Clinical manifestations, antibiotic susceptibility and molecular analysis of *Mycobacterium kansasii* isolates from a university hospital in Taiwan

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**Objectives:** *Mycobacterium kansasii* causes a variety of infections. Although previous reports on the prognosis of antimicrobial therapy have been mostly satisfactory, problems involving treatment failure or relapse have been encountered. The purpose of this study was to establish a relationship between the clinical treatment outcomes of *M. kansasii* infections and bacterial drug susceptibility, and their clonality.

**Methods:** A total of 37 *M. kansasii* clinical isolates and clinical information on 34 patients were retrospectively collected in a tertiary medical centre in Taiwan. Bacterial drug susceptibility was determined by the microdilution method. The phylogenetic relationship was analysed by PFGE analysis.

**Results:** Results of PFGE typing revealed a major cluster (cluster I) and eight other divergent patterns. Two/three strains leading to treatment failure were also multidrug resistant and belonged to cluster I.

**Conclusions:** A relationship between high drug resistance and genetic relatedness of some *M. kansasii* strains was established. This was associated with clinical treatment failure.

Keywords: non-tuberculous mycobacteria, susceptibility tests, multidrug resistance, clonality

**Introduction**

*Mycobacterium kansasii* is a slow-growing non-tuberculous mycobacterium (NTM).¹ Current treatment of *M. kansasii* infection is based on the criteria defined by the American Thoracic Society (ATS) and involves long-term antibiotic therapy.² Although previous reports noted low failure rates in treatment of *M. kansasii* infection,² treatment failure or relapse did occur in our clinical experience. So far, no reports have assessed the relationship between characteristics of *M. kansasii* strains and clinical treatment outcomes in Taiwan. In the present study, we set out to understand the recent situation regarding *M. kansasii* infections in Chang Gung Memorial Hospital, by reviewing patients’ medical records and treatment history, assessing the drug susceptibility of clinical isolates, evaluating the clonal spreading possibility and comparing these characteristics with the clinical outcomes.

**Patients and methods**

**Clinical review, case definition and classification of outcomes**

The study was conducted on 37 *M. kansasii* clinical isolates that had been retrospectively collected from 36 patients from August 2000 to January 2004 at a tertiary hospital medical centre, Chang Gung...
Memorial Hospital. Patients had been diagnosed as having M. kansasii pulmonary infection on the basis of the criteria of the ATS.2

Bacterial strains and chemicals
All M. kansasii isolates were collected from the Department of Laboratory Medicine, Chang Gung Memorial Hospital. Bacteria were identified by hsp65 gene polymorphism analysis, as previously described.4 The reference strain M. kansasii ATCC 12478 was from Union Biotech Company (Taiwan). Chemicals were from Sigma (USA).

Drug susceptibility test
The MICs of several antibiotics for M. kansasii were determined by a microdilution assay according to the standard protocol of the CLSI (formerly the NCCLS).5 Drugs tested included the first-line antibiotics isoniazid, rifampicin and ethambutol (Sigma), and the second-line antibiotics clarithromycin (Abbott), moxifloxacin (Bayer), rifabutin, streptomycin, amikacin, ciprofloxacin and sulfamethoxazole (Sigma). Susceptible and resistant breakpoints were determined according to the CLSI standard.5

Molecular typing by restriction digestion PFGE
All M. kansasii isolates were subtyped by restriction digestion PFGE as described by Zhang et al.6 Isolates were grouped as clonal if six or fewer band differences were observed.7 Isolates with more than six distinct bands were designated as unrelated (non-clonal).

Results and discussion
Demographic data and clinical aspects
A total of 37 M. kansasii strains were isolated from 36 patients. Among these, 34 complete medical charts were available for review. Thirty patients had evidence of pulmonary infection, three patients had soft tissue infection and one patient had disseminated infection (Table 1). The mean (±SD) age of patients was 59.9 ± 19.7 years. Twenty-four (70.6%) of the 34 patients were male. Besides M. kansasii infection, the most common co-morbidity factor was chronic obstructive pulmonary disease (9/34; 26.5%), followed by malignancy (6/34; 17.6%). Both immunocompetent and immunocompromised patients had been infected by M. kansasii, with a relative ratio of 52.9% (18/34) and 47.1% (16/34), respectively. The most common presenting symptoms were cough (17/34; 50%), followed by dyspnoea on exertion (10/34; 29.4%), fever (10/34; 29.4%) and weight loss (10/34; 29.4%). All patients were treated with isoniazid (5 mg/kg/day), rifampicin (10 mg/kg/day) and ethambutol (20 mg/kg/day), with or without clarithromycin (600 mg/day). During treatment, one patient had persistent and progressive soft tissue pus discharge. Three patients died, including one with AIDS, one having pneumonia with septic shock and the other having disseminated infection. The importance of M. kansasii infection was also reported from another medical centre in Taiwan, in which mortality was reported to be highest (100%) in patients with disseminated NTM infections.8

Drug susceptibility pattern is related to clinical significance
The quality control strain (M. kansasii ATCC 12478) was resistant to streptomycin and was susceptible to all of the other nine drugs. The susceptibility of the distinct clinical isolates is summarized in Table 2. A trend was found that the 37 M. kansasii strains were moderately resistant to isoniazid (27/37; 73% susceptibility) and rifampicin (29/37; 78.4% susceptibility), and highly resistant to ethambutol (10/37; 27% susceptibility). Also, six isolates (6/37; 16.2% susceptibility) were resistant to isoniazid, rifampicin and ethambutol, and six other strains were resistant to both isoniazid and rifampicin. The four strains causing treatment failure (strain nos 4, 14, 23 and 31) were resistant to one or more of the first-line antimicrobial agents rifampicin, isoniazid and/or ethambutol. Notably, two strains isolated from the same patient from different timepoints showed a rifampicin-susceptible and a high-level rifampicin-resistant (MIC=16 mg/L) phenotype, respectively, suggesting induced or selected drug resistance. For the second-line antibiotics, M. kansasii strains were susceptible to rifabutin (36/37; 97.3% susceptibility), clarithromycin (37/37; 100% susceptibility), streptomycin (35/37; 94.6% susceptibility) and amikacin (36/37, 97.3% susceptibility). Moderate resistance to ciprofloxacin (26/37; 70.3% susceptibility) and sulfamethoxazole (30/37; 81.1% susceptibility) was also observed. Comparatively, strains showed a higher resistance pattern to moxifloxacin (22/37; 59.5% susceptibility).

While high drug resistance to first-line antibiotics may be one of the contributing factors leading to treatment difficulty in M. kansasii infection, previous reports indicated low rifampicin resistance in many other countries.9 However, a high rifampicin resistance rate (12%) was also reported by da Silva Telles et al.10 in Brazil. Besides, while M. kansasii strains were basically susceptible to second-line antibiotics in Western countries,9 resistance to ciprofloxacin and sulfamethoxazole, and to a high degree resistance to moxifloxacin, were also observed in this study (Table 2). This is similar to a report from da Silva Telles et al.,10 who observed a high ciprofloxacin resistance rate (66%) in Brazil. Thus, the phenomenon of high resistance to
**Table 2.** *In vitro* drug susceptibilities of 37 *M. kansasii* isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Resistance (%)</th>
<th>Susceptibility (%)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>27.0</td>
<td>73.0</td>
<td>1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>21.6</td>
<td>78.4</td>
<td>0.5</td>
</tr>
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<td>Rifabutin</td>
<td>2.7</td>
<td>97.3</td>
<td>0.125</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>73.0</td>
<td>27.0</td>
<td>8</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0</td>
<td>100.0</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>29.7</td>
<td>70.3</td>
<td>1</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>40.5</td>
<td>59.5</td>
<td>2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>5.4</td>
<td>94.6</td>
<td>4</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2.7</td>
<td>97.3</td>
<td>8</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>18.9</td>
<td>81.1</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 1.** Phylogenetic analysis of *M. kansasii* isolates. Chromosomal DNA extracted from *M. kansasii* cells was digested by restriction endonuclease AseI followed by separation by PFGE with a CHEF Mapper system (Bio-Rad, USA). Gels were stained with ethidium bromide, photographed under UV light illumination and analysed using Scanalytics software (Fairfax, USA). Patterns with at least one PFGE band difference were considered significant. Isolates were grouped as clonal if six or fewer band differences were observed; otherwise, unrelated (non-clonal). ATCC, *M. kansasii* ATCC 12478 as a standard; open circle, treatment failure case; asterisk, isoniazid- and rifampicin-resistant case.
ethambutol, rifampicin, isoniazid and ciprofloxacin is similar to that reported in Brazil and significantly different from that reported in Western countries.9–11

We hypothesize that the high drug resistance rate is associated with the widespread use of antitubercular drugs due to the high prevalence of tuberculosis in Taiwan.

**Cluster I strains are identified worldwide**

The clonality of 32 *M. kansasii* strains was successfully analysed by PFGE. Twenty-two isolates showed a band pattern with six or fewer distinct bands, and were grouped as genetically related cluster I, including three subclusters (Figure 1).7 The remaining 10 isolates were divergent in PFGE patterns and were classified as clusters II (3/32), III (1/32), IV (1/32), V (1/32), VI (1/32), VII (1/32), VIII (1/32) and IX (1/32).

A major *M. kansasii* clone has spread throughout Japan, Europe and the USA.6,12,13 Using a similar approach, we identified 22 cluster I strains (8 Ia, 6 Ib and 8 Ic, 22/32; 68.8% of isolates) showing a conserved pattern indistinguishable from the common pattern seen in French isolates (Ia),12 42% of isolates (Aa) characterized in the USA7 and with only one band difference from the common pattern (type M) in Japan.13

**Drug resistance and clonality related to treatment difficulty**

Of the six strains showing both isoniazid and rifampicin resistance phenotypes, the PFGE pattern of four strains was available. They were grouped into clusters Ib, Ic and VIII (Figure 1; labelled with asterisks). Further analysis indicated that three cluster I strains resulted in treatment failure, indicating that strains resulting in treatment difficulty were mostly isoniazid and rifampicin resistant (2/3) and genetically related (3/3). In conclusion, the strains resulting in treatment difficulty that we encountered were phylogenetically related to those reported in many other countries. For future studies, continuous monitoring of drug resistance patterns and clonality of *M. kansasii* strains is warranted.

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**Transparency declarations**

None to declare.

**References**