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Rapidly growing mycobacteria in Singapore, 2006–2011

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Abstract

Nontuberculous mycobacteria infection is a growing global concern, but data from Asia are limited. This study aimed to describe the distribution and antibiotic susceptibility profiles of rapidly growing mycobacterium (RGM) isolates in Singapore. Clinical RGM isolates with antibiotic susceptibility tests performed between 2006 and 2011 were identified using microbiology laboratory databases and minimum inhibitory concentrations of amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, imipenem, linezolid, moxifloxacin, sulfamethoxazole or trimethoprim-sulfamethoxazole, tigecycline and tobramycin were recorded. Regression analysis was performed to detect changes in antibiotic susceptibility patterns over time. A total of 427 isolates were included. Of these, 277 (65%) were from respiratory specimens, 42 (10%) were related to skin and soft tissue infections and 36 (8%) were recovered from blood specimens. The two most common species identified were *Mycobacterium abscessus* (73%) and *Mycobacterium fortuitum* group (22%), with amikacin and clarithromycin being most active against the former, and quinolones and trimethoprim-sulfamethoxazole against the latter. Decreases in susceptibility of *M. abscessus* to linezolid by 8.8% per year (p 0.001), *M. fortuitum* group to imipenem by 9.5% per year (p 0.023) and clarithromycin by 4.7% per year (p 0.033) were observed. *M. abscessus* in respiratory specimens is the most common RGM identified in Singapore. Antibiotic options for treatment of RGM infections are increasingly limited.

Keywords: Antimicrobial susceptibility, *Mycobacterium abscessus*, *Mycobacterium fortuitum* group, Nontuberculous mycobacteria, Rapidly growing mycobacteria

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Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous organisms commonly found in the environment. Although they are traditionally not considered a major public health issue, as with tuberculosis, NTM is of emerging global interest and concern as its pathogenic potential becomes more apparent and diseases caused by NTM are increasingly prevalent. Incidence rates of 7.2 to 13.6 cases per 100 000 persons were recently reported [1,2]. Rapidly growing mycobacteria (RGM) are important causes of NTM infections, especially in Asia [3]. The proportion of NTM contributed by RGM has increased more than 2-fold to 35% in 2001 vs. 14% for the period 1992 through 1996 [4]. Among the RGM, *Mycobacterium abscessus*, *Mycobacterium fortuitum* group and *Mycobacterium chelonae* are most common.

Clinical presentations of RGM disease are highly varied and include infections of the respiratory tract (most common), skin and soft tissue structures, bone and joint, lymphadenitis, ophthalmic infections, otitis media and infection of the central nervous system. In addition, infective complications caused by RGM after surgical procedures and catheter use, as well as disseminated infections, especially in immunocompromised hosts, have been widely documented [5]. Treatment of serious RGM infections is challenging; drug therapy typically involves a multidrug regimen for a lengthy duration, is costly and is

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associated with drug-related toxicities. Moreover, response rates are highly variable, particularly in pulmonary RGM infections, with cure rates of only 30% to 50% [6].

The choice of antimicrobial therapy for RGM infections are primarily based on *in vitro* antimicrobial susceptibility testing, which in turn varies with the RGM species involved [7]. However, published reports on the epidemiology of NTM infections to date are derived mainly from continents other than Asia, and the possible geographical variation in the distribution of this group of environmental bacteria suggest that their data may not be directly relevant to the local context. Furthermore, we have observed possible increase in antimicrobial resistance among RGM species. We investigated the epidemiology and *in vitro* antibiotic susceptibility profiles of RGM species isolated in Singapore.

Methods

This observational cohort study was conducted involving RGM isolates recovered from patients at three major hospitals in Singapore: National University Health System (NUHS), Singapore General Hospital (SGH) and Tan Tock Seng Hospital (TTSH). The Central Tuberculosis Laboratory at SGH and the NUHS mycobacteriology laboratory are involved in the identification and antimicrobial susceptibility testing of all mycobacterial specimens in Singapore, and they provided the database of clinical RGM isolates for this study. Institutional review board approval was obtained at all study sites.

RGM isolates with antimicrobial susceptibility testing performed between January 2006 and December 2011 were included in this study. The types of clinical specimens from which the isolate was recovered were noted. Specimen site was classified as pulmonary if the RGM was isolated from sputum, lung tissue biopsy sample, pleural fluid or bronchoalveolar lavage; as skin and soft tissue structure if it was a culture of wound discharge or a biopsy specimen of a lesion involving skin, subcutaneous tissue, muscle, synovium or bone [8]; as a Tenckhoff catheter exit site if the specimen was of wound discharge from an exit site without a positive peritoneal dialysate (PD) culture; as PD peritonitis if RGM was isolated from PD fluid culture; and as lymphadenitis if a biopsy specimen or swab of a lymph node yielded a RGM [9].

Minimum inhibitory concentrations (MICs) of all antibiotics tested were recorded; only unique and nonduplicate isolates from the first culture of each patient were analysed. Linear regression analyses were performed by SPSS Statistics software, version 17.0 (IBM, Armonk, NY), to detect if there were any significant changes in antibiotic susceptibility over time. Results with a p value of <0.05 were deemed statistically significant.

RGM isolates at the Central Tuberculosis Laboratory in SGH were identified by negative DNA probe (AccuProbe; Gen-Probe Inc., San Diego, CA) and NAP (ρ-nitro-α-acetylamino-β-hydroxy-propiophenone) tests for *Mycobacterium tuberculosis* complex. Clinically significant isolates (determined by the attending physician in accordance with the criteria set out by the American Thoracic Society (ATS) [7]) were subsequently identified to species level by DNA reverse hybridization (INNO-LiPA MYCOBACTERIA v2, Innogenetics NV, Ghent, Belgium) and high-performance liquid chromatography. Discrepancies, if any, were resolved through 16S ribosomal RNA sequencing using primers 16S-27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 16S-907R (5′-CCG TCA ATT CMT TTR AGT TT-3′). The NUHS mycobacteriology laboratory identified RGM to species level by conventional biochemical methods (3-day arylsulphatase reaction, nitrate reduction, mannitol, inositol, sorbitol, and citrate utilization, tolerance to 5% NaCl, polymyxin susceptibility and presence of pigmentation) [10]. Microbroth dilution method was used in both institutions for antimicrobial susceptibility testing and MICs were determined and interpreted according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [11]. Antibiotics tested included amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, imipenem, linezolid, moxifloxacin, trimethoprim-sulfamethoxazole, tigecycline and tobramycin.

Results

A total of 427 RGM isolates from 392 patients were included in this study. The rate of positive RGM cultures requiring species identification and antibiotic susceptibility testing was fairly stable at an average of 14.6 isolates per 100 000 population each year [12]; there was also little variation in the proportion contributed by each RGM species (Fig. 1). *M. abscessus* was the most frequently recovered species (74%), followed by *M. fortuitum* complex (22%) and *M. chelonae* (3%); *Mycobacterium mucogenicum* was isolated in five cases and *Mycobacterium neoaurum* in one.

Approximately two-thirds of all isolates (n = 277) were from respiratory specimens; clinical samples from skin and soft tissue structures were the next most common but accounted for only 42 of cases (10%). *M. abscessus* was the predominant species identified across all sites, although *M. fortuitum* group was also equally important in lymphadenitis (Table 1). Of 11 *M. chelonae* isolates, six (55%) were recovered from the respiratory tract and three (27%) from the bloodstream. *M. mucogenicum* (n = 3) and *M. neoaurum* (n = 1) were largely implicated in central venous catheter–related bloodstream infections; *M. mucogenicum* was also identified from two respiratory specimens.

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