



## An emergence of *Mycobacterium abscessus* in blood cultures of Thai patients

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### ABSTRACT

**Aims:** Tuberculosis and other mycobacterial infections occur worldwide especially in patients with immunodeficiency. Typically, an empirical treatment for disseminated disease is required for initial therapy due to slow growing nature of most mycobacterial species. Therefore, species distribution and average time to positivity of blood culture is crucial. However, such information is limited for blood culture and, therefore, were determined.

**Methodology and results:** The blood culture data using the BACTEC FX system and drugs susceptibility testing (DST) pattern was recovered during 2012-2017 from a large teaching hospital in Bangkok, Thailand. Overall, 7.8% of 4,838 blood and 6.4% of 1,056 bone marrow (BM) samples were positive for mycobacterial growth. *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium avium*, and *Mycobacterium abscessus*, were the most three common species to be isolated from blood (3.8%, 2.1%, and 0.9%, respectively) and BM (2.4%, 2.4%, and 0.9%, respectively). The average time to positivity for MTBC, *M. avium*, and *M. abscessus* was 25.7, 16.1, and 3.8 days, respectively. From 209 antimycobacterial susceptibility testing (AST)-available MTBC strains, 6 (2.87%) strains were multi-drugs resistant (MDR-TB). From 35 AST-available *M. avium* complex (MAC) isolates, 6 (17.14%), 33 (94.29%), and 28 (80%) isolates were resistant to clarithromycin, moxifloxacin, and linezolid, respectively. BM MAC isolates were significantly more resistant to clarithromycin than the blood isolates (44.5% vs 7.69%;  $p=0.027$ ).

**Conclusion, significance and impact of study:** In summary, an emergence of *M. abscessus* and unusually high moxifloxacin and linezolid resistance of MAC isolates were reported in this study. Additional information of this study benefits physicians for anti-mycobacterial drug selection for initial treatment of mycobacteremia while blood and BM culture is pending.

**Keywords:** *Mycobacterium abscessus*, blood culture, bone marrow, detection time, susceptibility testing

### INTRODUCTION

Mycobacteria, is an acid-fast, Gram-positive, rod-shaped bacteria and is classified into two main groups, the slow and rapid grower (Gangadharam and Jenkins, 1997). The slow grower includes *Mycobacterium tuberculosis* complex (MTBC) and most species of non-tuberculous mycobacteria (NTM), such as *M. avium* complex (MAC). The rapid grower includes *M. abscessus*, *M. fortuitum* complex and *M. chelonae* complex. MTBC, a causative agent of tuberculosis, is primarily transmitted via aerosol droplets and commonly causes respiratory diseases (Kent, 1985).

Over the past decade, there has been a significant increase in reports of disseminated mycobacterial infection in immunocompromised patients (Bouza *et al.*, 1988). MAC is the most common cause of systemic mycobacterial infection in immunocompromised patients. The increasing trend of NTM infections especially in

immunocompromised patients, leads to the need of effectively identifying the etiologic agent for selecting the most appropriate treatment regimen (Chin *et al.*, 1994).

Due to the increased prevalence of systemic mycobacterial infections, several reports of mycobacteremia were published recently. For example, a previous study demonstrated detections of mycobacteria in the bloodstream of AIDS patients in a University Hospital in Brazil in 2001. With a total of 530 mycobacterial blood cultures incubated in the BACTEC 460 TB system, 41 (16%) patients were positive for mycobacteria. MTBC was recovered in 15 out of 41 (36.6%) patients and the average time to positivity of blood culture was 24 days. MAC was recovered in 22 out of 41 (53.6%) patients and the average time to positivity of blood culture was 15 days. Other species of mycobacteria were recovered in 4 out of 41 (9.8%) patients (Oplustil *et al.*, 2001). In Thailand, a surveillance for bloodstream infections in two rural Thailand provinces

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by the BacT/Alert MB system during 2005-2012 showed 78% and 22% of 354 mycobacterial isolates were identified as MTBC and NTM respectively (Jorakate *et al.*, 2015). In 2009, another report of 327 mycobacterial blood isolates from our hospital, Siriraj Hospital in Bangkok, showed MTBC (54.5%), *M. avium* (37.5%), and *M. intracellulare* was the most common (Foongladda *et al.*, 2009). No rapid grower was identified and the average time to detect positive of blood culture was not determined.

For an optimal treatment, species identification of mycobacteria, and antimycobacterial susceptibility are crucial. However, due to the long incubation time of most mycobacterial species, selection of definitive antibiotic is often delayed. The average time to detect positive blood culture of each mycobacterial species can be provisionally used to differentiate the slow grower from the rapid grower. However, information of the average time to detect positive of blood culture is lacking. Therefore, this study aimed to report the average time to detect positive of blood and bone marrow culture of each mycobacterial species. Species distribution and antibiotics susceptibility were also described.

## MATERIALS AND METHODS

### Mycobacterial strains, species identification, and detection time

All mycobacteria strains from blood and bone marrow (BM) cultures were identified in the Mycobacteriology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, during the period 2012-2017. The BACTEC FX System was used according to a manufacturer's instructions (Fuller *et al.*, 2001). The BACTEC Myco/F Lytic media was used. Time from an insertion of the bottle into the instrument until instrument detected positive were also calculated as the time to detect positive. Species identification was confirmed by using in-house real time PCR and Line probe assays according to previous published methods (Tortoli *et al.*, 2003, Foongladda *et al.*, 2009). The time to detect positive were available only in 2016-2017 and only mycobacterial species with least 3 strains were included in the calculation.

### Antimycobacterial susceptibility testing

The antimycobacterial susceptibility testing (AST) for *M. tuberculosis* complex was performed by using [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assays (Foongladda *et al.*, 2002), and the automated drug susceptibility using BACTEC™ MGIT 960 systems (Becton Dickinson, Sparks, MD, USA) (Scarpato *et al.*, 2004), according to the manufacturer's instructions. AST of MAC was performed by micro broth dilution using the TREK Diagnostic Systems (Thermo scientific, West Sussex, UK.), according to the manufacturer's instruction. Interpretation of susceptibilities were done according to the Clinical and Laboratory Standards Institute (CLSI)

standard for MGIT and according to the previous publication for MTT (Foongladda *et al.*, 2002). For MTBC, clinical break points for each drug were as follows: isoniazid, resistant,  $\geq 0.1 \mu\text{g/mL}$  (MGIT 960) or  $\geq 0.2 \mu\text{g/mL}$  (MTT); rifampicin, resistant,  $\geq 1 \mu\text{g/mL}$  (both methods); and ethambutol, resistant  $\geq 5 \mu\text{g/mL}$  (both methods). Except isoniazid resistant strains determined by the MGIT 960, all resistance strains were again confirmed by the agar proportional method according to the CLSI guideline (isoniazid, resistant,  $\geq 0.2 \mu\text{g/mL}$ ; rifampicin, resistant,  $\geq 1 \mu\text{g/mL}$ ; and ethambutol, resistant  $\geq 5 \mu\text{g/mL}$ ). For MAC, clinical breakpoints were interpreted only for clarithromycin, moxifloxacin and linezolid with cut-off points (CLSI, 2011), as follows: clarithromycin, susceptible,  $\leq 8 \mu\text{g/mL}$ ; intermediate,  $16 \mu\text{g/mL}$ ; resistant,  $\geq 32 \mu\text{g/mL}$ ; moxifloxacin, susceptible,  $\leq 1 \mu\text{g/mL}$ ; intermediate,  $2 \mu\text{g/mL}$ ; resistant,  $\geq 4 \mu\text{g/mL}$ ; and linezolid, susceptible,  $\leq 8 \mu\text{g/mL}$ ; intermediate,  $16 \mu\text{g/mL}$ ; resistant,  $\geq 32 \mu\text{g/mL}$ . This study was approved by Siriraj hospital ethic committee (Si 765/2017).

### Statistical analysis

Statistical analysis using mean, percentage, and t-test were calculated by Microsoft Excel enterprise 2007 (Microsoft Corporation, Redmond, WA, USA). Significance was defined as  $p \leq 0.05$ .

## RESULTS

During 2012-2017, 4,838 blood and 1,056 BM samples from patients suspected to have mycobacterial infection, were sent for mycobacterial cultures. 7.8% of the blood and 6.4% of the BM samples were positive for mycobacterial growth (Table 1). MTBC and *M. avium* were the most common mycobacterial species to be isolated from both blood (3.8% and 2.1%) and BM (2.4% and 2.4%). In addition, *M. abscessus* was the most common rapid growers isolated from blood (0.9%) and BM (0.9%), respectively. Distributions of other mycobacterial species were presented in Table 1.

Time to positivity for the mycobacteria from blood and BM culture were then evaluated. The average time to positivity for MTBC was 25.7 days, ranging from 8 to 38 days. For *M. avium*, the average time to positivity was 16.1 days, ranging from 8 to 38 days. While the average time to positivity of *M. abscessus*, a rapid grower, was 3.8 days, ranged from 3 to 7 days. Time to positivity for other mycobacterial species were indicated in Table 2.

From a total of 209 MTBC strains, 6 (2.87%) strains were multi-drugs resistant (MDR-TB) while 23 (11%) strains and 8 (3.82%) strains were mono-resistant to isoniazid and rifampicin, respectively. 184 (88.04%) strains were pan-susceptible to isoniazid, rifampicin and ethambutol. Percentages of susceptibility of each drug were shown in Table 3. No statistical difference in susceptibility profile between isolates from blood and BM isolates (Table 3).

A total of 35 *M. avium* complex isolates were investigated for susceptibility profile using micro broth

**Table 1:** Number of mycobacterial isolates from blood and BM during 2012-2017

Year	2012		2013		2014		2015		2016		2017		Total Blood (%)	Total BM (%)
Sample type	BI	BM	BI	BM	BI	BM	BI	BM	BI	BM	BI	BM		
Total No. of sample	989	245	925	76	816	126	808	238	826	150	474	221	4,838 (100)	1,056 (100)
Positive for mycobacteria	87	14	99	10	56	14	58	7	49	15	29	8	378 (7.8)	68 (6.4)
<i>M. tuberculosis</i> complex	48	6	45	4	23	7	32	2	22	5	14	1	184 (3.8)	25 (2.4)
- <i>M. avium</i>	29	3	19	4	20	5	17	2	13	7	5	4	103 (2.1)	25 (2.4)
- <i>M. intracellulare</i>	0	1	0	0	0	0	3	1	2	0	0	0	5 (0.1)	2 (0.2)
- <i>M. avium</i> complex (other)	2	1	4	0	4	0	4	2	2	3	3	0	19 (0.4)	6 (0.6)
- <i>M. simiae</i>	1	0	0	0	2	0	0	0	1	0	0	0	4 (0.1)	0 (0)
- <i>M. scrofulaceum</i>	0	0	0	1	2	1	1	0	3	0	0	0	6 (0.1)	2 (0.2)
- <i>M. kansasii</i>	0	0	0	1	1	1	0	0	0	0	0	0	1 (<0.1)	2 (0.2)
- <i>M. haemophilum</i>	2	0	1	0	0	0	0	0	1	0	2	1	6 (0.1)	1 (0.1)
- <i>M. abscessus</i>	4	3	24	0	4	0	1	0	5	0	4	1	42 (0.9)	4 (0.9)
- <i>M. fortuitum</i> complex	1	0	0	0	0	0	0	0	0	0	0	0	1 (<0.1)	0 (0)
- other <i>Mycobacterium</i> spp.	0	0	6	0	0	0	0	0	0	0	1	1	7 (0.1)	1 (0.1)
Negative for mycobacteria	902	231	826	66	760	112	750	231	777	135	445	213	4460	988

BI, blood; BM, bone marrow

**Table 2:** Average time to positivity of each mycobacterial species during 2016-2017 (n=101).

Mycobacterial species	No. of isolates		Average time to positivity in days (SD)		P-value
	Blood	BM	Blood	BM	
<i>M. tuberculosis</i> complex	36	6	26.4 (8.75)	27.4 (5.03)	0.789
<i>M. avium</i>	18	11	17.38 (9.64)	20.29 (12.43)	0.486
<i>M. avium</i> complex (other)	5	3	19 (2.58)	16 (1.41)	0.119
<i>M. scrofulaceum</i>	3	0	27 (4.36)	N/A	N/A
<i>M. haemophilum</i>	3	1	36.33 (2.08)	N/A	N/A
<i>M. abscessus</i>	9	1	4.75 (1.78)	N/A	N/A

N/A – not applicable due to insufficient isolate numbers or incalculable

**Table 3:** Antimycobacterial susceptibility patterns of 209 MTBC isolates.

Drug	% susceptibility (total number)		P-value
	Blood	BM	
Isoniazid			
Susceptible	89.67 (165)	84 (21)	0.491
Resistant	10.33 (19)	16 (4)	
Rifampicin			
Susceptible	95.65 (176)	100 (25)	0.599
Resistant	4.35 (8)	0 (0)	
Ethambutol			
Susceptible	100 (184)	100 (25)	1.000
Resistant	0 (0)	0 (0)	

**Table 4:** Antimycobacterial susceptibility patterns of 35 MAC isolates.

Drug	% susceptibility (total number)		P-value
	Blood	BM	
Clarithromycin			
Susceptible	92.31 (24)	55.56 (5)	0.027
Resistant	7.69 (2)	44.44 (4)	
Moxifloxacin			
Susceptible	7.69 (2)	0 (0)	1.000
Resistant	92.31 (24)	100 (9)	
Linezolid			
Susceptible	26.92 (7)	0 (0)	0.153
Resistant	73.08 (19)	100 (9)	

dilution. A total of 29 (82.86%) isolates were susceptible to clarithromycin, 33 (94.29%) isolates and 28 (80%) isolates were resistant to moxifloxacin and linezolid, respectively. Percentages of susceptibility of each drug were shown in Table 4. BM isolates (44.44%) were significantly more resistant to clarithromycin than the blood isolates (7.69%). There was significant association between sample type and susceptibility frequency to clarithromycin for MAC ( $p = 0.027$ , Table 4).

## DISCUSSION

In our study, a higher isolation rate of MTBC and MAC (includes *M. avium*, *M. intracellulare* and other *M. avium* complex) was shown in both blood and BM samples

similar to our previous study (Foongladda *et al.*, 2009). Surprisingly, unlike before 2010 when no *M. abscessus* was isolated from any of our blood specimens (Foongladda *et al.*, 2009), *M. abscessus* was the second most common NTM isolated from both blood and bone marrow in this study. Unfortunately, the outbreak of *M. abscessus* blood infection in 2013 was not detected until this study. Therefore, the source of this outbreak was unknown. This outbreak might be caused by injections of contaminated medical devices and drinking water system. However, evidences were absent and further investigations are needed. In fact, infections caused by *M. abscessus* in Thailand have been previously limited to only local infections such as skin infections and lymphadenitis (Sungkanuparph *et al.*, 2003,

Phowthongkum *et al.*, 2005). Systemic infection caused by *M. abscessus* in Thailand was considered very rare as only one such was evident in an extensive review of a large teaching Thai hospital, King Chulalongkorn Memorial Hospital during 1997-2003 (Phowthongkum *et al.*, 2005). The high prevalence of *M. abscessus* in this study is also in contrast to a meta-analysis in Iran which reported that *M. simiae*, *M. terrae*, *M. avium-intracellulare* were the most (44.2%), second most (18.3%), and third most (16.5%), respectively (Khaledi *et al.*, 2016). This suggests that the high prevalence of *M. abscessus* in Thailand might be unique to this part of Asia. The rise of *M. abscessus* infection was speculated by some studies to be due to either declining worldwide use of the Bacille Calmette-Guérin vaccine (Sabin *et al.*, 2017) or an emergence of a novel adult-onset immunodeficiency disorder (Lee *et al.*, 2015). However, these require further investigations before any solid conclusion to be made.

As expected, the average time to positivity for those slow growers was longer than the rapid growers. For example, it took 25.7 days for MTBC to be detected by the BACTEC FX system while only 4.75 days were required for *M. abscessus* to have such positivity. Comparing to the previous study from Brazil (Oplustil *et al.*, 2001), our average time to positivity for MTBC and MAC was slightly longer (24 versus 26.4 days for MTBC and 15 versus 17.38-19 days for MAC). This slight difference might be caused by different model of the blood culture system as the previous study use the BACTEC 460 TB, a radiometric detection, comparing to our BACTEC FX, a fluorometric detection. Other factors can also affect for example, volume of blood and loading of organisms that is different among patients. Though a direct comparison between both systems was not available, a meta-analysis of BACTEC MGIT 960, another fluorometric detection system, also showed slight superior sensitivity (85.8%) of the 460 TB system to the 81.5% sensitivity of the MGIT system (Cruciani *et al.*, 2004).

Similar to a previous study of MTBC (Chaiprasert *et al.*, 2013), the drug resistance was most commonly found with isoniazid (11%) and second most commonly found in rifampicin (3.82%). The result of MDR-TB in our study was 2.87%. However, these resistance figures were only half of the previous report (Chaiprasert *et al.*, 2013). This might be due to the fact that our MTBC was isolated from blood but MTBC of the previous study were isolated from sputum. This was supported by another report from Thailand showing lower drug-resistance rate of extrapulmonary MTBC isolates than that of pulmonary MTBC isolates (Boonsarngsuk *et al.*, 2018).

For MAC, the most and second most common drug resistance was found in moxifloxacin (94.29%) and linezolid (80%), respectively. This is in contrast to a recent study of blood MAC isolates from Taiwan showing that only 21% were resistant to moxifloxacin and more than 90% of the isolates were susceptible to linezolid (Lee *et al.*, 2017). However, the high susceptibility to clarithromycin, the first-line drug for MAC treatment, was found in both ours and the Taiwan study. Interestingly, significantly higher rate of clarithromycin resistance was

found in BM MAC isolates but the number of BM isolates was small. As a specific correlation between BM and mycobacterial susceptibility has never been reported, more BM MAC isolates are needed before drawing any conclusion.

## CONCLUSION

This study provides average time to positivity of blood and BM cultures for both slow and rapid growers. Moreover, specific patterns of species distribution and antimycobacterial susceptibility pattern of blood and BM isolates were also reported. These data will benefit clinical practitioners for antimycobacterial drug selection while mycobacterial blood or BM culture is pending.

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