Mycobacterium arupense: A review article on an emerging potential pathogen in the Mycobacterium terrae complex

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1. Introduction

Nontuberculous mycobacteria (NTM) are, by definition, mycobacteria unrelated to the Mycobacterium tuberculosis complex and Mycobacterium leprae. NTM are an ineluctable part of the natural environment. Isolates of NTM have been recovered from soil, surface and tap water, animals and even food products [1,2]. More than 170 species of NTM have been described in the literature [3]. The Mycobacterium terrae complex (MTC) is a group of environmental mycobacteria most commonly implicated as causative agents in bone and joint infections arising from direct environmental inoculation injuries [4]. Prior to the introduction of more sophisticated means of species identification, the MTC was initially identified phenotypically and comprised of non-chromogenic, slowly-growing species – M. terrae, and M. nonchromogenicum [5]. The advent of gene sequencing leads to further delineation and new species recognition within the MTC. Currently, the MTC has expanded to include other genetically similar mycobacteria: M. kumamotonense [6], M. senuense [7], M. paraterre [8], Mycobacterium strain JDM601 [9], M. engbaekii [10], M. longobardum [10], M. heraklionense [10], M. virginiense [4], and M. arupense [11].

Since its first isolation in 2006 by Cloud et al., at least ten clinical reports (Table 1) of M. arupense and numerous more reports on its isolation from environmental and clinical samples have been reported in the English literature. In a 2016 study by Varsredy et al., 26 clinical isolates associated with tenosynovitis or osteomyelitis from 1984 to 2014, previously diagnosed by nonmolecular methods as M. terrae and M. nonchromogenicum, underwent molecular genetic testing by 16S ribosomal RNA (16S rRNA), heat-shock protein 65 (hsp65), and RNA polymerase subunit B (rpoB) sequencing [4]. In this report, 10 of the mycobacteria were now identified as M. arupense. The authors also evaluated 14 previously published cases of tenosynovitis due to MTC, and identified 4 of these as a 100% identity with M. arupense type strain, and an additional 4 with greatest identity with M. arupense type strain. These findings highlight the need for species identification using gene sequencing methods, and suggests that a significant portion of previously MTC related infections diagnosed by culture and phenotypic means could be now classified as possible M. arupense.

1.1. Mycobacterium arupense

M. arupense was first described in 2006 by Cloud et al. at the Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology from a human tendon specimen [11]. The mycobacterium is phenotypically indistinguishable from M. nonchromogenicum or M. terrae within the MTC. The mycobacterium has been noted to cause a variety of clinical presentations such as disseminated infection in an immunocompromised host [12], osteoarticular joint infection [13], pulmonary infection [14–16] and, most commonly, tenosynovitis [17–22].

1.2. Epidemiology

M. arupense has been noted to be commonly isolated from the environment and clinical samples. In the environment, the organism has been isolated from surface water [21], soil [1], fish tanks [23], animal urine samples [1], and bioaerosols in duck houses [24]. Interestingly, the mycobacterium has a predilection for small rodents such as voles, house mice and the Gambian pouched rat [1]. Despite its wide presence in the environment and in clinical isolates, M. arupense is considered a rare NTM infection. The true epidemiology of M. arupense infection is unknown, and will be better elucidated with more common use of sequencing methods for identification of NTM infections.

2. Microbiology

2.1. Phenotypic and biochemical characterization of M. arupense

The organism grows slowly on Lowenstein–Jensen agar at 37 °C and rapidly at 30 °C, taking 10–12 days and 5–7 days respectively [11]. Although generally nonchromogenic, the mycobacterium has been noted to have the potential to form light pink-colored colonies after 3–4 weeks on Middlebrook 7H10/7H11 solid medium biplates [20]. It does not have any accumulation of niacin, iron uptake or urease activity.
2.2. Molecular testing and identification of *M. arupense*

*M. arupense* has a unique genetic signature in the helix 18 of the 16S rRNA gene, a two-nucleotide insertion that is uniform across the remainder of the members of the MTC. This simple two-nucleotide insertion remains one of the most important supportive features to help determine whether a mycobacterium is related to the MTC [25].

Prior to *M. arupense* description, partial 16S rRNA gene sequencing did not distinguish *M. arupense* from the previously known members of MTC. Once the nearly full 16S rRNA gene sequencing was performed, the results showed a close match to *M. nonchromogenicum* (99.6% identity) [11]. In addition, sequencing of the 16S-23S internal transcribed spacer (ITS) region and the *hsp65* gene confirmed, that *M. arupense* was a novel species, as described by Cloud et al. Cloud et al. noted that the ITS data for *M. arupense* showed intra-species heterogeneity, and ITS sequencing for identification of *M. arupense* and other members of the MTC is not recommended. In this paper, the *hsp65* gene (401 nt region) revealed a sequence significantly different from those of strains of the *M. terrae*. It is most closely related to *M. nonchromogenicum*, with 25 nt mismatches [11]. The improved sequencing technology led to the identification of the novel species of mycobacterium, and following this identification came clinical descriptions of case reports and case series of infections attributed to the newly described mycobacterium.

3. Clinical manifestations

*M. arupense* has been reported to cause tenosynovitis, osteoarticular joint infection, pulmonary infection, and disseminated disease. A summary of all reported PubMed indexed cases of *M. arupense* infection is included in Table 1.

3.1. Tenosynovitis and osteoarticular joint infection

*M. arupense* tenosynovial and osteoarticular infections typically have quite an insidious course, presenting as long-standing pain and swelling [17–22]. They are difficult to diagnose with symptoms persisting for months [17–19,21,22] to years [20] prior to definitive diagnosis in many of the described cases. History may be suggestive of direct penetrating injury to the area affected [19,21], and trauma to affected area is commonly reported [14,17,20], and upper extremities the common site of infection [17–22]. Treatment involves surgery in form of tenosynovectomy, or bone resection in case of osteoarticular involvement, and prolonged combination antimicrobial treatment. Histologic examination of the tissue may or not reveal granulomatous changes or acid-fast bacilli depending on organism burden. The mycobacterium grows slowly on Lowenstein–Jensen agar, sometimes requiring extended incubation (i.e. several weeks) for a positive culture. Once diagnosed, combination antimicrobial regimen is often administered in conjunction with surgical intervention. The prognosis is usually favorable, with improvement or complete resolution of symptoms if appropriate therapy is instituted. Duration of therapy varies significantly in the outlined reports as result of co-morbidities, likely aggressiveness of surgical intervention, clinical response to treatment and the immune status of the patient (Table 1).
| Study and year | Percent susceptible % (No. of susceptible isolates/No. of total isolates)* |
|---------------|--------------------------------------------------------------------------------|
|               | Amikacin | Ciprofloxacin | Clarithromycin | Ethambutol | Linezolid | Moxifloxacin | Rifampin | Rifabutin | TMP/SMX | Doxycycline | Minocycline | Streptomycin** |
| Cloud et al. 2008 | 75 (6/8) | 0 (0/8) | 87.5 (7/8) | 100 (8/8) | 0 (0/8) | 0 (0/8) | 0 (0/8) | 100 (8/8) | 25 (2/8) | NA | NA | 0 (0/4) |
| Neonakis et al. 2010 | 100 (1/1) | 0 (0/1) | 100 (1/1) | 100 (1/1) | 0 (0/1)** | 0 (0/1) | 0 (0/1) | 100 (1/1) | NA | 0 (0/1) | NA | NA | 100 (1/1) |
| Senda et al. 2011 | NA | 0 (0/1) | 100 (1/1) | NA | 0 (0/1) | NA | 100 (1/1) | NA | NA | NA | NA | 0 (0/1) |
| Heidarieh et al. 2013 | NA | 0 (0/2) | 100 (2/2) | 0 (0/2) | 0 (0/2) | 0 (0/2) | 0 (0/2) | 100 (2/2) | NA | NA | NA | 0 (0/2) |
| Beam et al. 2014 | 62.5 (25/40) | (0/40) | 97.5 (39/40) | 62.5 (25/40) | 0 (0/40) | 0 (0/40) | 20 (0/40) | 97.5 (39/40) | 45 (16/40) | 21.7 (5/23) | 33 (2/6) | 0 (0/8) |
| Lee et al. 2014 | 0 (0/1) | 100 (1/1) | 100 (1/1) | 0 (0/1) | 0 (0/1) | 0 (0/1) | 0 (0/1) | NA | NA | NA | NA | NA |
| Vasireddy et al. 2016 | 33.3 (3/9) | (0/10) | 100 (10/12) | 100 (8/8) | 10 (1/1) | 100 (10/12) | 0 (0/10) | 100 (9/9) | 62.5 (5/8) | 0 (0/7) | 0 (0/3) | NA |
| Lopez et al. 2016 | 100 (1/1) | (0/1) | 100 (1/1) | 0 (0/1) | 0 (0/1) | 0 (0/1) | 17.2 (11/64) | 98.4 (59/60) | 43.1 (25/58) | 16.7 (5/30) | 22.2 (2/9) | 6.3 (1/16) |
| Sum of all isolates | 60 (36/60) | 0 (0/64) | 98.4 (63/64) | 98.4 (63/64) | 58.3 (35/60) | 0 (0/62) | 17.2 (11/64) | 98.4 (59/60) | 43.1 (25/58) | 16.7 (5/30) | 22.2 (2/9) | 6.3 (1/16) |

*For our breakpoints in drug sensitivity testing, data for *M. kansasii* and *M. marinum* were used as surrogate. Interpretive criteria for susceptibility and resistance is based on CLSI document M24-A2 [26]. Neonakis et al. performed Epsilometer testing to determine MICs. Lopez et al. and Senda et al. reported MICs without describing the method of DST. All other studies performed broth microdilution.

**Streptomycin is a non-CLSI standard drug.

***Susceptibilities were performed on levofloxacin and not moxifloxacin. Results suggested resistance. NA—not available.
3.2. Pulmonary infections

The diagnosis of definite NTM pulmonary infection requires exclusion of other possible disorders and compelling evidence of clinical and microbiologic findings of pulmonary infection, in accordance with the published American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) guidelines [2]. The criteria should be applied to all NTM cases, despite possible difference in virulence of the organisms.

In 2015, Al Hamal et al. prospectively reviewed a total of 53 cancer patients with positive cultures of M. arupense from respiratory specimens – 28 patient specimens were collected from BAL/BW, 24 from at least one sputum sample, and 1 from pleural fluid. The mycobacterium was identified by 16S rRNA and hsp65 gene sequencing. Per ATS/IDSA criteria, seven patients met the criteria for a definitive diagnosis of M. arupense infection, fourteen as probable infection, and twenty-nine as possible infection. Out of the seven definitive cases, two had received treatment. Out of the fourteen probable cases, four had received treatment. Lastly, out of the twenty-nine probable cases, seven had received treatment. Treatment was based on antimicrobial susceptibility and included a regimen of clarithromycin, rifabutin and ethambutol. A total of 13 patients received treatment and 40 patients did not receive treatment. Duration of treatment varied between 3 and 295 days with a median of 38 days. No significant difference in outcomes was observed with or without treatment. The investigators concluded that isolation of M. arupense from respiratory specimens, even if consistent with ATS/IDSA guideline definition is still likely to be representative of respiratory sample contamination, rather than true infection [16].

Neonakis et al. in Greece published a case report describing a possible infection by M. arupense. The patient presented with signs and symptoms suggestive of acute pneumonia and had a chest radiograph which showed bilateral consolidations. The case fulfilled "probable" diagnostic criteria as per ATS/IDSA guidelines. The patient clinically improved on antimicrobial therapy [14].

Similarly, Heidari et al. in Iran described another case of pulmonary infection in an HIV positive individual with CD4+ count of 80, with a history significant for pulmonary tuberculosis who presented with chronic cough and mild fevers. Chest radiograph was found to be unremarkable. Multiple sputum cultures and a BAL specimen were positive with what was later identified to be M. arupense by sequencing. The patient improved on antimicrobial therapy and cART [12].

Varghese et al. includes a case of M. arupense pulmonary infection while conducting a study of emerging rare species of NTM in Saudi Arabia. The patient suffered from Hodgkin’s lymphoma, HIV, and COPD. The mycobacterium was isolated from BAL and diagnosis was confirmed by 16S rRNA and rpoB testing. It was regarded as a clinically relevant infection as per ATS/IDSA guidelines. Antimicrobial therapy with clarithromycin, rifampin and ethambutol was initiated. Further details regarding the case are unavailable [15].

Literature shows M. arupense [1,4,11,16,20], among other nontuberculous mycobacteria such as M. gordonae [2] and M. mucogenicum [2], as a commonly isolated organism from respiratory specimens. The cases of possible pulmonary infection due to M. arupense need to be weighed against the likelihood of mere contamination.

3.3. Other infections

There has only been one case reported of disseminated infection, described in a HIV positive individual. He was markedly immunocompromised with a CD4+ count of 18 at presentation. Two separate blood cultures were notable for delayed growth of M. arupense. The patient was initiated on combination therapy for disseminated MAC with clarithromycin, ethambutol and rifabutin pending mycobacterial identification and susceptibilities, however patient succumbed to his disease weeks after initiating treatment. He was diagnosed posthumously with detection of M. arupense by complete 16S rRNA, partial hsp65 gene, and partial rpoB gene sequencing [12]. In vitro antimicrobial susceptibility testing revealed that the isolate was susceptible to all three antimicrobials administered and resistant to quinolones, linezolid, rifampin, and streptomycin [12].

4. Susceptibility testing

M. arupense isolated from infectious and non-infectious isolates are generally susceptible to rifabutin, clarithromycin, ethambutol, and partially susceptible to trimethoprim-sulfamethoxazole (TMP-SMX). Isolates appear to be resistant to rifampin, tetracycline, and quinolones (Table 2).

5. Discussion

Due to the small number of reported cases, currently no standard guidelines exist for treating M. arupense infection. Antimicrobial therapy is typically determined by in vitro susceptibility testing and combination therapy continued for the duration of treatment. Although there is a lack of data which would show correlation with clinical outcomes, treatment duration is varied, with reports ranging from as little as 6 months [17] to as many as 14 months [18]. Most of treatment and duration literature is based on bone and joint infections, and is limited for other organs system involvement. The majority of patients suffering from localized tenosynovitis or osteo-articular M. arupense infection have resolution of infection with combination of surgical and antimicrobial therapy. In cases of disseminated infection, with single report identified in literature, fatal outcome is reported. Empiric therapy with a three-drug regimen (combination of rifabutin, clarithromycin, and ethambutol) seems appropriate for non-pulmonary, non-disseminated M. arupense infection while awaiting susceptibility data [20]. In terms of M. arupense pulmonary infection, current data suggests that the mycobacterium is most commonly a contaminant or that the infection is self-limiting in nature. For disseminated disease, as this tends to occur in immune-compromised individuals, prolonged treatment is recommended with at least 12 months of antimicrobial therapy with clinical and microbiological response. Treatment should include a 6–12 month course of antimicrobial therapy after immunologic reconstitution in the setting of HIV/AIDS.

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Conflict of interest

none.

Supplementary materials

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