Thesis progression

Thesis title: Comparative genotypic and phenotypic drug susceptibility analysis of the *Mycobacteroides abscessus* complex using whole-genome sequencing

Thesis progression title: Mobile genetic elements detection and genotypic drug susceptibility testing analysis

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1. Introduction

The *Mycobacteroides abscessus* complex (MABC) is a group of rapidly growing nontuberculous mycobacteria (NTM) that has emerged as a significant opportunistic pathogen worldwide. MABC is responsible for a wide range of pulmonary and extrapulmonary infections, particularly among immunocompromised individuals. Infections caused by MABC are notoriously difficult to treat due to the organism's high intrinsic resistance to a broad spectrum of drugs, complex pathogenic mechanisms, and substantial genetic diversity. Moreover, MABC exhibits distinct, region-specific patterns of drug susceptibility and virulence across different geographic settings. Although drug therapy remains the cornerstone of treatment, clinical outcomes are frequently unsatisfactory, with high rates of treatment failure and relapse. This limited therapeutic success is largely attributed to the bacterium's persistent nature and multiple resistance mechanisms, including its lipid-rich cell wall and its ability to form biofilm. Consequently, the rising global incidence of MABC infections represents an increasing public health concern (Jin et al., 2022).

NTM are broadly classified into two groups: rapid-growing mycobacteria, such as MABC, which typically form visible colonies within seven days, and slow-growing mycobacteria, such as the *Mycobacterium avium* complex, which may require several weeks for growth. Members of MABC are acid-fast, rod-shaped bacteria ubiquitously distributed in environmental reservoirs, including soil and water. Clinically, MABC is associated with a broad spectrum of disease manifestations, ranging from pulmonary infections to skin and soft tissue infections, lymphadenitis, bone-joint infections, and, in severe cases, disseminated infections (Pennington et al., 2021).

Taxonomically, the MABC comprises three closely related subspecies: *M. abscessus* subsp. *abscessus* (*M. abscessus*), *M. abscessus* subsp. *massiliense* (*M. massiliense*), and *M. abscessus* subsp. *bolletii* (*M. bolletii*). These subspecies exhibit distinct drug susceptibility profiles, further complicating clinical management and treatment selection. Diagnosis of MABC infections remains challenging due to non-specific clinical presentations that vary by subspecies. In addition, standardized treatment guidelines regarding optimal drug regimens, dosing strategies, and treatment duration are constrained by limited and heterogeneous drug susceptibility data. Consequently, molecular methods are increasingly essential for accurate subspecies identification and genomic characterization, both of which are fundamental for effective diagnostic and therapeutic decision-making (Griffith et al., 2007; Pennington et al., 2021).

Whole-genome sequencing (WGS) has emerged as a powerful approach for comprehensive genomic analysis, enabling the identification of genotypic variants and novel mutations associated with drug resistance. Compared with conventional culture-based methods, WGS offers the potential to predict drug susceptibility profiles more rapidly and with greater resolution (Realegeno et al., 2021). Some previous studies had conducted comparative genomic analysis on MABC in antibiotic resistance, genome annotations, mobile genetic elements analysis, and core-SNPs phylogenetic analysis from whole-genome sequencing technologies and bioinformatics analysis, which revealed valuable insights on the diversity, virulence, and resistance nature of the MABC, including significant variation within species in genes encoding antimicrobial resistance, virulence, mobile genetic elements, and sequence typing (Jin et al., 2022). In Thailand, studies on *Mycobacteroides abscessus* complex are still limited, including a low number of Thai MABC isolates, and the intraspecies genomic diversity and resistance mechanisms of MABC remain poorly understood and have primarily focused on targeted analyses of known resistance-associated genes, including *erm*(41), *rrl*, *rrs*, *rplC*, *rplD*, *gyrA*, and *gyrB*. Although several studies have demonstrated partial concordance between phenotypic and genotypic drug susceptibility, known resistance-conferring mutations have often failed to explain phenotypic resistance to key drug classes, such as aminoglycosides, macrolides, linezolid, and fluoroquinolones. Many isolates exhibit elevated minimum inhibitory concentration (MIC) values despite lacking established resistance markers, and synonymous mutations have been observed in both susceptible and resistant phenotypes. These findings suggest that resistance may involve uncharacterized regulatory pathways or novel genetic mechanisms (Kaewprasert et al.,

2022; Prommi et al., 2025; Sukmongkolchai et al., 2023). Furthermore, existing studies in Thailand have not yet incorporated comprehensive genomic analyses, including genome annotation, sequence typing, systematic screening for virulence factors, mobile genetic elements, and mutations in regulatory genes and other determinants (e.g., *eis2*, *aac(2')*, and *whiB7*). These knowledge gaps limit current understanding of the resistance landscape and hinder accurate prediction of phenotypic drug susceptibility based on genotypic data.

Therefore, the present study aimed to perform a comprehensive genomic characterization of clinical MABC isolates in Thailand, with a focus on genomic diversity and the identification of novel mutations in resistance-associated genes. By integrating WGS data with phenotypic drug susceptibility profiles, this study sought to enhance diagnostic accuracy and support more informed therapeutic decision-making for MABC infections.

2. Objective

2.1 To investigate the genetic mutation that confers drug resistance in MABC Thai isolates using whole-genome sequencing analysis.

2.2 To study the relationship between phenotypic and genotypic drug susceptibility patterns in MABC.

3. Conceptual framework

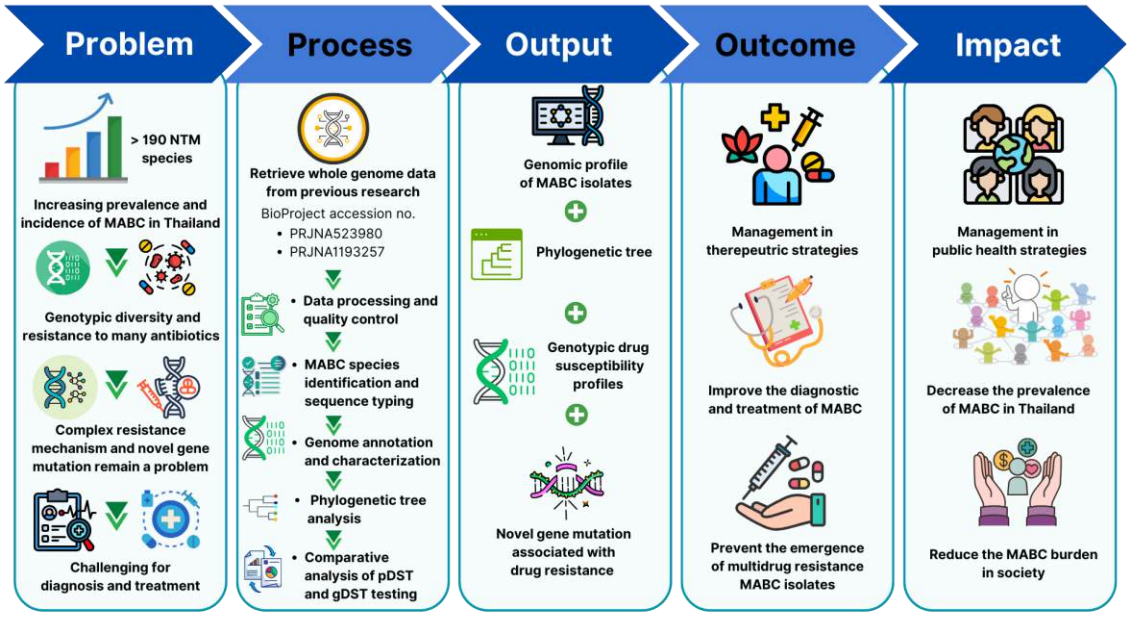

Figure 1 The conceptual framework of this research

The *Mycobacteroides abscessus* complex (MABC) is increasingly prevalent worldwide, with a high potential for both intrinsic and acquired drug resistance, limited options for effective therapeutic regimens, and a persistent nature and substantial diversity between isolates, often leading to prolonged treatment and unsatisfactory outcomes. This project aims to conduct a comparative analysis of phenotypic and genotypic drug susceptibility in MABC, applying whole-genome sequencing technologies and bioinformatics pipelines to study bacterial isolate diversity, genomic characterization, and to detect genomic mutations in genes associated with drug resistance that affect phenotypic drug susceptibility. The findings could support diagnosis and treatment management and potentially lead to a decrease in the prevalence of MABC in Thailand (**Figure 1**)

4. Materials and methods

4.1 Study design

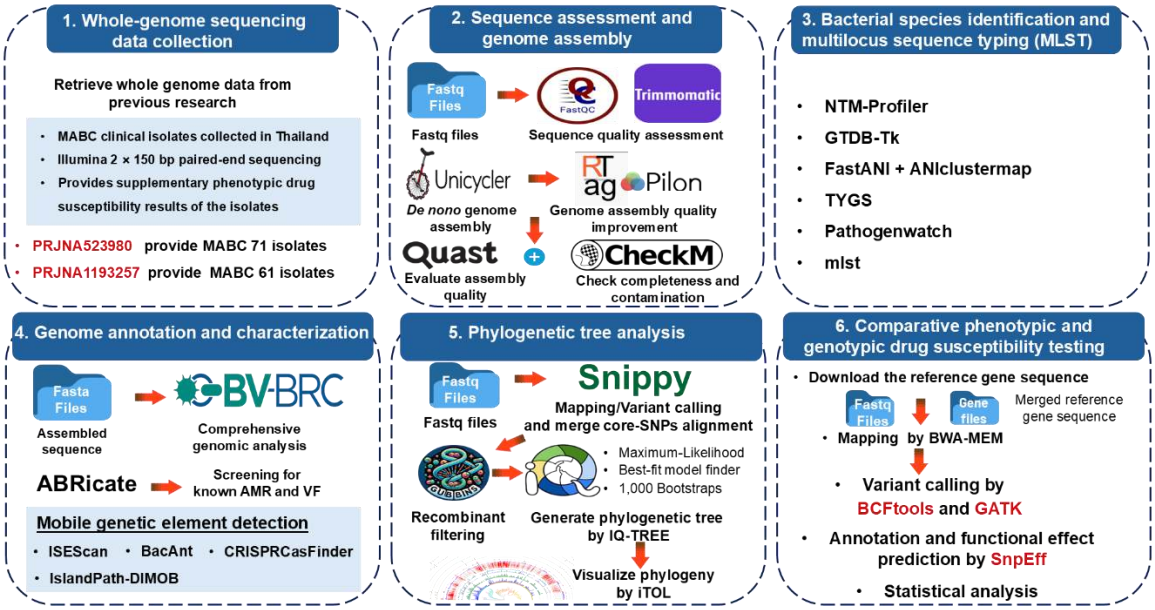

Figure 2 The study design of this research

4.2 Bacterial isolates

This study analyzed Illumina 2 × 150 bp paired-end short read sequencing data (FASTQ files) obtained from previous investigations. A total of 132 publicly available MABC isolates

were retrieved from the Sequence Read Archive (SRA) under BioProject accession no. PRJNA523980 (Kaewprasert et al., 2022) and PRJNA1193257 (Prommi et al., 2025). These isolates were originally recovered from patients with NTM-infections at Srinagarind Hospital, Khon Kaen University, and King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok, Thailand. Clinical specimens were obtained from both pulmonary and extrapulmonary sources.

Phenotypic drug susceptibility testing (pDST) had been previously performed using the broth microdilution method with the Sensititre™ Rapid Growing Myco RAPMYCOI plate (TREK Diagnostic Systems, West Sussex, UK). Minimum inhibitory concentration (MIC) values were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) M24, 3rd edition guidelines. These pDST data were used for comparative phenotypic and genotypic analyses in the present study. This study was approved by the Institutional Review Board of the Khon Kaen University Ethics Committee in Human Research (No. HE681705; approved on 20 November 2025). All procedures were conducted in accordance with the Declaration of Helsinki and the International Council for Harmonisation (ICH) Good Clinical Practice guidelines.

4.3 Sequencing quality assessment and genome assembly

Raw sequencing read quality was assessed using FastQC version 0.12.1 (Andrews, 2010). Low-quality reads (length <75 bp) and adapter sequences were trimmed using Trimmomatic version 0.40 (Bolger et al., 2014) with the following parameters: LEADING:3, TRAILING:3, SLIDINGWINDOW: 4:15, and MINLEN:75. High-quality filtered reads were retained for downstream analyses.

De novo genome assembly was performed using Unicycler version 0.5.1 (Wick et al., 2017). Assembly quality was further improved using RagTag version 2.1.0 (Alonge et al., 2022) and polished with Pilon version 1.24 (Walker et al., 2014). Assembly metrics were evaluated using QUAST version 5.3.0 (Gurevich et al., 2013), while genome completeness and contamination were assessed using CheckM version 1.2.3 (Parks et al., 2015). Only assemblies exhibiting >95% completeness and <5% contamination were retained for subsequent analyses.

4.4 Subspecies identification and multilocus sequence typing (MLST)

Initial species identification of MABC isolates from filtered FASTQ files was performed using NTM-Profiler version 0.6.1 (Phelan, 2021) and validated at the assembly level using GTDB-Tk version 2.5.2 (Chaumeil et al., 2020). Subspecies identification was conducted using FastANI version 1.34 (Jain et al., 2018), and pairwise average nucleotide identity (ANI) values were visualized as a heatmap using ANIclustermap version 2.0.1 (Shimoyama, 2022). ANI comparisons were performed against reference genomes, including *M. abscessus* subsp. *abscessus* ATCC 19977 (NCBI accession number: GCA_000069185.1), *M. abscessus* subsp. *massiliense* CCUG 4484 (GCA_002086375.1), and *M. abscessus* subsp. *bolletii* BD (GCA_003609715.1). Additional subspecies validation was performed using the Type Strain Genome Server (TYGS) version 402 (Meier-Kolthoff & Göker, 2019), applying a digital DNA-DNA hybridization (dDDH) threshold of >70% for subspecies delineation. Sequence types (STs) were determined using Pathogenwatch version 23.5.0 (available on https://pathogen.watch/) and mlst version 2.23.0 (Seemann, 2017).

4.5 Genome annotation and characterization

Assembled genomes were uploaded to the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) for comprehensive genomic analysis (Olson et al., 2022). Genome annotation was performed using the RAST toolkit (RASTtk) pipeline to characterize protein-coding genes and functional subsystems.

Antibiotic resistance genes (ARGs) and virulence factors were identified by screening against multiple databases, including the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017), the Virulence Factor Database (VFDB) (Chen et al., 2016), National Center for Biotechnology Information (NCBI) (NCBI Resource Coordinators, 2016), ResFinder (Florensa et al., 2022), ARG-ANNOT (Gupta et al., 2014), and MEGARes (Lakin et al., 2017). Screening was performed using ABRicate version 1.2.0 (Seemann, 2020) with a default parameters.

4.6 Mobile genetic elements analysis

Mobile genetic elements (MEGs) were identified using multiple bioinformatics tools. Insertion sequences were detected using ISEscan version 1.7.3 (Xie & Tang, 2017). Transposable elements were identified using BacAnt version 3.4.0 (Hua et al., 2021) with a minimum identity threshold of 90% and a coverage cutoff of 60%. Clustered regularly interspaced short palindromic

repeats (CRISPR) and CRISPR-associated (Cas) systems were identified using CRISPRCasFinder version 1.1.2 (Couvin et al., 2018). Genomic islands were detected using IslandPath-DIMOB version 1.0.6 (Bertelli & Brinkman, 2018) by using the annotated GenBank files generated by the BV-BRC pipeline as the input.

4.7 Single-nucleotide polymorphisms (SNP)-based phylogenetic tree analysis

Filtered reads were mapped to the *M. abscessus* ATCC 19977 reference genome (NC_010397.1). Haploid variant calling and core-genome alignment were performed using Snippy version 4.6.0 (Seemann, 2020), generating a core alignment of 5,067,172 bp. Putative recombination regions were identified and removed using Gubbins version 3.4.1 (Croucher et al., 2015), resulting in a recombination-free SNP alignments of 224,443 bp. Maximum-likelihood phylogenetic reconstruction was performed using IQ-TREE version 2.4.0 (Nguyen et al., 2015) with the best-fit nucleotide substitution model and 1,000 ultrafast bootstrap replicates. Phylogenetic tree was visualized using iTOL version 7 (Letunic & Bork, 2007). Pairwise SNP distances were calculated using snp-dists version 0.8.2 (Seemann, 2021).

4.8 Detection of genetic mutations associated with drug resistance

This study focused on four drugs with defined CLSI M24 breakpoints and have both susceptible and non-susceptible isolates: amikacin, clarithromycin, linezolid, and moxifloxacin. Resistance-associated genes analyzed included *erm*(41), *rrs*, *rrl*, *rplC*, *rplD*, *rplV gyrA*, *gyrB, eis1, eis2, aac(2'), tetR,* and *whiB7*. Reference sequences for these genes were obtained from the *M. abscessus* ATCC 19977.

Sequencing reads were mapped to reference gene sequences using BWA-MEM version 0.7.19 (Li & Durbin, 2009). Resulting SAM files were converted to BAM format, sorted, and indexed using SAMtools version 1.23 (Danecek et al., 2021). GATK version 3.4.0 (McKenna et al., 2010) was used for sequence realignment and evaluation of coverage depth and mapping quality. Variant calling was performed using both BCFtools version 1.23 (Danecek et al., 2021) and GATK to identify SNPs and insertions/deletions (indels). Variants were filtered, normalized, and re-headered using BCFtools, and SNP variant annotation and functional effect prediction were performed using SnpEff version 5.4.0 (Cingolani et al., 2012). Amino acid substitutions were identified, and variants enriched among drug-resistant isolates were evaluated. Phenotype–

genotype correlations were assessed to identify potential novel mutations associated with drug resistance.

4.9 Statistical analysis

Continuous variables are presented as the mean ± standard deviation, followed by the range (minimum–maximum). Categorical variables are reported as frequencies and percentages. Comparisons of drug resistance rates among MABC subspecies and associations between phenotypic drug susceptibility and specific genetic variants were analyzed using the Chi-squared test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

5. Results

5.1 Study population and phenotypic drug susceptibility testing

Of the initial 132 isolates, 124 met the inclusion criteria, with each genome exhibiting >95% completeness and <5% contamination. Taxonomic identification using NTM-Profiler and GTDB-Tk, and Pathogenwatch confirmed that all isolates belonged to the MABC. Further genomic analyses using FastANI (ANI >98%) and TYGS (dDDH >89%) consistently classified the isolates into three subspecies: *M. abscessus* (n = 65/124, 52.42%), *M. massiliense* (n = 53/124, 42.74%), and *M. bolletii* (n = 6/124, 4.84%).

Multilocus sequences typing (MLST), performed using PubMLST via the mlst tool, yielded multiple sequence types (STs). We can assign sequence types to 51 of 124 isolates. The most prevalent groups included ST37 (n = 11), ST39 (n = 11), and ST38 (n = 6)

pDST data were available for 117 of the 124 isolates. Comparative analyses focused on four clinically relevant antimicrobials: amikacin, linezolid, clarithromycin, and moxifloxacin. High susceptibility was observed for amikacin (100/117, 85.47%), whereas resistance was predominant for linezolid (64/117, 54.70%) and moxifloxacin (112/117, 95.73%). Clarithromycin resistance increased markedly from Day 3 (22/117, 18.80%) to Day 14 (71/117, 60.68%), with inducible resistance detected in 46 isolates (39.32%).

Based on subspecies distribution, the highest susceptibility was still obtained from amikacin, shown in *M. bolletii* (100%), *M. massiliense* (93.48%), and *M. abscessus* (78.46%). linezolid resistance is quite high among the subspecies, except for *M. bolletii.* For clarithromycin,

the *M. massiliense* isolates tend to be more susceptible (73.91%) compared to the other two subspecies. Finally, all three subspecies display high moxifloxacin resistance; both *M. massiliense* and *M. bolletii* isolates indicate 100% resistance, while *M. abscessus* shows 92.30% resistance among the isolates.

5.2 Mobile genetic elements analysis

Analysis of mobile genetic elements (MGEs) identified 15 insertion sequence (IS) families, comprising a total of 676 IS elements across the 124 genomes. Within the subspecies *M. abscessus*, *M. massiliense*, and *M. bolletii* there are 408, 250, and 18 IS families, respectively. The most prevalent families were IS21 (175 elements), IS481 (136 elements), and IS701 (125 elements). No transposable elements met the stringent BacAnt filtering criteria (≥90% identity and ≥60% coverage); however, several low-confidence transposable fragments were detected.

Clustered regularly interspaced short palindromic repeat (CRISPR-Cas) system analysis revealed a heterogeneous distribution, with 0 to 4 CRISPR loci predicted per genome. In addition, a total of 592 genomic islands (an average of 4.77 ± 1.93 per genome) with the size of individual genomic islands ranging from 3,402 bp to 93,927 bp were identified across the isolates, except for isolate AB826491, in which no genomic islands were detected (**Tables 1 and 2**).

5.3 Mutations in genes associated with phenotypic drug resistance

For amikacin, the resistance-associated genes include *rrs*, *eis1* (MAB_4124), *eis2* (MAB_4532c), *aac(2')* (MAB_4395), and *whiB*7 (MAB_3508c). Only *rrs* gene mutation was detected, an A1382G mutation found in three non-susceptible (intermediate and resistant) *M. abscessus* isolates.

For linezolid, *rrl*, *rplC*, *rplD*, *rplV*, and MAB_2885 genes were investigated. Several *rrl* gene mutations were detected uniquely in non-susceptible isolates, including C1558A, C1831T, A2272G, A2293C, C2570T, and C2697T in *M. abscessus* isolates and A843T, A2272G, G2651C, and C2785T in *M. massiliense* isolates. But no mutations were detected for *M. bolletii* isolates. Additionally, mutations in the *rplC* gene were detected, including two non-synonymous mutations, G150C (Ala51Pro) and C177A (Pro60Thr), and one synonymous mutation, C324A. In *rplD,* a C150A non-synonymous mutation (Gln51Lys) was found in three non-susceptible *M. abscessus* isolates. No *rplV* mutation was found. Several mutations were also found in the

MAB_2885 gene locus, including Gly9Glu, Ala129Thr, Glu170Gln, and His182Tyr found in M. abscessus isolates, and His141Tyr mutation was found in *M. massiliense* isolates.

For clarithromycin, several non-synonymous mutations were detected in the *erm*(41) genes, unique to non-susceptible *M. bolletii* isolates, including Cys78Arg, Gly84Arg, Pro85His, Ala86Ser, Val90Ala, Ser105Arg, and Ser139Gly, and only one mutation was detected in *M. abscessus*. Mutations in *rrl* genes include A843T, A2272G, and A2273C. Several *whiB7* non-synonymous mutations were detected, including Gly36Ser and Arg93Cys mutations in *M. abscessus* isolates and two mutations in *M. bolletii* isolates, which are Ala87Thr and Arg89Cys

For moxifloxacin, fluoroquinolone resistance determinant *gyrA* and *gyrB* were analyzed. Several non-synonymous mutations were found (**Table 3**).

6. Conclusion

This study provides valuable insight into the distribution details of mobile genomic elements and nucleic acid substitution among the MABC clinical isolates. The multiple IS families in total 676 were identified across 124 isolates, with the detection of 592 genomic islands (an average of 4.77 ± 1.93 per genome), and the presence of CRISPR sequences, which may influence genetic rearrangement associated with bacterial diversity and adaptability. The detection of nucleic acid and protein substitution in genes associated with antimicrobial resistance is crucial for genotypic drug susceptibility analysis, providing evidence of the antimicrobial resistance mechanism of the bacteria, which could be useful for the selection of effective drugs and preventing the occurrence of resistance isolates.

Furthermore, the detection of multiple nucleic acid substitutions with mutations that cause a change to an amino acid (non-synonymous mutation) is potentially strong evidence of the genotypic drug resistance determinant, but to confirm if there is any association of these mutations with resistance phenotypes and concordance with phenotypic drug susceptibility profiles, further proof with statistical analysis is required.

Table 1 The insertion sequences (IS) families identified across 124 MABC clinical isolates.

IS families	Total (n = 124)	*M. abscessus* (n = 65)	*M. massiliense* (n = 53)	*M. bolletii* (n = 6)
IS701	125	66	53	6
IS21	175	71	98	6
IS481	136	74	58	4
IS3	67	50	15	2
IS110	31	20	11	0
IS256	91	89	2	0
ISNCY	6	6	0	0
ISL3	7	5	2	0
IS30	6	6	0	0
IS1380	18	18	0	0
IS200/IS605	7	1	6	0
new	2	2	0	0
IS5	3	0	3	0
IS1634	1	0	1	0
IS1182	1	0	1	0
Total	**676**	**408**	**250**	**18**

Table 2 Summarize table for mobile genetic element (MGEs) analysis.

Mobiles genetic element	Total (n = 124)	*M. abscessus* (n = 65)	*M. massiliense* (n = 53)	*M. bolletii* (n = 6)
IS families	676	408	250	18
Transposons	NA	NA	NA	NA
Genomic island	592	302	268	22
CRISPR	0-4	0-3	0-4	0-3

Table 3 Detected single-nucleotide polymorphism (SNPs) variant mutation associated with amikacin (AMK), linezolid (LZD), clarithromycin (CLA), and moxifloxacin resistance in *Mycobacteroides abscessus* complex (MABC).

Antibiotics	**Genes**	**Nucleic acid substitution found in a non-susceptible isolate (Position inside a reference gene)[a]**		
		***M. abscessus* (n = 65)**	***M. massiliense* (n = 46)**	***M. bolletii* (n = 6)**
Amikacin	*rrs*	A1382G	Not found	Not found
	eis1	Not found	Not found	Not found
	eis2	Not found	Not found	Not found
	aac(2')	Not found	Not found	Not found
	WhiB7	Not found	Not found	Not found
Linezolid	*rrl*	C1558A, C1831T, A2272G, A2293C, C2570T, C2697T	A843T, A2272G, G2651C, C2785T	Not found
	rplC	G150C, C177A, C324A	Not found	Not found
	rplD	C150A	Not found	Not found
	rplV	Not found	Not found	Not found
	MAB_2885	G25A, C282T, G384A, G507C, C543T	C420T	Not found
Clarithromycin	*erm*(41)	A120G, G158A, C419T	Not found	T231C, G249A, C253A, G255T, T268C, A312C, A414G
	rrl	A2272G, A2273C	A843T, A2272G	Not found
	WhiB7	G105A, C222T, C276T	Not found	G258A, C264T
Moxifloxacin	*gyrA*	C201G, C231A, G286A, C384T, T502C, G579A, T651C, C690T, G798A, C864G, A957G, T969C, G990A, G1071A, G1074A, T1077G, G1158A, C1191T, G1233T, G1269A, C1500T, C1503T, C1518T, T1545G, C1647T, T1662C, T1737C, G1818C, C1842T, C1884T, G1992C, T2016C, T2160C, A2319G, C2331T, A2349T, A2349C, G2373A	T108C, C132G, C141G, C147T, G243A, T258C, G378A, C381T, C486T, C585T, T591C, C747T, C864G, C900T, C939T, A957G, C972T, C999T, G1017A, G1071A, C1251T, C1287G, C1341T, C1458T, C1485T, C1599T, C1629T, T1662C, G1695A, T1737C, G1749A, T1752C, C1827G, G1866A, G2004A, T2160A, T2160C, C2166T, C2176T, A2349C, C2352T, C2358T, G2373A, G2430A	C129T, T252C, T258C, C864G, C867T, C999T, G1071A, C1182A, C1434T, T1662C, C1707T, T1897C, C2034T, T2097C, T2103G, C2136T, C2148T, T2241C, T2247G, A2349C, G2373A, C2427T

	gyrB	G12A, T57C, C69T, C87G, G126A, G171C, G381A, A513C, C657T, G684A, T711C, C745A, C753T, C783T, C828G, C1014T, C1101T, C1113G, T1263C, C1599T, G1743A, C1818G, C1893T, C1893G, A1911G, C1917T	T42C, C69G, T75C, C90G, T132C, C159T, C405T, G435A, C510G, A513C, G606A, C678T, T768G, A990G, G1002A, G1005A, G1029C, C1041G, C1053T, C1125T, G1152A, C1182T, G1260A, T1263C, C1287T, A1356G, C1401G, T1410C, C1425T, G1437C, C1446A, G1533C, G1569C, C1599T, T1659C, T1702C, C1727G, G1734A, C1785A, C1818T, G1839A, A1896G, C1923T	G20A, T132C, G273C, T418C, G477A, A513C, G573A, C579G, C600G, C657G, T711C, T855C, A990G, G1005A, G1029C, T1089C, T1263C, G1311A, A1344G, T1407C, C1413T, G1533C, C1572T, C1599T, T1702C, G1782A, C1794T, C1815T, T1827C, G1839A, C1842T, T1851C, G1890T, A1911G, T1959C
Antibiotics	**Protein**	**Amino acid substitution profiles in resistance isolates (Position inside a reference gene)**		
		***M. abscessus* (n = 65)**	***M. massiliense* (n = 46)**	***M. bolletii* (n = 6)**
Linezolid	50S ribosomal protein L3	Ala51Pro, Pro60Thr	Not found	Not found
	50S ribosomal protein L4	Gln51Lys	Not found	Not found
	MAB_2885	Gly9Glu, Ala129Thr, Glu170Gln, His182Tyr	His141Tyr	Not found
Clarithromycin	Erm(41)	Arg41Gly	Not found	Cys78Arg, Gly84Arg, Pro85His, Ala86Ser, Val90Ala, Ser105Arg, Ser139Gly
	WhiB	Gly36Ser, Arg93Cys	Not found	Ala87Thr, Arg89Cys

Moxifloxacin	GyrA	Pro68Ala, Leu78Ile, Arg96Gln, His129Tyr, Val168Ala, Ala194Thr, Ser218Pro, Pro231Ser, Gly267Arg, His289Asp, Thr320Ala, Cys324Arg, Glu331Lys, Ala358Thr, Ala359Thr, Ser360Ala, Glu387Lys, Gly412Cys, Asp424Asn, His501Tyr, Leu502Phe, Arg507Cys, Arg550Trp, Ser580Pro, Gly607Arg, His610Asp, Pro615Ser, Ala623Thr, His629Tyr, Leu633Pro, Gly665Arg, Gly669Ser, Ser673Pro, Ile774Val, Arg778Cys, Arg784Trp, Ala792Thr	Arg24Gly, Cys37Arg, Arg45Gly, Arg48Gly, Pro50Ser, Asp82Asn, Ser87Pro, Gly127Ser, Arg128Cys, His163Tyr, Pro196Ser, Pro250Ser, His289Asp, His301Tyr, Pro314Ser, Thr320Ala, Arg325Cys, Ala340Thr, Ala358Thr, His418Tyr, Arg430Gly, Pro448Ser, Pro496Ser, Ala566Thr, Ser580Pro, Val584Ile, His610Asp, Ala623Thr, Gly669Ser, His723Tyr, Ala726Val, Arg785Cys, Pro787Ser, Ala792Thr, Gly811Arg	Pro44Ser, Ser87Pro, His289Asp, Arg290Cys, Ala358Thr, Arg479Cys, Pro570Ser, Leu633Pro, Pro679Ser, Phe700Leu, Cys702Gly, Leu713Phe, Trp748Arg, Ala792Thr
	GyrB	Glu5Lys, Ser20Pro, Pro30Ala, Ala43Thr, Asp58His, Gly128Ser, Thr172Pro, Gly229Ser, Cys238Arg, Pro249Gln, Pro252Ser, Arg277Gly, Gln372Glu, Val582Ile, His607Asp, Arg632Cys, Arg632Gly, Thr638Ala	Cys26Arg, Pro30Ala, Arg31Gly, Ala43Thr, Ser45Pro, Arg54Cys, Val146Met, Arg171Gly, Thr172Pro, Gly203Arg, Arg227Cys, Ser257Ala, Ile331Val, Glu335Lys, Glu336Lys, Glu344Gln, Arg348Gly, Arg376Trp, Ala385Thr, Ala421Thr, Ile453Val, Gln468Glu, Ser471Pro, Pro476Ser, Ala480Pro, Gly512Arg, Val524Leu, Ser554Pro, Val568Ala, His576Gln, Gly579Arg, Gln596Lys, His607Tyr, Gly614Arg, Thr633Ala, Arg642Cys	Ser45Pro, Gly92Arg, Leu140Pro, Glu160Lys, Thr172Pro, Ala192Thr, Pro194Ala, Gln201Glu, Arg220Gly, Cys238Arg, Ile331Val, Glu336Lys, Glu344Gln, Ala438Thr, Arg449Gly, Trp470Arg, Gly512Arg, Pro525Ser, Val568Ala, Ala595Thr, Arg606Trp, Gly614Arg, Cys618Arg, Ala631Ser, Thr638Ala

[a] Position on the gene of a reference based on *Mycobacteroides abscessus* ATCC 19977 (NC_010397.1).

Not found, no nucleotide mutation that is unique only to non-susceptible *M. abscessus* complex isolates was found.

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