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Clinical features of patients with bacteraemia caused by *Mycobacterium avium* complex species and antimicrobial susceptibility of the isolates at a medical centre in Taiwan, 2008–2014

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ABSTRACT

Advanced molecular typing methods have greatly expanded the taxonomy of *Mycobacterium avium* complex (MAC) species; however, little is known about the epidemiology and clinical features of bacteraemia caused by different MAC species. In this study, the clinical characteristics of patients treated for MAC bacteraemia in a tertiary-care centre in northern Taiwan during 2008–2014 were investigated. Isolates were identified to species level by *rpoB* gene and 16S–23S rRNA internal transcribed spacer region sequencing. Among 30 patients with bacteraemia due to MAC, the majority ($n = 26$) had concomitant human immunodeficiency virus (HIV) infection. Of the 30 blood isolates obtained from patients, 24 were *M. avium* subsp. *hominissuis*, 4 were *Mycobacterium colombiense* and 2 were *Mycobacterium intracellulare*. All four *M. colombiense* isolates were from HIV-infected patients. Bacteraemia due to *M. colombiense* was associated with higher 30-day mortality than bacteraemia due to *M. avium* subsp. *hominissuis* [2/4 (50%) vs. 1/24 (4%); $P = 0.045$, Fisher's exact test]. All four *M. colombiense* isolates were susceptible to clarithromycin, moxifloxacin and linezolid. Among the five patients who received ethambutol treatment and four patients who received fluoroquinolone treatment for various durations between positive MAC cultures, two and three patients, respectively, had isolates with significantly increased (≥4-fold) ethambutol and fluoroquinolone minimum inhibitory concentrations. *M. colombiense* was the second leading causative pathogen of MAC bacteraemia, comprising 15% of all MAC isolates obtained from HIV-positive patients. Monitoring the susceptibility of MAC isolates to ethambutol and fluoroquinolones is warranted in patients with persistent MAC bacteraemia.

1. Introduction

Species of the *Mycobacterium avium* complex (MAC) remain the most frequently isolated nontuberculous mycobacteria (NTM) species and are responsible for various human infections including pulmonary disease, skin and soft-tissue infection and disseminated infections [1–3]. Although originally considered to consist of only *M. avium* and *Mycobacterium intracellulare*, MAC now comprises more than 10 species or subspecies [2]. Disseminated MAC infections commonly occur in patients with acquired immunodeficiency syndrome (AIDS) and have been reported to develop in up to 25% of AIDS patients with severely compromised immune systems [2,4].

Advances in molecular typing techniques have resulted in the discovery of novel NTM species, of which *Mycobacterium colombiense* and *Mycobacterium chimaera* have attracted the most attention among new MAC species [5,6]. *M. colombiense* was first isolated in 2006 from human immunodeficiency virus (HIV)-positive prisoners in Colombia and has recently been shown to be associated with lymphadenopathies and cutaneous infections [5,7–9]. *M. chimaera* was first isolated from patients who had undergone open heart surgery, although the infections only became apparent after a time lag of months to years. It was later determined that transmission was from the heater–cooler units of heart–lung machine [6,10]. A recent study found that *M. chimaera* accounted for 28.4% of all pulmonary diseases due to MAC in the USA [11].

Traditionally, detailed species identification of MAC isolates was considered unnecessary because it was believed to provide little

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prognostic benefit [12]. However, a number of recent studies have shown that the knowledge gained from species identification is very beneficial in providing appropriate treatment for patients with infections due to NTM species [11,13,14]. Boyle et al reported that patients with infections due to *M. avium* or *M. chimaera* were at higher risk of clinical relapse/re-infection than those with infections due to *M. intracellulare* [11]. In addition, Koh et al found that patients with pulmonary infection caused by *M. intracellulare* had more severe clinical disease and worse prognosis than patients with pulmonary infection due to *M. avium* [14].

In this study, bacteraemic isolates of MAC were identified to species level in order to better understand the epidemiology of these pathogens. Whether there were differences in clinical course and outcomes among different MAC species causing bacteraemia was also investigated.

2. Patients and methods

2.1. Hospital setting and patients

This study was conducted at National Taiwan University Hospital (NTUH), a 2900-bed tertiary-care centre in northern Taiwan. Clinical manifestations, microbiological data, antimicrobial therapy and outcome of all patients with bacteraemia due to MAC during the period from January 2008 to December 2014 were retrospectively analysed.

2.2. Definition

A persistent MAC-positive blood culture was defined as the presence of at least two separate positive cultures within an interval of ≥7 days regardless of antimicrobial treatment. Standard anti-MAC therapy comprised at least a macrolide and ethambutol as per 2007 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines [12]. Crude mortality was calculated on Days 14 and 30 after the onset of bacteraemia. Patients were followed until death or loss to follow-up.

2.3. Isolation and identification of M. avium complex to species level

The procedures for preparing clinical specimens for culture of mycobacteria at the NTUH Mycobacteriology Laboratory have been reported previously and followed recommended guidelines [15]. Blood samples tested for mycobacteria were inoculated directly in BACTEC 9240 MYCO/F Lytic bottles (Becton Dickinson Microbiology Systems, Sparks, MD) and were incubated for 6 weeks. NTM isolates were identified to species level using conventional biochemical methods as previously described [15]. For accurate identification of MAC isolates to subspecies level, the *rpoB* gene and the internal transcribed spacer (ITS) region of the 16S–23S rRNA gene were sequenced as previously described [11,16–18]. The primers used for *rpoB* gene sequencing were 5′-GGCAAGGTCACCCCGAAGGG-3′ and 5′-AGCGGCTGCTGGGTGATCATC-3′, and the primers used for sequencing the 16S–23S rRNA ITS region were 5′-TTGTACACACCGCCCGTCA-3′ and 5′-TCTCGATGCCAAGGCATCCACC-3′. Sequence comparisons were performed by BLAST (Basic Local Alignment Search Tool) analysis using sequences in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) database. To differentiate between *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*, duplex PCR was performed to detect the insertion sequences IS*901* and IS*1245* as previously described [19].

2.4. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of 13 antimicrobial agents against all MAC blood isolates recovered from patients with MAC bacteraemia were determined using the SLOMYCO Sensititre® panel (TREK Diagnostic Systems, Cleveland, OH). The agents included in the panel were clarithromycin, rifabutin, ethambutol, isoniazid, moxifloxacin, rifampicin, trimethoprim/sulphamethoxazole (SXT), amikacin, linezolid, ciprofloxacin, streptomycin, doxycycline and ethionamide. In brief, 50 μL of the organism suspension was transferred into 11 mL of Sensititre® Mueller–Hinton broth with 5% (v/v) OADC (oleic acid–albumin–dextrose–catalase) growth supplement (TREK Diagnostic Systems). The final bacterial inoculum of the positive control well was 5×10^5 CFU/mL (range 1×10^5–1×10^6 CFU/mL). Positive growth control wells (bacterial inoculum without adding any antibiotic agent) and negative growth control wells (without bacteria inoculum or any antibiotic agent) for susceptibility testing were incorporated in every agent–isolate combination in each. Plates were incubated at 35 °C in a non-CO_2 incubator and were read after 7 days. If growth was sufficient in the positive growth controls and there was no growth in the negative growth controls, the results were read; otherwise the plates were re-incubated for up to 14 days until the positive growth controls grew sufficiently (turbid) [20]. MICs were read using a Sensititre® manual viewer (TREK Diagnostic Systems) through an inverted mirror as the lowest concentration of antibiotic showing complete growth inhibition, except for SXT where the MIC was read as the lowest concentration that inhibited 80% growth compared with the positive growth control [20,21]. MIC breakpoints were interpreted according to 2012 Clinical and Laboratory Standards Institute (CLSI) guidelines as follows: clarithromycin, susceptible (S), ≤8 mg/L; intermediate (I), 16 mg/L; resistant (R), ≥32 mg/L; and linezolid, S, ≤8 mg/L; I, 16 mg/L; R, ≥32 mg/L [21]. A ≥4-fold increase in MIC between the first MAC blood isolates and the subsequent (last) isolates was defined as a significant decrease in susceptibility to a given antibiotic.

2.5. Statistical analysis

The χ^2 test or Fisher's exact test was used for categorical comparison of data, and differences in means of continuous variables were tested by one-way analysis of variance (ANOVA). A *P*-value of <0.05 was considered to indicate statistical significance; all tests were two-tailed. All statistical analyses were performed using the statistical package SAS for Windows v.9.3 (SAS Institute Inc., Cary, NC).

3. Results

During the period from January 2008 to September 2014, a total of 42 MAC isolates were recovered from 30 patients with bacteraemia at NTUH. These isolates were identified to species level by sequencing the *rpoB* gene and the 16S–23S rRNA ITS region.

Species identification results were 100% concordant between the *rpoB* and 16S–23S rRNA ITS genes sequencing methods. The 42 isolates included *M. avium* subsp. *hominissuis* ($n = 35$), *M. colombiense* ($n = 4$) and *M. intracellulare* ($n = 3$). No isolates of *M. chimaera* or *M. avium* subsp. *avium* were identified. Bacteraemia was caused by *M. avium* subsp. *hominissuis* in 24 patients, *M. colombiense* in 4 patients and *M. intracellulare* in 2 patients. In addition, six of the patients had two MAC blood isolates, one patient had five blood isolates (all *M. avium* subsp. *hominissuis*) and one patient had three blood isolates. All patients with MAC bacteraemia were infected with one single MAC species.

The clinical characteristics of the 30 patients with MAC bacteraemia are summarised in Table 1. Briefly, the median patient age was 40 years and the majority (26/30; 87%) were men. Most patients (26/30; 87%) had concomitant HIV infection and all four non-HIV-infected patients had underlying co-morbidities including diabetes ($n = 4$), end-stage renal disease ($n = 1$) and cancer ($n = 2$).