Pulmonary and extrapulmonary infection caused by *Mycobacterium conceptionense*: the first report from Iran

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We report what we believe to be the first clinical isolation of *Mycobacterium conceptionense* in a developing country.

**Case reports**

**Case one**

A 38-year-old man presented to hospital with high fever, cough, chest pain and 10 kg of weight loss in 6 weeks. His medical record showed that he was a narcotic addict, IV drug user and infected with HIV during a course of imprisonment in 2007. He was negative for tuberculin test and had no history of infection with mycobacteria. One year earlier, he had developed cytomegalovirus disease and candidosis. At admission, laboratory testing revealed an elevated C-reactive protein (CRP) of 50 mg/L; erythrocyte sedimentation rate (ESR) of 63 mm/h; a viral load of 5000 copies/mL; and a CD4 T-cell count of 40 cells/mm³. His chest X-ray was normal.

Direct microscopic examination of blood specimen for acid fast bacilli was negative, but culture on Löwenstein-Jensen (LJ) slant yielded colonies of rapidly growing mycobacterium. Three weeks after the initiation of amikacin therapy, the patient showed marked improvement and lived for one more year before he died of hepato-renal failure.

**Case two**

A 57-year-old man admitted to hospital because of urinary tract infection with chronic flank pain. Her major symptoms included urgency, frequency, dysuria and moderate fever. Gram staining on mid-stream urine sample revealed numerous polymorphonuclear leukocytes. Culture of specimen on blood agar and MacConkey agar was negative. The laboratory examination results were as follows: haemoglobin 16 gm/dL; white cell count 8.7 × 10⁶/cu.mm; ESR 58 mm/h; CRP 30.9 mg/L. Evaluation of sono-graphic imaging revealed inflammation of the lower urinary tract. Kidney stones were ruled out by computed tomography (CT) scan.

She had no history of infection with mycobacteria and was negative for HIV and other immunosuppressive diseases. The acid fast staining of three consecutive 24-hour mid-stream clean catch urine specimens were positive, and the culture on LJ medium yielded pure rapidly growing *mycobacterium*. The patient became afebrile on amikacin therapy.

**Case three**

A 62-year-old woman was admitted to hospital because of urinary tract infection with chronic flank pain. Her major symptoms included urgency, frequency, dysuria and moderate fever. Gram staining on mid-stream urine sample revealed numerous polymorphonuclear leukocytes. Culture of specimen on blood agar and MacConkey agar was negative. The laboratory examination results were as follows: haemoglobin 16 gm/dL; white cell count 8.7 × 10⁶/cu.mm; ESR 58 mm/h; CRP 30.9 mg/L. Evaluation of sono-graphic imaging revealed inflammation of the lower urinary tract. Kidney stones were ruled out by computed tomography (CT) scan.

She had no history of infection with mycobacteria and was negative for HIV and other immunosuppressive diseases. The acid fast staining of three consecutive 24-hour mid-stream clean catch urine specimens were positive, and the culture on LJ medium yielded pure rapidly growing *mycobacterium*. The patient became afebrile on amikacin therapy.
The clinical isolates, namely M71 (case 1), M89 (Case 2), and M137 (case 3), were sent to our research laboratory in a period of two to three months after initial isolation for definitive identification. The isolates were subjected to preliminary identification and susceptibility to common antimycobacterial agents for rapidly growing mycobacteria according to standard procedures.1,2 They were then subjected to molecular identification which included PCR restriction fragment length polymorphism analysis (PRA algorithm) of a 644-bp fragment of the hsp65 and direct sequencing analysis of almost full length of 16S rDNA and 16S–23S internal transcribed spacer (ITS) as well as partial sequencing of hsp65 and rpoB genes as previously described.3–7

GenBank accession numbers for 16S rDNA, ITS, rpoB, and hsp65 sequences as a representative isolate determined in this study strain M137 are GU142926, HM536981, HM536982 and HM536983, respectively.

Based on phenotypic characteristics, the isolates were non-pigmented mycobacterial species growing at 25˚C and 37˚C but not at 42˚C. They grew on LJ medium with 5% NaCl and MacConkey agar without crystal violet, were positive for semi-quantitative and heat-stable catalase, nitrate, tween hydrolysis, and iron uptake tests; and were negative for niacin, urease and D-sorbitol tests. They were susceptible to amikacin, streptomycin, clarithromycin, doxycycline, sulfamethoxazole, imipenem and ciprofloxacin.

In PRA analysis all isolates produced fragments of 480/199, 270/161/117 and 254/97 base pairs in AvaII, HpaII, and HphI digestion, respectively. The PRA profiles were distinct from those of previously characterized mycobacteria (Figure 1).3 The reproducibility of PRA assay was evaluated by including M. fortuitum (ATCC 49403) as a positive internal control. A tube without DNA template was also included in all PCR and PRA reactions as a negative control. Restriction fragments shorter than 97 bp were not considered to reduce confusion with primer-dimer bands.3

The 16S rDNA sequence (1459 bp) of three isolates were identical and showed the highest similarity of 99.86% with that of M. conceptionense and 99.59% with those of type strains of M. fortuitum, M. porcinum and M. farcinogenes. These values correspond to two and six nucleotide differences. The hypervariable signature sequences of the investigated isolates were 100% identical to those of the type strain of M. conceptionense (Figure 2).

The hsp65 sequences of the isolates were identical and showed 98.53%, 97.96% and 97.42% similarities with those of the type strains of M. conceptionense, M. farcinogenes and M. fortuitum, respectively. These values correspond to five, eight and nine nucleotide differences. The ITS sequences of the isolates were identical and showed 100%, 93.16% and 71.11% similarities with those of the type strains of M. conceptionense, M. senegalense and M. fortuitum, respectively. These values correspond to 0, 16 and 65 nucleotide differences. The rpoB sequences of the investigated isolates were identical and showed 98.84% and 95.71% similarities with those of the type strains of M. conceptionense and M. fortuitum, respectively. These values correspond to 5 and 29 nucleotide differences.

Discussion

M. conceptionense was first isolated from a wound infection of a healthy young woman and described as a novel rapidly growing mycobacterium in 2006.8 To the best of our knowledge, there have been only two further reports on isolation of the organism from clinical specimens in the world literature.9,10
Definitive identification of the Iranian isolates as *M. conceptionense* was achieved by taking into account phenotypic and molecular characteristics and in particular the sequencing of key genetic markers. The etiologic role of the isolates might be inferred from the fact that acid fast bacilli were microscopically observed in two out of three different clinical specimens. Furthermore, all three isolates were recovered from pure culture. Also, no such isolate was made in our laboratory in the past and during the same period of time. On the other hand, the isolates were made from more than one diseased specimen in the patients.

In summary, the present study together with the previous findings show that *M. conceptionense* is capable of infecting a variety of organs in humans.

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