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Short Communication

Drug susceptibility profile of *Mycobacterium kansasii* clinical isolates from Brazil

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ABSTRACT

Objectives: Mycobacterium kansasii (*M. kansasii*) pulmonary infection can cause disease with clinical and radiological features similar to tuberculosis. Failure to treat *M. kansasii* infection is usually associated with resistance; to increase the chance of successful treatment it is important to identify the species and know the susceptibility profile. This study aimed to evaluate the antimycobacterial susceptibility profiles of *M. kansasii* isolates from Brazil.
Methods: Sixty-nine *M. kansasii* isolates from 69 patients were identified by partial sequencing of the *hsp65* gene, and their susceptibility profiles were analysed by minimal inhibitory concentration (MIC) assays.
Results: From 69 isolates, 68 showed susceptibility to clarithromycin, amikacin, and moxifloxacin. Most strains showed high rates of resistance to trimethoprim–sulfamethoxazole and ciprofloxacin. Resistance to rifampicin and ethambutol was found in 12% and 25% of isolates, respectively.
Conclusions: Worrying results were found regarding susceptibility to some drugs used as first-line agents in the treatment of diseases caused by *M. kansasii*.

1. Introduction

Mycobacterium kansasii (*M. kansasii*) is a photochromogenic mycobacteria, first described by Buhler and Pollack in 1953, whose pathogenic potential has been increasingly recognised, especially in pulmonary disease. Recent studies indicate that *M. kansasii* could be the first or second cause of non-tuberculous mycobacteria (NTM) infection in some countries, after *Mycobacterium avium* complex species [1–5]. An increased infection rate caused by *M. kansasii* has been reported in Brazil [6].

Mycobacterium kansasii pulmonary infection can cause disease with clinical and radiological features similar to tuberculosis (TB), and the majority of patients present with symptoms like cough, chest pain, dyspnoea, and nonmassive haemoptysis [7,8].

The drugs generally used for treating diseases caused by *M. kansasii* are rifampicin, isoniazid and ethambutol. Because rifampicin is a critical component for successful treatment of infections caused by *M. kansasii,* chemotherapy becomes problematic when resistance to these drug occurs, usually leading to treatment failure. In these cases, it is vital to determine the drug susceptibility profile for appropriate treatment [9,10]. In vitro drug susceptibility tests are standardised for only a few species of NTM, such as *M. kansasii* [10]. This species seems to present a clinical response to antimicrobial susceptibility correlated with the in vitro activity [7].

The current study aimed to analyse the antimycobacterial drug susceptibility profiles of *M. kansasii* clinical isolates, since such studies are very scarce in Brazil.

2. Material and methods

2.1. Mycobacterial isolates

Sixty-nine *M. kansasii* isolates from 69 patients were included in this study. Patients fulfilled the microbiological diagnosis criteria of NTM pulmonary disease in accordance to American Thoracic Society guidelines [7]. The strains were isolated from

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Table 1
In vitro susceptibility to antibiotics for 69 *M. kansasii* isolates.

Antimicrobial agent	Breakpoint (CLSI)	MIC (μg/mL) range in triplicates	Total (%)	
			Susceptibility	Resistance
Ethambutol	>4	0.5–8	17 (25)	52 (75)
Isoniazid	– [a]	0.156–2.5	–	–
Rifampicin	>1	0.062–4	61 (88)	8 (12)
Streptomycin	– [a]	0.312–10	–	–
Moxifloxacin	>2	0.062–0.125	69 (100)	0
Clarithromycin	>16	0.25–2	68 (99)	1 (1)
Amikacin	>32	1–8	69 (100)	0
Trimethoprim–sulfamethoxazole	>2/38	8/152–16/304	3 (4)	66 (96)
Ciprofloxacin	>2	1–16	34 (49)	35 (51)

– [a]There are no breakpoints established to determine the susceptibility and resistance of NTM to these antibiotics.
Abbreviations: CLSI, Clinical and Laboratory Standards Institute; NTM, non-tuberculous mycobacteria.

pulmonary sites and obtained between 2008–2016 from the collection of the National Reference Laboratory for Tuberculosis, Centro de Referência Professor Hélio Fraga (ENSP/Fiocruz), in Rio de Janeiro, Brazil. All isolates were identified as *M. kansasii* by partial sequencing of the *hsp65* gene.

2.2. Antimycobacterial drug susceptibility testing

Antimycobacterial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. Nine antimycobacterial agents (streptomycin, isoniazid, ethambutol, rifampicin, amikacin, clarithromycin, moxifloxacin, trimethoprim-sulfamethoxazole, and ciprofloxacin) were tested to determine susceptibility following the breakpoints recommended by the CLSI (Table 1) [10].

3. Results

In order to assure consistency of results, triplicates were used for the MIC determination of all the studied isolates. The results of the same sample in all cases showed 100% concordance for resistance or susceptibility, with small variations in their values (Table 1).

Two of the nine tested antimicrobials – streptomycin and isoniazid – have no cut-off point established by the CLSI, so it was not possible to classify the samples as susceptible or resistant. However, the MIC values (μg/mL) of the triplicate samples of each strain ranged from 0.156–2.5 μg/mL for isoniazid and 0.312–10 μg/mL for streptomycin. From the 69 strains on which MIC tests were carried out, 17 (25%) showed resistance to ethambutol, eight (12%) to rifampicin, and one strain (1%) showed resistance to clarithromycin. No resistance for amikacin and moxifloxacin was observed. Ciprofloxacin and trimethoprim–sulfamethoxazole showed high rates of resistance (51% and 96%, respectively) (Table 1).

4. Discussion

The susceptibility patterns for all antimycobacterial agents were precisely determined and showed high reproducibility in all strains used in this study.

Brazil is a country with a high rate of TB [11]. Once patients are diagnosed as positive acid-fast bacilli, established TB treatment is initiated. As such, species-level identification rarely occurs and patients with *M. kansasii* disease are initially treated for TB. The treatment regimen for TB includes four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol). The three most effective regimens for the elimination of *M. kansasii* infection, as recommended by the American Thoracic Society, are rifampicin and ethambutol in combination with other drugs, depending on the treatment regimen used. Due to exposure to these two drugs in TB treatment, these isolates can acquire resistance, mostly to rifampicin and ethambutol, when subsequently treated for *M. kansasii*. The current study found resistant profiles to these two drugs in analysed isolates. The same was reported in studies conducted in other countries. Very high rates of rifampicin resistance (around 50%) were found in China and Iran. As in the current study, a high rate of resistance to ethambutol (20.5%) was also found in China. Studies conducted in São Paulo, Brazil and Taiwan showed extremely high rates of resistance to this drug (95% and 73%, respectively) [5,12–14]. However, in the United Kingdom, The Netherlands and Spain, the rates of resistance of *M. kansasii* to these two antimicrobials were low [15–17].

Clarithromycin showed excellent activity against tested strains (99%), in accordance with previous studies that also showed a high level of in vitro susceptibility (99–100%) [12–17]. High resistance rates of *M. kansasii* to clarithromycin have only been reported in China (20.5%). This high prevalence of clarithromycin-resistant *M. kansasii* isolates was attributed to the misuse of macrolides in China [5].

In case of treatment failure associated with rifampicin resistance, the CLSI recommends that secondary antimicrobial agents should be tested [10]. The secondary agents amikacin and moxifloxacin have been shown to be very promising antimicrobials against *M. kansasii*, since studies in many countries of the world report good in vitro activity [6,12–15,17]. The same occurred in the current study, with 100% of the tested isolates displaying sensitivity to these drugs.

Notably, the current study found a very high rate of resistance to ciprofloxacin and trimethoprim–sulfamethoxazole. Rates similar to these were also obtained in a study conducted in the state of São Paulo, Brazil, where 66% of strains showed resistance to ciprofloxacin. In Taiwan and Iran, resistance was also observed in many isolates [12–14]. Despite using these antimicrobials to mostly treat rapidly growing mycobacteria, the CLSI recommends that they should be tested as secondary agents for *M. kansasii*. However, there is a high rate of resistance associated with them, especially in Brazil, probably caused by its widespread use for controlling other microorganisms. This result is in contrast with those observed in The Netherlands, United Kingdom and China, which report low rates of resistance to ciprofloxacin and trimethoprim–sulfamethoxazole [16,17].

5. Conclusions

The results obtained in this study show that clarithromycin, amikacin and moxifloxacin are effective drugs against *M. kansasii* in vitro. The high resistance rates to rifampicin and ethambutol are of concern, mainly because rifampicin is the main drug used in

M. kansasii infection treatment, and both are used as first-line agents. The data suggest that more studies should be conducted to evaluate the susceptibility profile of *M. kansasii*, due its importance in the treatment of these infections.

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Competing interests

None.

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Not required.

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