High rate of reinfection and possible transmission of Mycobacterium avium complex in Northeast Thailand

Wicharajit Boonjetsadaruhk, Oraweew Kaewprasert, Arnone Nithichanon, Pimjai Ananta, Prajuab Chaimane, Kanin Salao, Wisitsak Phoksavat, Marut Laohaviroj, Auttawit Sirichot, Yang Fong, Suwin Wongwajana, Wises Namwat, Ploenchon Chetchotisakd, Kiatichai Faksri

1. Introduction

Nontuberculous mycobacteria (NTM) are aerobic, acid-fast bacilli belonging to the family Mycobacteriaceae. The M. avium complex (MAC) is among the disease-causing NTM [1]. Members of the MAC are slow-growing opportunistic pathogens [2] that can cause human diseases including pulmonary disease, skin and soft-tissue infection and disseminated infections [3]. The two recognized species in the complex, M. avium and Mycobacterium intracellulare, can be found in common environments such as soil and natural waters [1,4]. M. avium is further subdivided into four subspecies: M. avium subsp. avium, M. avium subsp. hominisuis, M. avium subsp. paratuberculosis and M. avium subsp. silvaticum [1]. Identification of MAC species and strains is important for definite diagnosis and patient management. MAC is associated with antibiotic resistance [5]. Treatment of MAC infections is complicated and expensive [1]. Treatment results are poor with success rates about 40% [6]. However, the factors associated with MAC treatment difficulty are still unclear.

Keywords: Genotyping MLST Mycobacterium avium complex Phylogenetic Reinfetion

ABSTRACT

The Mycobacterium avium complex (MAC) includes two main species of non-tuberculous mycobacteria (NTM), M. avium and Mycobacterium intracellulare. These can cause serious disease, especially in immunocompromised patients. Little information is available concerning genetic diversity of NTM. We used multilocus sequence typing (MLST) based on a highly discriminative gene set to analyze MAC serially isolated from patients to determine the rate of MAC reinfection. Genomic DNA was sequenced from 49 MAC isolates (15 cases comprised of 11 true reinfections and 4 instances of colonization). More than half of the MAC isolates tested were found to be multidrug resistant. The discriminatory power was assessed of 24 house-keeping genes (fusA, gnd, lipT, pepB, glpK, argH, rpoB, murC, cya, rrl, 16S rRNA ITS, hsp65, recF, sodA, est, 16S-23S rRNA ITS, pta, rrl, rrs, hsp65, rpoB, est) previously used for genotyping of MAC and other NTM. Seven genes (fusA, secA, rpoB, hsp65, 16S rRNA, 23S rRNA, 16S-23S rRNA ITS) had a discriminatory power index higher than 0.9 and were included in the optimized gene set for MAC species and clonal strain analysis could be further applied for the diagnosis and patient management.

* Corresponding author at: Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: kiatichai@kku.ac.th (K. Faksri).

https://doi.org/10.1016/j.onehlt.2022.100374
Received 4 October 2021; Received in revised form 12 December 2021; Accepted 6 February 2022
Available online 11 February 2022
2352-7714/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license.
Multilocus sequence typing (MLST) is a common tool used to genotype MAC isolates. Various combinations of genes have been used in the past for this purpose. These include a four-gene set (rpoB, hsp65, 16S rRNA, 23S rRNA ITS) [7], five-gene set (recF, lipT, pepB, gnd1, est) [8] and a nine-gene set (recF, lipT, pepB, gnd1, est, aspB, sodA, groEL1, hsp65) [9]. However, these gene sets have never been assessed for their discriminatory power or evaluated using samples serially isolated from the same patient. One previous study investigated serial isolates of MAC from 49 patients in Korea based on the four-gene set and reported a high reinfection rate (73%) [7]. Such a high reinfection rate needs to be confirmed in a different population.

Distinguishing between the two species of MAC is generally based on differences in the rRNA genes. A commercial line-probe assay kit is the GenoType Mycobacterium Assay, which is based on 23S rRNA and 16S-23S rRNA ITS also showed a high utility for MAC species identification [7,11]. Since MAC can show genetic differences in different geographical regions [12], it is necessary to evaluate these approaches in Southeast Asia.

Exposure to environmental sources has been suggested as the main route of MAC infection [13]. Transmission of other NTM between humans may be possible [14,15], but has not yet been reported for MAC.

We aimed to optimize MLST, testing the utility of different sets of genes to identify MAC. We then wished to use the optimized set to analyze the genetic diversity of MAC in Thailand and to characterize serial isolates of MAC from the same patient. We also aimed to evaluate the performance of MLST based on various gene sets for species identification relative to results from the GenoType Mycobacterium Assay.

### 2. Methods

#### 2.1. Study population and classification

Forty-nine serial isolates of *M. avium* complex (MAC) came from 15 patients at Siriraj Hospital, Khon Kaen Province, Northeast Thailand during the period 2012 to 2016. Age, gender, locations (provinces) and other details for all 15 patients are summarized in Table 1. Ages of patient ranged from 27 to 91 years (with an average of 55 years). Seven were men (four <60 and three ≥60 years of age) and
Fig. 1. Phylogenetic trees based on 5-gene set (248 SNPs) (A), 4-gene set (457 SNPs) (B), 9-gene set (476 SNPs) (C) and optimized 7-gene set (925 SNPs) (D). These bootstrap consensus trees were inferred from 1000 replicates. Different highlight colors represent the isolates from each patient. One isolate, 9 isolates, 8 isolates and 9 isolates were identified as examples of reinfection (red stars) based on the trees inferred from the 5-gene, 4-gene, 9-gene and the optimized 7-gene sets. Reinfection was identified when serial isolates collected from the same patient fell on different branches in the tree (refer to SNP distances shown in Fig. 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
eight were women (five <60 and three ≥60 years of age). The patients were from many provinces in the region: Khon Kaen, Kalasin, Nong Khai, Nong Bua Lamphu, Yasothon, Mahasarakham, Buriram and Nakhon Phanom. The isolates were taken from specimens such as sputum, tracheal suction, neck (pus), stool, synovial fluid, skin, cheek (pus) and other tissues. Cases of true infection were identified on the basis of isolation of NTM from sterile sites (i.e., bone joint samples and pus) and other tissues. Cases of true infection were identified on the basis of isolation of NTM from sterile sites (i.e., bone joint samples and other tissues). Cases of true infection were identified on the basis of isolation of NTM from sterile sites (i.e., bone joint samples and other tissues). Cases of true infection were identified on the basis of isolation of NTM from sterile sites (i.e., bone joint samples and other tissues).

### Table 2

Pairwise comparisons of the phylogenetic interpretations based on 4-gene, 5-gene and 9-gene sets, and the optimized 7-gene set.

| Characteristics | 4 genes vs. 5 genes | 4 genes vs. 7 genes | 4 genes vs. 9 genes | 5 genes vs. 7 genes | 5 genes vs. 9 genes | 7 genes vs. 9 genes |
|-----------------|----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Species identification | 58.62% (17/29 isolates) | 96.67% (29/30 isolates) | 96.67% (29/30 isolates) | 60% (18/30 isolates) | 60% (18/30 isolates) | 100% (30/30 isolates) |
| Mycobacterium intracellulare | 45% (9/20 isolates) | 100% (19/19 isolates) | 100% (19/19 isolates) | 47.57% (9/19 isolates) | 47.57% (9/19 isolates) | 100% (19/19 isolates) |
| Mycobacterium avium | 53.06% (26/49 isolates) | 97.96% (48/49 isolates) | 97.96% (48/49 isolates) | 55.10% (27/49 isolates) | 55.10% (27/49 isolates) | 100% (49/49 isolates) |
| Total | | | | | | |
| Reinfection | 11.11% (1/9 isolates) | 100% (9/9 isolates) | 88.89% (8/9 isolates) | 11.11% (1/9 isolates) | 11.11% (1/9 isolates) | 88.89% (8/9 isolates) |

* Agreement between the two methods in recognition of reinfection cases.

2.6. Multilocus sequence typing (MLST)

Sequences from 24 housekeeping genes (fusA, atpD, pheT, glnA, topA, secA, argH, gdpK, murC, cya, pta, rrl, rrs, hsp65, rpoB, 16S-23S rRNA ITS, recF, lipT, pepB, gnd, aspB, groEL, sodA and est) were used for genetic analysis of MAC and other NTM based on findings from previous studies [7,9,11,24,25]. Characteristics of these genes and the primers used to amplify them are described in Supplementary Table 1. Each gene sequence from the 49 MAC isolates was extracted from the aligned mapped sequences. The gene sequences of reference strains M. avium subsp. avium (SRA accession number CP028731), M. avium subsp. hominis (CP018363), M. avium subsp. paratuberculosis (NC_002944), M. intracellulare ATCC 13950 (CP003322) and Mycobacterium chelonae CCUG 47445 (NZ_CP007220) were used for comparisons.

2.7. Phylogenetic analysis

Phylogenetic analysis was done using MEGA-7 [26] based on a four-gene set (16s rRNA, hsp65, rpoB, 16S-23S rRNA ITS) [7], a five-gene set (recF, lipT, pepB, gnd1, est) [8], a seven-gene set (fusA, secA, 16S-23S rRNA ITS, rpoB, hsp65, 16S rRNA, 23S rRNA) and a nine-gene set (hsp65, recF, lipT, pepB, gnd1, aspB, sodA, groEL, est) [9]. The maximum-likelihood method was employed using the most suitable model of sequence evolution (GTR) and 1000 bootstrap replications. M. chelonae CCUG 47445 was used as an outgroup and M. intracellulare ATCC 13950, M. avium subsp. avium, M. avium subsp. hominis (M. avium) subsp. paratuberculosis were used as reference strains.

2.8. Data analysis

The discriminatory power (D) of each gene for classification of MAC strain was calculated based on the number of unrelated strains tested (N), the number of different types identified (S) and N × S × X100 [27].

3. Results

3.1. Study population and characteristics

All 49 MAC isolates were from 15 patients from Srinagarind Hospital, a super-tertiary hospital located in Northeast Thailand. Eleven cases (41 isolates) were defined as true NTM infections and 4 cases (8 isolates) were regarded as examples of colonization (Table 1). Almost half of the isolates from patients with true infections (41.46%, n = 17/41) caused pulmonary disease. The remainder of such isolates (58.54%, n = 24/41) had caused extra-pulmonary infection including disseminated infection using for sorting and indexing of mapped sequences. Local realignment of the mapped reads was performed using GATK version 3.4.0 [23].
A high-resolution gene set for MLST is necessary to determine susceptibility and resistance have not been established. Interestingly, 27 of the 49 MAC isolates in our study were multidrug resistant (three or more antibiotic categories) and six MAC isolates were highly resistant to all four antibiotics tested (amikacin, clarithromycin, linezolid and moxifloxacin).

All 49 MAC isolates were identified to the species level using GenoType Mycobacterium line-probe assay (LPA): 34/49 were M. intracellulare and 15/49 were M. avium.

3.2. Analysis of discriminatory power of house-keeping genes and comparisons among 4-gene-, 5-gene-, 7-gene- and 9-gene-based MLST

The discriminatory power of MLST for classification of MAC strain using various combinations of 24 house-keeping genes was analyzed. Seven genes individually had a discriminatory power index higher than 0.9 (Supplementary Table 1) and were combined into a set (the optimized 7-gene set) that was then compared with other previously reported gene sets (4-gene, 5-gene and 9-gene). The phylogenetic trees constructed based on the various gene sets are shown in Fig. 1.

The 4-gene, 7-gene and 9-gene sets agreed equally well (83.67% of cases; M. intracellulare = 28/49, M. avium = 13/49) with the GenoType Mycobacterium line-probe assay (LPA) for species identification (data not shown). The 5-gene set had lowest agreement with the LPA (55.10%, 27/49, M. intracellulare = 20/49, M. avium = 7/49). There was 83.67% (41/49 isolates) concordance between LPA and the 7-gene set (Fig. 1). Among the isolates with discordant results, 2 isolates (patients#15.1 and #11.3) were identified by LPA as M. avium, but the 7-gene set as M. intracellulare. Further, 6 isolates (patients #3.2, #3.3, #3.4, #13.1, #13.2, #5.2) were identified as M. intracellulare by LPA but as M. avium by the 7-gene set.

In pairwise comparisons among the four different gene sets, the 5-gene set agreed least well with the others in terms of species identification and recognition of reinfection (Table 2). One isolate was identified as resulting from reinfection based on the 5-gene tree whereas 9, 8 and 9 isolates were identified as due to reinfection based on the 4-gene, 9-gene and the optimized 7-gene sets, respectively (Fig. 1).

3.3. Ability to distinguish between reinfection and persistent infection of MAC

Identification of reinfection and persistent infection was based on different cut-off levels according to the optimized 7-gene set (>87 SNPs for reinfection and ≤ 41 SNPs for persistent infection) (Fig. 3) and concordance of species identification based on LPA (Table 2). Reinfection rate was estimated to be 54.55% (6/11 true reinfection cases) based on both the optimized 7-gene set and the 4-gene set (Table 2). Different strains were recovered from one colonization case (patient#12), a situation analogous to reinfection. The interval time between samples during which reinfection occurred ranged from 8 to 296 days with an average of 97.9 days (Fig. 3). Reinfection in one patient (patient#9) was not identified by the 9-gene set. The 5-gene set identified only one reinfection case.

3.4. Cluster analysis for possible transmission between patients

Cluster analysis of MAC infections showed two possible clonal transmission clusters (cluster 1 = P#11, P#12 and cluster 2 = P#5, P#12 and P#13), based on distances ≤41 SNPs (Fig. 2). Cluster 1 was supported by a geographical link (the same province) and collection time (16 months apart). Cluster 2 was supported by their occurrence in adjacent provinces (Khon Kaen and Mahasarakham) and collection time (7 months apart) (Fig. 3 and Table 1).

4. Discussion

MAC infection is a public health problem worldwide and an important cause of morbidity and mortality. The two recognized species with the MAC are M. avium and M. intracellulare: both can infect humans. MAC infection is usually chronic and is highly associated with drug resistance and treatment failure [1]. More than half of the MAC isolates (55%) in this study were multidrug resistant, which is a major public health concern [28,29]. M. avium and M. intracellulare cannot be differentiated through conventional microbiological tests and their clinical features are often considered indistinguishable [1]. However, a study in Korea found that patients with M. intracellulare lung disease exhibited a more severe illnesses and worse prognosis than patients with M. avium lung disease [30]. A previous study using an animal model also suggested that M. intracellulare was the more virulent species [31]. Therefore, identification of the exact species involved is of clinical importance. Some serious MAC infections and treatment failures are associated with antibiotic resistance. In addition, during the course of antibiotic treatment, the MAC isolates sampled might be derived from the same clone that initially infected the patient (persistent infection) or may represent a new clone (reinfection). The extent to which reinfection by MAC can influence the apparent treatment failure rate is still unclear. Here, we optimized a gene set that can be used for species and strain classification. Use of this gene set demonstrated a high reinfection rate of MAC in the Thai population. Our optimized gene set also allowed us to explore relationships among serial isolates, making it possible to infer instances of human-to-human transmission of MAC.

Molecular typing is a useful tool to discriminate between reinfection and persistent infection cases, allowing us to investigate below the species level [15]. A high-resolution gene set for MLST is necessary to distinguish whether MAC isolates are from the same clone or different clones. Such information can be used for molecular epidemiology and diagnosis. Here, we analyzed the discriminatory power of 24 house-keeping genes selected from the various genotyping sets used in previous studies on MAC [7–9,11,25] and other NTM [24,25]. We showed that our 7-gene set has the highest discriminatory power, best resolution to differentiate reinfection from persistent infection, and the highest concordance with LPA for species identification. We also used this gene set to demonstrate possible human-human transmission of MAC.

MLST is the genetic analysis tool most commonly used to genotype MAC [9,32]. Many house-keeping genes have been used for...
mycobacterial identification, such as the 16S and 23S rRNA genes, hsp65, rpoB, superoxide dismutase gene, and internal transcribed spacer (ITS) region [33]. Previous studies have simply adopted a convenient gene set reported by others or have failed to optimize for the most suitable gene set for genetic analysis of MAC [7–9, 11, 25]. We determined the discriminatory power of 24 house-keeping genes using serial isolates of MAC from the same patients, in whom treatment had not apparently been successful. Such isolates could include examples of persistent infection (infection by the same clone across different time points) and/or reinfection (infection due to the acquisition of a new clone of bacteria). Here, we showed that 7 of the 24 genes (fusA, secA, 16S-23S rRNA ITS, rpoB, hsp65, 16S rRNA, 23S rRNA) had a

Fig. 3. Phylogenetic tree of 925 SNPs from the optimized 7-gene set (fusA, secA, 16S-23S rRNA ITS, rpoB, hsp65, 16S rRNA, 23S rRNA) of MAC isolates using the maximum likelihood method. All 49 strains were identified as either Mycobacterium intracellulare or Mycobacterium avium subsp. avium. This bootstrap consensus tree was inferred from 1000 replicates. Blue circles represent bootstrap values and the size of each circle is proportional to its value (the largest blue circle indicates a value of 100%). M. avium subsp. avium, M. avium subsp. hominisuis, M. avium subsp. paratuberculosis and M. intracellulare ATCC 139950 (accession numbers CP028731, CP018363, NC_002944 and CP003322, respectively) were used as reference strains. (D = Disseminated, P = Pulmonary, S = Skin, T = True, C = Colonization, E = Extra-pulmonary, Pink colour = M. avium, Dark pink = M. intracellulare). C1 = cluster 1 (Patient 11.2 and 12.2), C2 = cluster 2 (Patient 5.1–5.2, 13.1 and 12.1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isolates of MAC from the same patients, in whom treatment had not apparently been successful. Such isolates could include examples of persistent infection (infection by the same clone across different time points) and/or reinfection (infection due to the acquisition of a new clone of bacteria). Here, we showed that 7 of the 24 genes (fusA, secA, 16S-23S rRNA ITS, rpoB, hsp65, 16S rRNA, 23S rRNA) had a
discriminatory power index higher than 0.9 for differentiating serial isolates of MAC. We compared this optimized 7-gene set with previously used gene sets. The discriminatory power of each was proportional to the number of SNPs in each. The 7-gene set (925 SNPs) is better than the 4-gene (457 SNPs), 9-gene (476 SNPs) and 5-gene (248 SNPs) sets. The optimized 7-gene set had the highest concordance (but comparable to the 4-gene and 9-gene sets) for species identification of MAC compared to LPA.

A study from Korea using the 4-gene set reported a high reinfection rate of MAC (73%) and suggested that this might be a factor contributing to chronic infection that creates treatment difficulties [7]. In our study, the 4-gene set identified the same proportion (54.55%) of reinfection cases as did the optimized 7-gene set. We confirmed a high rate of reinfection due to MAC in Thailand, in agreement with the previous report from Korea. We also found one out of four cases of MAC colonization included a change of clone through time, a situation analogous to reinfection and possibly due to independent acquisitions from the environment. As MAC infection usually occurs in immunocompromised hosts [34], the reacquisition of MAC from environmental exposure during treatment might explain the high reinfection rate observed, leading to chronic infection and treatment difficulties.

Using the number of SNPs differing between serial isolates of MAC from individual patients, we identified cut-off values to distinguish between reinfection and persistent infection. For the high-resolution 7-gene set, reinfection was identified based on ≥87 SNP differences between sequential isolates and persistent infection was identified based on ≤41 SNPs. The high average interval time (98 days), and high number of SNPs, separating the reinfection isolates also supported the identification of reinfection. The results of the same 4-gene set compared to the previous study [7] also support the validity of both the optimized 7-gene set and the reinfection rate identified from this study.

We used the cut-off values based on the optimized 7-gene set to identify possible clonal transmission clusters of MAC. There were 2 clusters found. Cluster 1 comprised two cases from Khon Kaen occurring two years apart. However, the social data and exposure history from cluster 1 are not available for analysis. Cluster 2 comprised 4 isolates from 3 cases from the adjacent provinces of Mahasarakham and Khon Kaen within the same time period in February–June 2016. Such clusters could be a result of exposure to the same environmental source, such as soil [35]. There is increasing speculation that human-to-human transmission of some NTM infections can occur [36,37]. Such transmission has never been reported for MAC but cannot be excluded. Additional study that includes the social links and exposure history is needed to confirm human transmission. This should also include analysis of MAC environmental isolates, such as from soil and the household environment.

Many molecular methods can be used to identify NTM. The line-probe assay is the most widely used. This method enables simultaneous detection and identification of different mycobacterial species using house-keeping genes and DNA sequences such as the 16S-23S rRNA gene spacer, 23S rRNA gene and rpoB gene [7]. The LPA assay we used (GenoType Mycobacterium CM) has 97% and 92.4% sensitivity and specificity, respectively, compared to biochemical methods, HPLC, INNO-LIPA MYCOBACTERIA (Innogenetics NV) and 16S rRNA gene sequencing [10]. The LPA has 98.23% and 50% specificity and sensitivity compared to HPLC [30]; MLST using our 7-gene set achieved a high concordance with LPA (83.67%). As no bacterial taxon other than MAC was analyzed, specificity cannot be calculated. Since the LPA uses only a single gene (23S rRNA), the higher discriminatory power of the 7-gene set might lead to some discordance. However, biochemical tests were not available to us for comparison. Therefore, we cannot conclude whether the 7-gene set has a higher performance for MAC species identification. At least, the 7-gene set was comparable with the previous 4-gene and 9-gene sets for MAC species identification. Given its higher overall discriminatory power, the 7-gene set is the optimal set for genetic analysis of MAC.

MAC can infect many organs, especially in HIV patients, and is considered the most common cause of chronic lung infection [39]. A previous study reported that MAC causes pulmonary infection far more frequently than extrapulmonary [34]. In our study, M. intracellulare was the major species isolated from both pulmonary and extrapulmonary sites. However, no significant difference was seen between the two species comparing pulmonary and extrapulmonary sites. Also, we found no evidence to support an association between the number of mutations and the time interval between serial isolates, nor any association between the number of mutations and site of infection. Our sample size limited the power of statistical analysis. A correlation analysis between site of infection and sub-specific strains was not done due to the limited range of sample sites and because serial isolates from the same patient could not be regarded as independent samples. [36,37]

5. Conclusion

We evaluated a 7-gene set for MLST analysis that provided high discriminatory power and diagnostic performance for the genetic study of MAC. MLST analysis using this gene set can be used for MAC species identification. The results we obtained indicated that the rate of reinfection was 54.55%. Two small clusters of possible transmission of MAC between humans were found.

Funding

This study was supported by General Supportive Grant (IN63217), Khon Kaen University, Thailand 2019 and Research and Diagnostic Center for Emerging Infectious Diseases (RCEID), Khon Kaen University, Thailand.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2022.100374.

CRediT authorship contribution statement

Wicharajit Boonjetsadarukh: Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft. Orawee Kaewprasert: Formal analysis, Investigation. Arnone Nitchanon: Supervision. Pimjai Ananta: Resources. Prajub Chaimaneet: Resources. Kanin Salao: Supervision. Wisitsak Phoksawat: Methodology. Marut Laohaviroj: Supervision. Attawit Sirichoot: Data curation, Formal analysis, Supervision, Writing – review & editing. Yang Fong: Supervision. Suwin Wongwajana: Supervision. Wises Namwat: Supervision. Viraphong Lutiltanond: Supervision. Ploench Chetchotisakd: Resources, Supervision. Kiatichai Faksri: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare there are no competing interests.

Acknowledgments

This study was supported by General Supportive Grant (IN63217), Khon Kaen University, Thailand 2019 and Research and Diagnostic Center for Emerging Infectious Diseases (RCEID), Khon Kaen University, Thailand. We would like to acknowledge Prof. David Blair for editing the manuscript via Publication Clinic KCU, Thailand.

References

[1] S.M. Akram, F.N. Attia, Mycobacterium avium intracellulare, in: StatPearls, StatPearls Publishing LLC, 2021.
I. Kham-Ngam, P. Chetchotisakd, P. Ananta, et al., Differentiation between W. Boonjetsadaruhk et al.

J.M. Bryant, D.M. Grogono, D. Greaves, et al., Whole-genome sequencing to

A.S. Lee, P. Jelfs, V. Sintchenko, et al., Identification of non-tuberculous C.Y. Turenne, D.M. Collins, D.C. Alexander, et al.,

E. Richter, S. Rusch-Gerdes, D. Hilleman, Evaluation of the GenoType

H. Whiley, A. Keegan, S. Giglio, et al., Clinical features of patients with M. Lee, J.Y. Chien, Y.T. Huang, et al., Clinical characteristics, treatment outcomes, S.M. Moon, H.Y. Park, S.Y. Kim, et al., Clinical characteristics, treatment outcomes, treatment outcomes for

J. Ichikawa, J. van Ingen, W.J. Koh, et al., Genetic diversity of clinical H.B. Xu, R.H. Jiang, L. Li, Treatment outcomes for

K. Ichikawa, J. van Ingen, W.J. Koh, et al., Genetic diversity of clinical Mycobacterium avium subsp. hominisuis and Mycobacterium intracellulare isolates causing pulmonary diseases recovered from different geographical regions, Infect. Genet. Evol. 36 (2015) 293–310, https://doi.org/10.1016/j.meegid.2018.12.001.

Y. Nishiuchi, T. Iwamoto, F. Maruyama, Infection sources of a common non-tuberculous mycobacterial pathogen, Mycobacterium avium complex, Front. Med. (Lausanne) 4 (2017) 27, https://doi.org/10.3389/fmed.2017.00027.

J.M. Bryant, D.M. Grogono, D. Greaves, et al., Whole-genome sequencing to identify transmission of Mycobacterium avcusis between patients with cystic fibrosis: a retrospective cohort study, Lancet 381 (2013) 1551–1560, https://doi.org/10.1016/S0140-6736(13)60632-7.

I. Kham-Ngam, P. Chetchotisakd, P. Ananta, et al., Differentiation between persistent infection/colonization and re-infection/re-colonization of Mycobacterium avcusis isolated from patients in Northeast Thailand, Infect. Genet. Evol. 68 (2019) 35–42, https://doi.org/10.1016/j.meegid.2018.12.001.

D.E. Griffith, T. Akramit, B.A. Brown-Elliott, et al., An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases, Am. J. Respir. Crit. Care Med. 175 (2007) 367–416, https://doi.org/10.1164/rccm.200704-471ST.

CLSI (Ed.), Susceptibility testing of mycobacteria, Nocardia spp., and other aerobic actinomycetes, 3rd ed, CLSI standard document M24, Clinical and Laboratory Standards Institute, Wayne, PA, 2018.

L.N. De Almeida, W. Da Silva Carvalho, M.L. Rossetti, et al., Evaluation of six different DNA extraction methods for detection of Mycobacterium tuberculosis by means of PCR-IS6110: preliminary study, BMC Res. Notes 6 (2013) 561, https://doi.org/10.1186/1756-0500-6-561.

A.M. Somily, M.A. Barry, H.A. Habib, et al., Evaluation of GenoXpert MTB/RIF for detection of Mycobacterium tuberculosis complex and rpo B gene in respiratory and non-respiratory clinical specimens at a tertiary care teaching hospital in Saudi Arabia, Saudi Med. J. 37 (2016) 1404–1407, https://doi.org/10.15537/ smj.2016.12.15056.

A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.

H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM, arXiv (2013) 1–3.

H. Li, B. Handaker, A. Wysoker, et al., The sequence alignment/map format and SAMtools, Bioinformatics 25 (2009) 2078–2079, https://doi.org/10.1093/bioinformatics/btp352.

A. McKenna, M. Hanna, E. Banks, et al., The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data, Genome Res. 20 (2010) 1297–1303, https://doi.org/10.1101/gr.107524.110.

D.C. Alexander, T.K. Marras, J.H. Ma, et al., Multilocus sequence typing of Mycobacterium xenopi, J. Clin. Microbiol. 52 (2014) 3973–3977, https://doi.org/10.1128/JCM.01601-14.

E. Macheras, J. Konjek, A.L. Roux, et al., Multilocus sequence typing scheme for the Mycobacterium avcusis complex, Res. Microbiol. 165 (2014) 82–90, https://doi.org/10.1016/j.resmic.2013.12.003.

S. Kumar, G. Stecker, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874, https://doi.org/10.1093/molbev/msw054.

P.R. Hunter, Reproducibility and indices of discriminatory power of microbial typing methods, J. Clin. Microbiol. 28 (1990) 1903–1905, https://doi.org/10.1128/jcm.28.9.1903-1905.90.

H. Parker, R. Lorenz, J. Ruelas Castillo, et al., Mechanisms of antibiotic tolerance in Mycobacterium av cusis complex: lessons from related mycobacteria, Front. Microbiol. 11 (2020), 573983, https://doi.org/10.3389/fmicb.2020.573983.

S. Saxena, H.P. Spaink, G. Forn-Cuni, Drug resistance in nontuberculous mycobacteria: mechanisms and models, Biology (Basel) 10 (2021), https://doi.org/10.3390/biology10020096.

W.J. Koh, B.H. Jeong, K. Jeon, et al., Clinical significance of the differentiation between Mycobacterium avium and Mycobacterium intracellulare in M avcusis complex lung disease, Chest 142 (2012) 1482–1488, https://doi.org/10.1378/chest.12-0494.

H. Tomioka, H. Saito, K. Sato, et al., Comparison of the virulence for mice of Mycobacterium avium and Mycobacterium intracellulare identified by DNA probe test, Microbiol. Immunol. 37 (1993) 259–264, https://doi.org/10.1111/j.1348-0421.1993.tb02081.x.

M.C. Maiden, J.A. Bygraves, E. Feil, et al., Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms, Proc. Natl. Acad. Sci. U. S. A. 98 (1998) 3140–3145, https://doi.org/10.1073/pnas.95.6.3140.

G.J. Njas, L.M. Ng, R. Juren, et al., Development of multiplex PCR assays based on the 16S rRNA internal transcribed spacer for the detection of clinically relevant nontuberculous mycobacteria, Lett. Appl. Microbiol. 52 (2011) 546–554, https://doi.org/10.1111/j.1472-765X.2011.03045.x.

C.L. Daley, Mycobacterium avcusis complex disease, Microbiol. Spectr. 5 (2017), https://doi.org/10.1128/microbiolspec.TM7-0045-2017.

D.M. Yajko, D.P. Chin, P.C. Gonzalez, et al., Mycobacterium av cusis complex in water, food, and soil samples collected from the environment of HIV-infected individuals, J. Acquir. Immune Defic. Syndr. Hum. Retrovir. 9 (1995) 176–182.

D. Jeon, Infection source and epidemiology of nontuberculous mycobacterial lung disease, Tuberc. Respir. Dis. (Seoul). 82 (2019) 94–101, https://doi.org/10.4046/tuberd.2018.0026.

M. Zulu, N. Monde, P. Nkhoma, et al., Nontuberculous mycobacteria in humans, animals, and water in Zambia: a systematic review, Front. Trop. Dis. 2 (2021), https://doi.org/10.3389/ftd.2021.679501.

G. Sebastian, S.B. Nagaraja, T. Vishwanatha, et al., Identification of non-tuberculous mycobacterium by LPA (CM/AS) assay, HPLC and biochemical test: which is feasible for RNTCP? Indian J. Tuberc. 65 (2018) 329–334, https://doi.org/10.1016/j.jitb.2018.08.003.

C.B. Inderfied, C.A. Kemper, L.E. Bermudez, The Mycobacterium avcusis complex, Clin. Microbiol. Rev. 6 (1993) 266–310, https://doi.org/10.1128/CMR.6.3.266.