacin. As far as could be ascertained, this is the first case of isolation of MDR strain of *M. tuberculosis* from a HIV-negative but immunosuppressed patient who had an infection with the same, but susceptible strain 6 months before.

As evident from the study, the chance of exogenous reinfection with the same but drug-resistant strain is the most likely explanation. Exogenous reinfection with the same strain is possible and the *strain* could be MDR from some undetected source within the endemic community. This can better explain the acquisition of resistance against all those drugs, to which the patient was not exposed earlier. Reinfection 6 months after starting of treatment is not common as it is usually reported to occur after first two to five years in immunocompetent persons, but supportive evidences showed that reinfection along with progression to active disease might occur at any time after treatment has been discontinued, and even during treatment for active tuberculosis. Moreover, an ongoing tuberculous infection and simultaneous immunosuppressive therapy might significantly divert the immune response, thereby increasing the overall susceptibility to ‘superinfection’, as suggested by Warren *et al.* with the same but MDR strain. The chances of ‘simultaneous infection’, as documented by Braden *et al.* with both the strains (susceptible and MDR) could be another possibility. Drug-susceptibility testing could discriminate simultaneous infection with different susceptibility profiles if individual colonies from the isolate were tested, which was not in our case. It might be the possibility that the sensitivity pattern of the susceptible strain was obtained at the first attempt. Even RFLP and phage typing were not enough to determine whether there was simultaneous infections or reinfection (superinfection) with *M. tuberculosis*. There is a remote possibility of endogenous
reactivation with multi- “drug resistance in previously treated case”, as suggested by Van Rie et al. 12 Studies suggested that the rate of endogenous reactivation far exceeded the rate of exogenous reinfection in endemic countries. 13,14 The history of regular medication in this case might be notoriously misleading as it was in majority of the patients with multidrug resistance. 12 However, it is difficult to explain the acquisition of multidrug resistance of the strain in such a short period against those drugs, to which the patient was not exposed.

The unexpected susceptibility results in second episode were unlikely due to a switch in specimens or cross-contamination of cultures in laboratories; no other samples to switch or contaminate were identified. The drug susceptibility test has its limitations with an overall accuracy ranging between 84-100%. 15 Since mycobacteria often clump, it is difficult to obtain homogenous inoculum suspensions and this may affect results. We tried to overcome this problem with thorough vortexing with glass beads. Niacin test (95% positive16) and nitrate reduction test (97% positive16) helped in differentiating M. tuberculosis from other mycobacteria in M. tuberculosis complex. RFLP and phage typing were also useful in excluding atypical mycobacteria, like M. kansasii and M. scrofulaceum which are the most common organisms causing cutaneous involvement in immunosuppressed patients.

Isolation of MDR M. tuberculosis showed that the lesions of the skin were not a tuberculid type as there was true bacterial invasion of the skin. The cutaneous abscesses were of various sizes both over the flexor and the extensor surfaces of the limbs and the trunk with no muscle involvement and no sinus formation. It was a rare case of miliary TB of non-reactive type17 as the biopsy of the axillary lymph node showed non-specific necrosis containing disintegrating polymorphonuclear leukocytes and enormous numbers of AFB. In a typical case, granulomas and epithelioid cells are lacking. Miliary TB of non-reactive type, though often seen in severe HIV infection 18 was found in patient on immunosuppressive therapy. A similar case study has been documented in another patient with dermatomyositis under immunosuppressive therapy. 19 The liver and the spleen are the most commonly involved organs followed by the lung, the bone marrow and the kidney. 17 The chest x-ray shows inconspicuous diffuse mottling. In our case, there was no other organ involvement.
The rapid clinical progression of the skin infection indicated the poor cellular immune response due to immunosuppressive therapy with corticosteroid and methotrexate as reported earlier. The patient was not HIV-positive but developed MDR TB rapidly within 6 months, involving lungs as well as skin due to the immunosuppressed condition. We think that the treatment was adequate in our case but the development of **new skin abscesses** with initial regression of disease suggests that the tubercle bacilli in the cutaneous and subcutaneous tissue were somehow protected by the action of the antituberculous drugs. This could be the ‘paradoxical expansion of disease during therapy’, the drugs could not penetrate well in these areas probably due to polymyositis as reported in a case of dermatomyositis-associated subcutaneous calcification, or the large mass of bacilli present during the acute dissemination phase of the infection might have lodged in the small arterial vessels and caused obstruction of these vessels, which might have led to necrosis and abscess formation in the surrounding areas.

This is probably the first case reported where all first and most of the second line drugs with a total of 9 antitubercular drugs were found to be resistant and yet the patient was **cured**. The patient was treated with 6 antitubercular drugs, 5 conventional and 1 new one ie. sparfloxacin for 1.5 years. Out of 6 drugs, 4 first line drugs showed high-level resistance; amikacin and kanamycin was very closely related and chances of cross-resistance could not be ignored and resistance to sparfloxacin was likely, since the patient was highly resistant to ciprofloxacin. Therefore, drugs might not be considered as the only reason for cure. The immunosuppressive therapy was stopped before starting antitubercular therapy with 6 drugs and the patient might have a reconstitution of the immune system along with the therapy. It might explain the recovery of the patient. Complete drainage of the abscesses might be an important adjunct to the treatment with better penetration of drugs. The clinical progress of the patient during therapy was slow and the patient was microbiologically cured for the first time only after 1 year. The treatment was continued for another 6 months. The active lesions on the chest **radiograph** diminished gradually only after 10 months.
Conclusions

Severe immunosuppression may lead to disseminated TB such as miliary TB or other rare types of extra-pulmonary TB such as cutaneous abscesses. Follow-up of patients is important, and if response to treatment is poor, adherence to treatment, drug resistance and other possible reasons such as continuation of immunosuppressive therapy should be considered. In this case intervention by drainage of abscesses, discontinuation of immunosuppressive treatment and possibly long-term treatment with additional second line antituberculosis drugs eventually lead to cure.
Competing interests

• We did not receive any reimbursement, fee, funding, or salary from an organization that may in any way gain or lose financially from the publication of this paper in the past five years, or is such an organization financing the present article.
• We did not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this paper.
• We do not have any other financial competing interests.
• There is no non-financial competing interest to declare in relation to this paper.
Authors' contributions

CM carried out the case study and follow-up, in the clinical as well as microbiological aspects, study design, sensitivity of the organism, participated in the FAST plaque assay and molecular identification method and drafted the manuscript.

AG carried out FAST plaque assay and molecular identification method.

AA conceived the study, participated in the design of the study and acted as an overall supervisor.

All authors read and approved the final manuscript.
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References


Figure 1
Sample figure title
The PCR amplified product for devR gene (513 bps) of M. tuberculosis was visualized after staining with ethidium bromide.

Figure legend text
(From left to right) Lane M; 100bp ladder Bangalore Genei (India), Lane 1; Negative control, Lane 2; M. tuberculosis H37Rv, Lane 3; Patient’s isolate of sensitive M. tuberculosis strain (on first admission), Lane 4; Patient’s isolate of MDR M. tuberculosis strain (on readmission), Lane 5; M. kansasii, Lane 6; M. scrofulaceum.