Department of Microbiology, Faculty of Medicine, Khon Kaen University

GENOMIC CHARACTERIZATION AND DRUG SUSCEPTIBILITY PROFILES OF *MYCOBACTERIUM KANSASII* : INSIGHTS FROM WHOLE-GENOME SEQUENCING STUDIES IN ASIA

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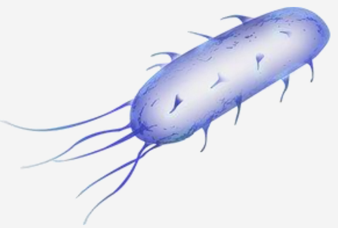
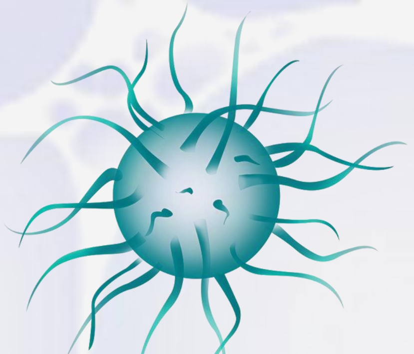
Date: 18 February 2026





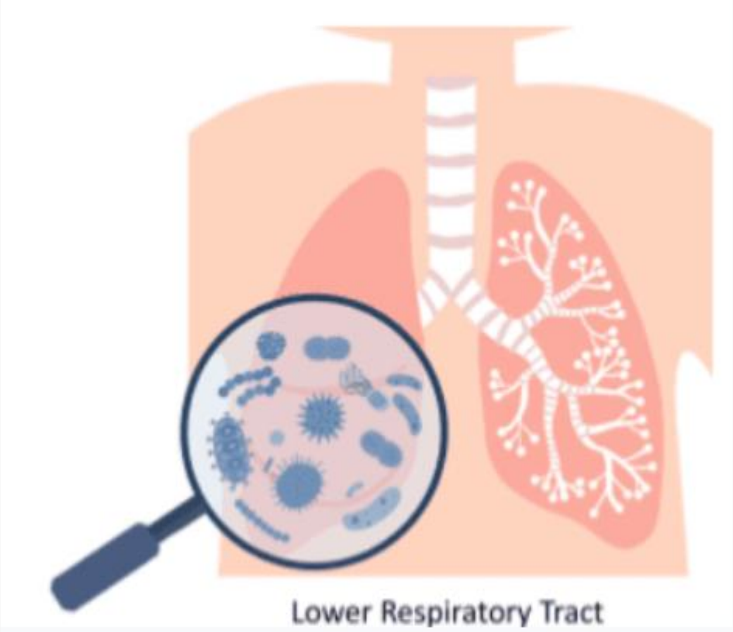
Contents

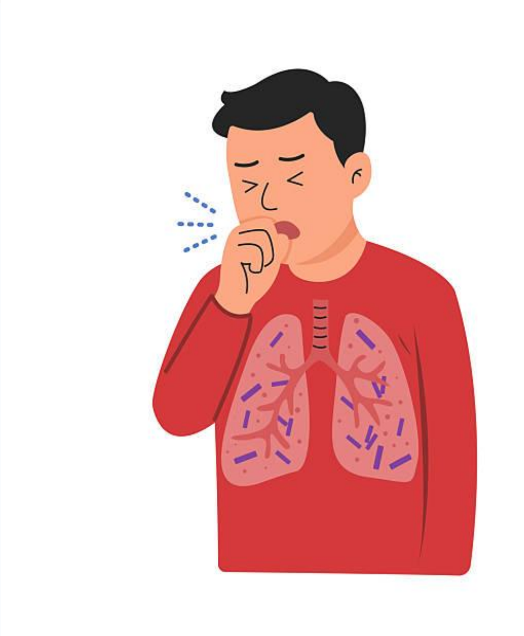
Introduction

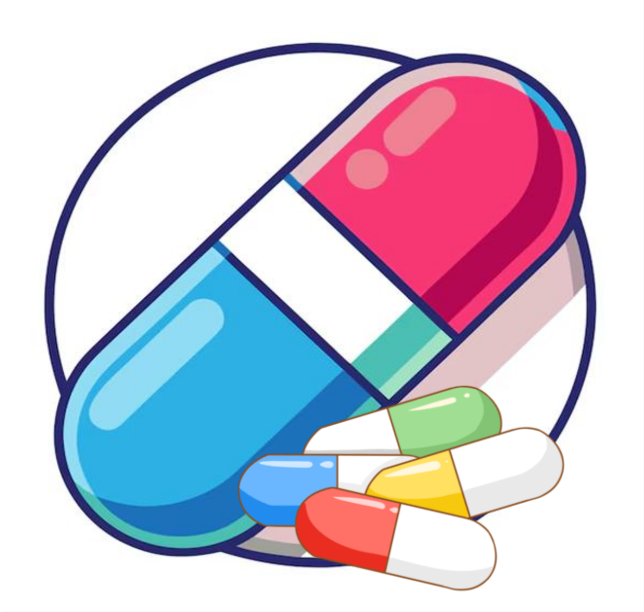
NON-TUBERCULUS MYCOBACTERIA (NTM)

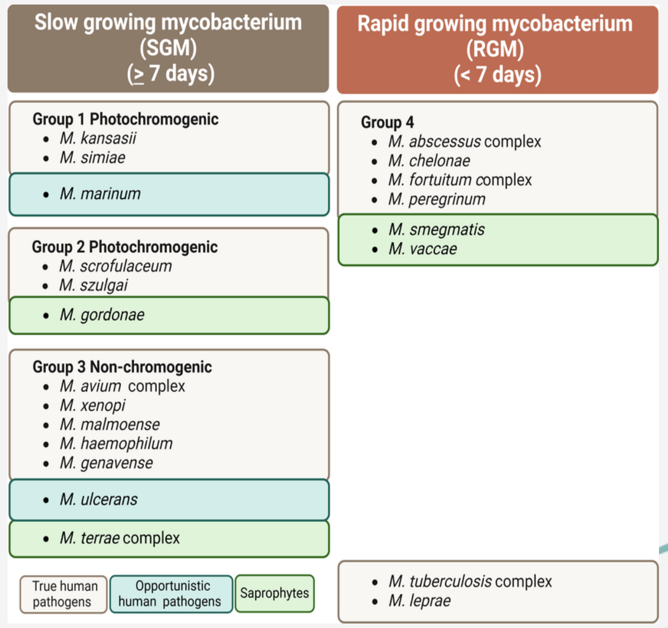
- Aerobic, Non-motile
- Acid-fast Bacilli
- >180 species
- Rapid growing, Slow growing
- Found in environment
- Difficult to diagnosis
- Multi-drugs resistant
- Pneumonia
- Opportunistic infection

(Parte et al., 2020)







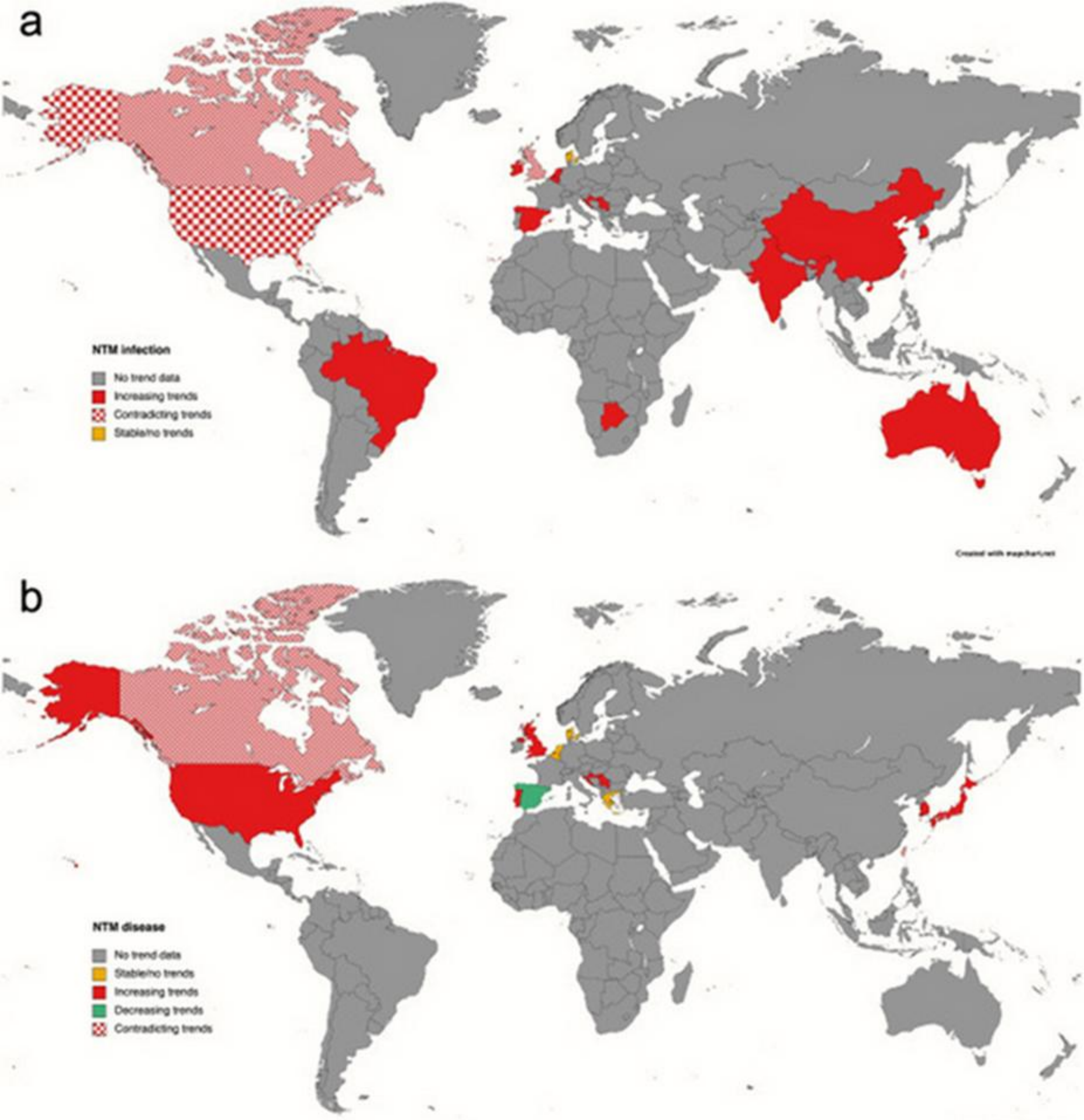


Slow growing mycobacterium (SGM) (≥ 7 days)	Rapid growing mycobacterium (RGM) (< 7 days)
Group 1 Photochromogenic • *M. kansasii* • *M. simiae*	**Group 4** • *M. abscessus* complex • *M. chelonae* • *M. fortuitum* complex • *M. peregrinum*
• *M. marinum*	• *M. smegmatis* • *M. vaccae*
Group 2 Photochromogenic • *M. scrofulaceum* • *M. szulgai*	
• *M. gordonae*	
Group 3 Non-chromogenic • *M. avium* complex • *M. xenopi* • *M. malmoense* • *M. haemophilum* • *M. genavense*	
• *M. ulcerans*	
• *M. terrae* complex	
True human pathogens Opportunistic human pathogens Saprophytes	• *M. tuberculosis* complex • *M. leprae*

(RUNYON, 1959)

Introduction

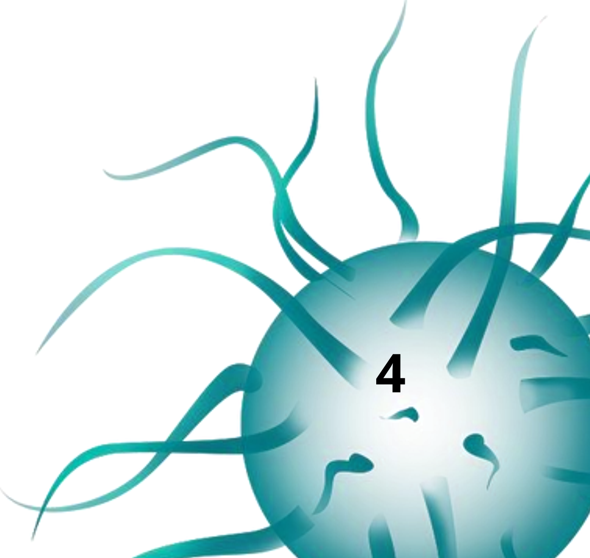
Trend of NTM in global



(Dahl et al. 2022)

Global rise in reported NTM-associated for both infection and disease (NTM infection 4.0%, NTM disease 4.1%)

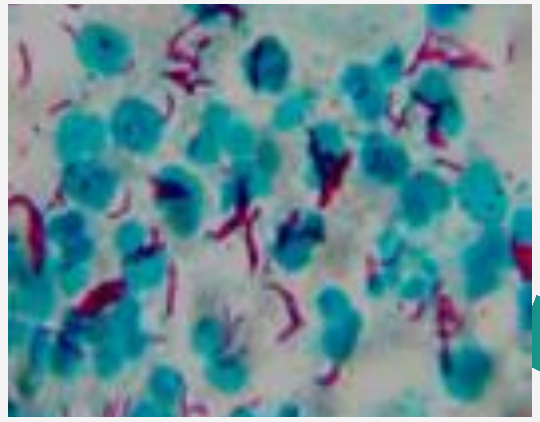
(Dahl et al.2022)



Introduction

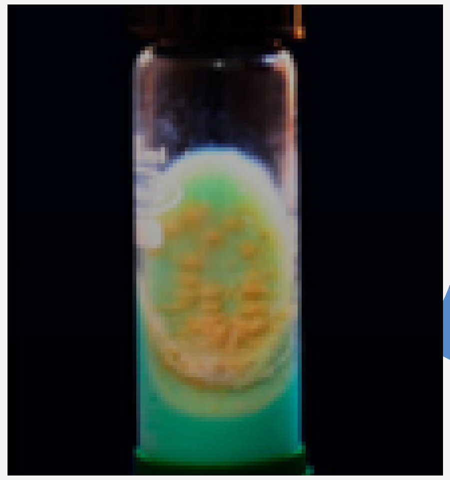
Mycobacterium kansasii

- 01 Slow-growing NTM
- 02 *Mycobacterium kansasii* complex (MKC) member
- 03 Commonly found in tap and municipal water systems (Vaerewijck et al., 2005).
- 04 First isolation location in Kansas City, USA (Hauduroy, 1955)
- 05 Causing pulmonary infections similar to tuberculosis



***Mycobacterium* kansasii**

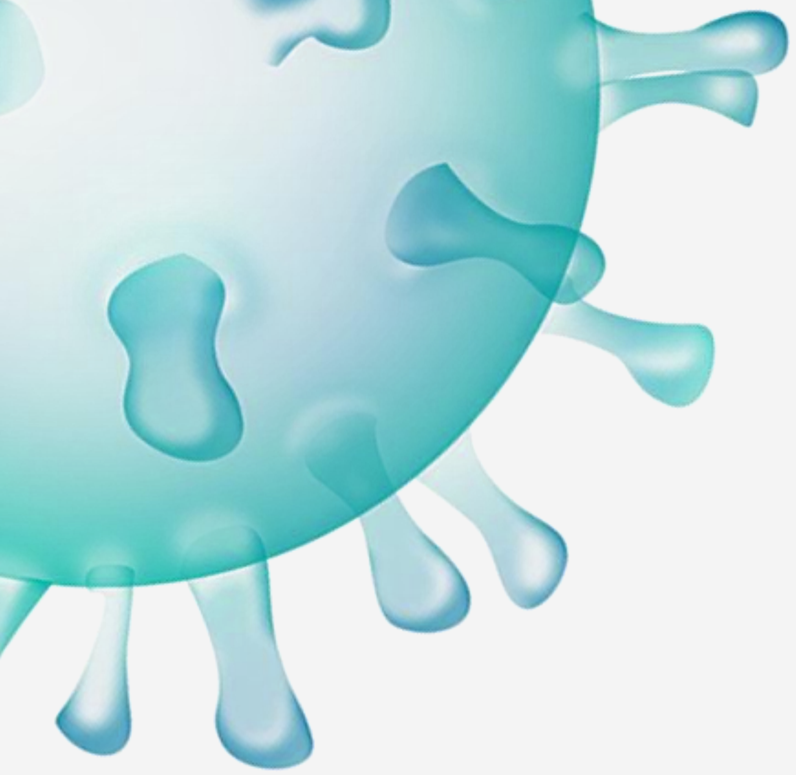
https://encyclopedia.pub/entry/1567

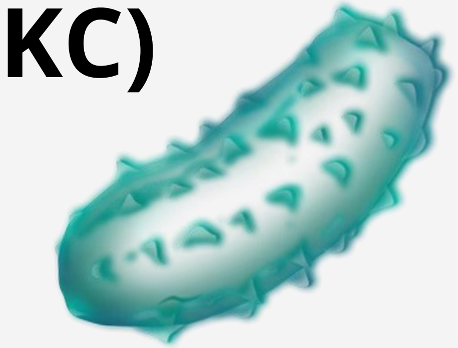


***M.kansasii* strains grown on Middelbrook 7H11 agar**

https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/mycobacterium-kansasii

***M.kansasii* causing severe pneumonia in a patient with leukemia**

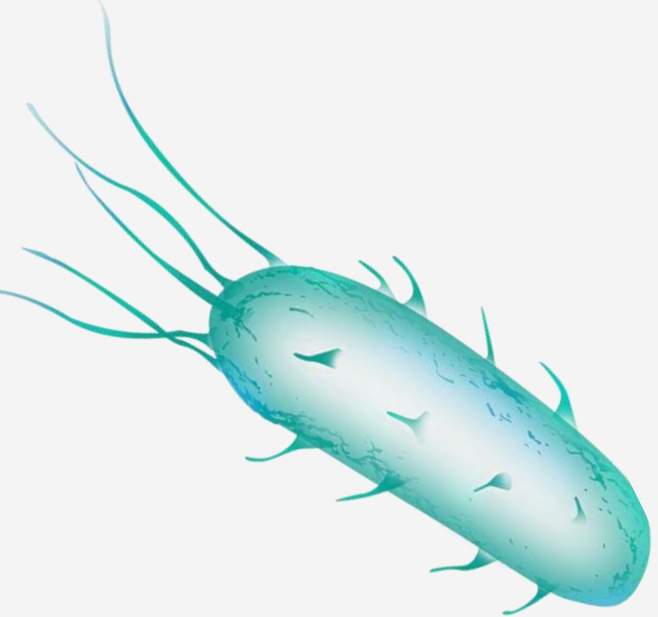


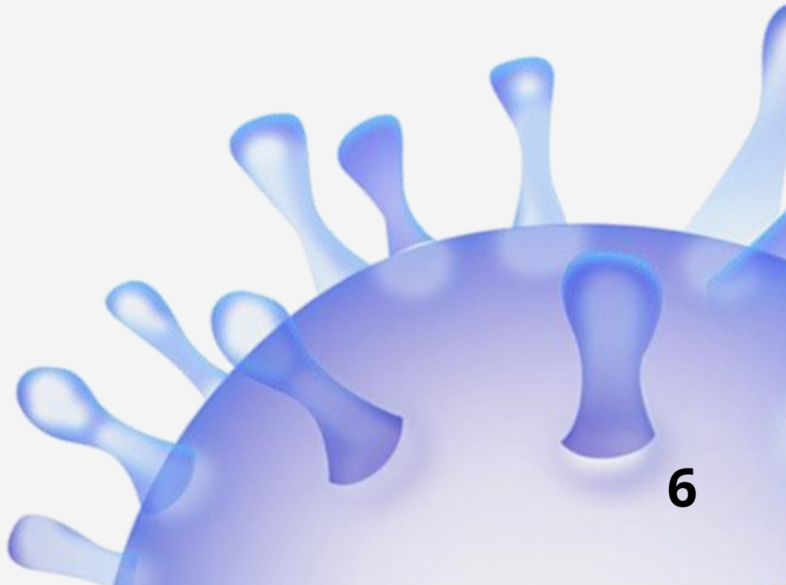


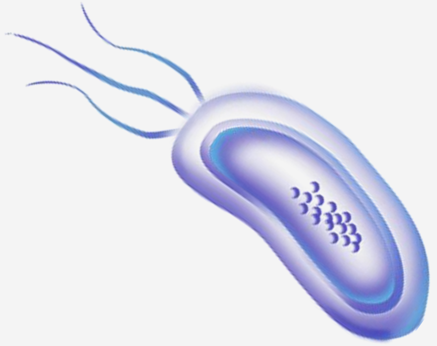
Member of the *Mycobacterium kansasii* complex (MKC)

- ***Mycobacterium kansasii* (subtype I) (major human pathogen)**
- ***Mycobacterium persicum* (subtype II)**
- ***Mycobacterium pseudokansasii* (subtype III)**
- ***Mycobacterium ostraviense* (subtype IV)**
- ***Mycobacterium innocens* (subtype V)**
- ***Mycobacterium attenuatum* (subtype VI)**
- ***Mycobacterium gastri***

(Luo et al., 2021)

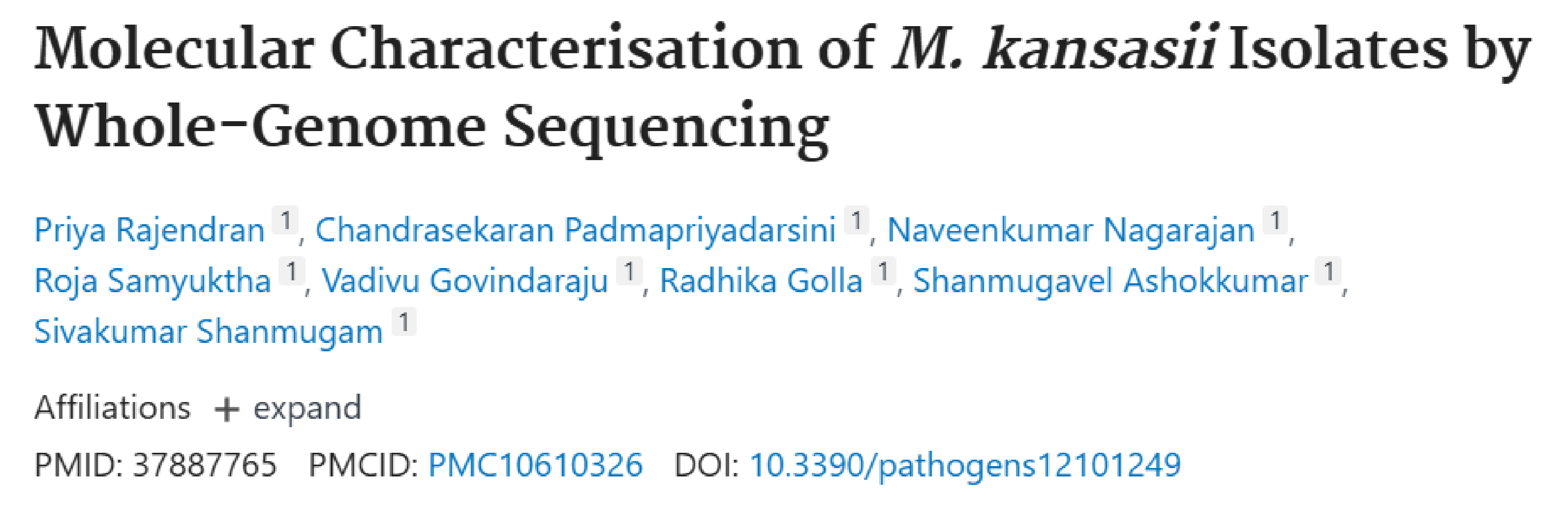






Seminar papers

1st Paper



> Pathogens. 2023 Oct 17;12(10):1249. doi: 10.3390/pathogens12101249.

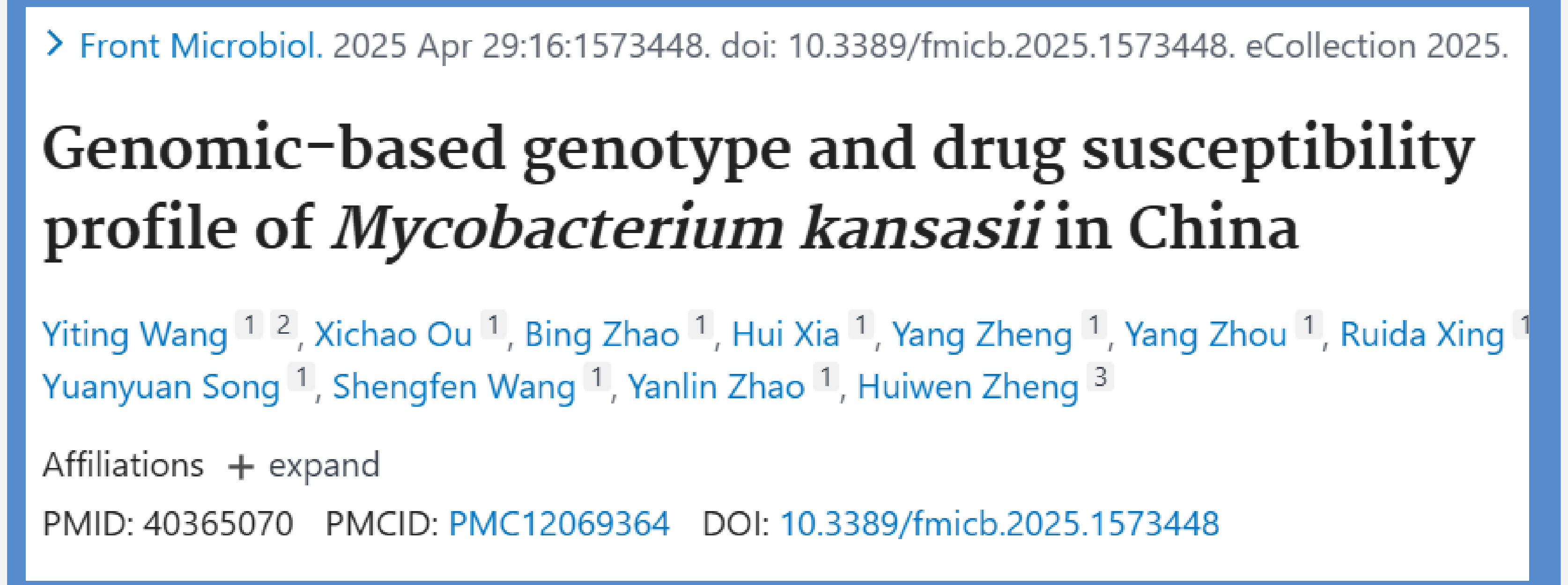
Molecular Characterisation of *M. kansasii* Isolates by Whole-Genome Sequencing

Priya Rajendran [1], Chandrasekaran Padmapriyadarsini [1], Naveenkumar Nagarajan [1], Roja Samyuktha [1], Vadivu Govindaraju [1], Radhika Golla [1], Shanmugavel Ashokkumar [1], Sivakumar Shanmugam [1]

Affiliations + expand

PMID: 37887765 PMCID: PMC10610326 DOI: 10.3390/pathogens12101249

2nd Paper



> Front Microbiol. 2025 Apr 29:16:1573448. doi: 10.3389/fmicb.2025.1573448. eCollection 2025.

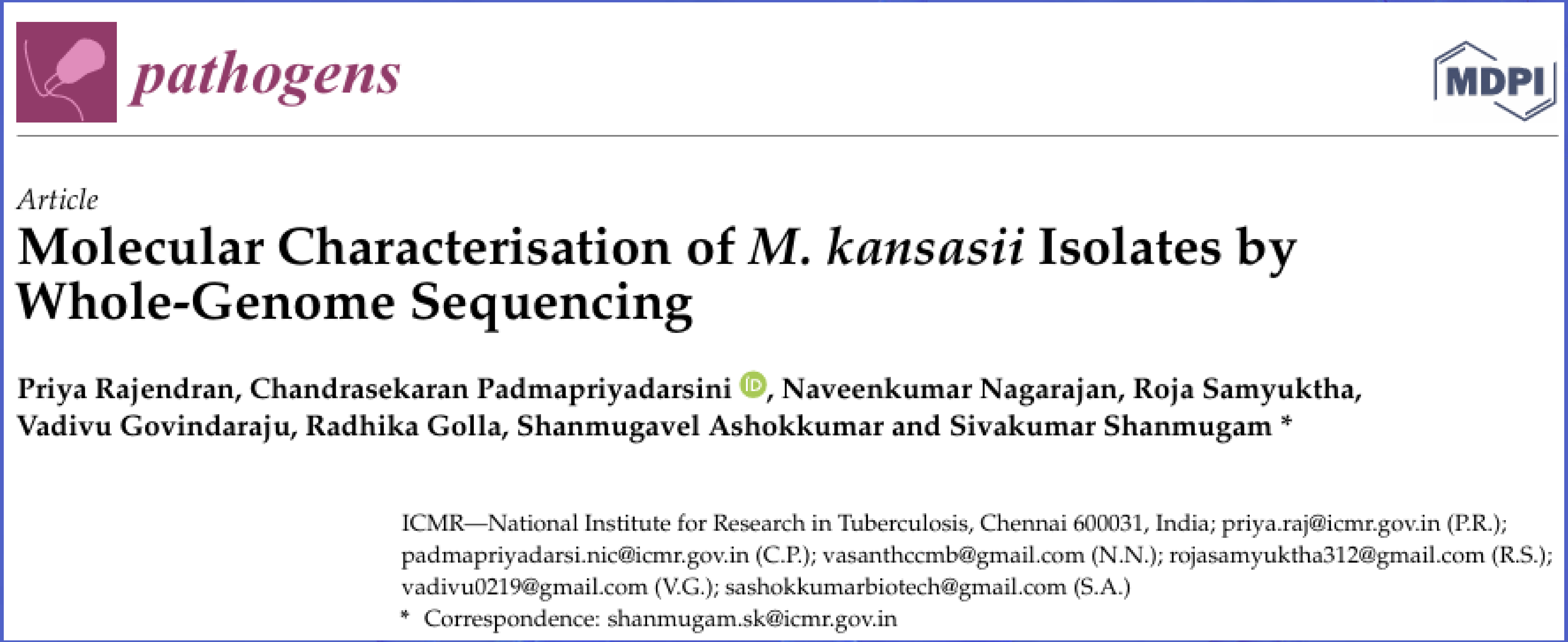
Genomic-based genotype and drug susceptibility profile of *Mycobacterium kansasii* in China

Yiting Wang [1 2], Xichao Ou [1], Bing Zhao [1], Hui Xia [1], Yang Zheng [1], Yang Zhou [1], Ruida Xing, Yuanyuan Song [1], Shengfen Wang [1], Yanlin Zhao [1], Huiwen Zheng [3]

Affiliations + expand

PMID: 40365070 PMCID: PMC12069364 DOI: 10.3389/fmicb.2025.1573448

1st Paper



pathogens

MDPI

Article

Molecular Characterisation of *M. kansasii* Isolates by Whole-Genome Sequencing

Priya Rajendran, Chandrasekaran Padmapriyadarsini, Naveenkumar Nagarajan, Roja Samyuktha, Vadivu Govindaraju, Radhika Golla, Shanmugavel Ashokkumar and Sivakumar Shanmugam *

ICMR—National Institute for Research in Tuberculosis, Chennai 600031, India; priya.raj@icmr.gov.in (P.R.); padmapriyadarsi.nic@icmr.gov.in (C.P.); vasanthccmb@gmail.com (N.N.); rojasamyuktha312@gmail.com (R.S.); vadivu0219@gmail.com (V.G.); sashokkumarbiotech@gmail.com (S.A.)

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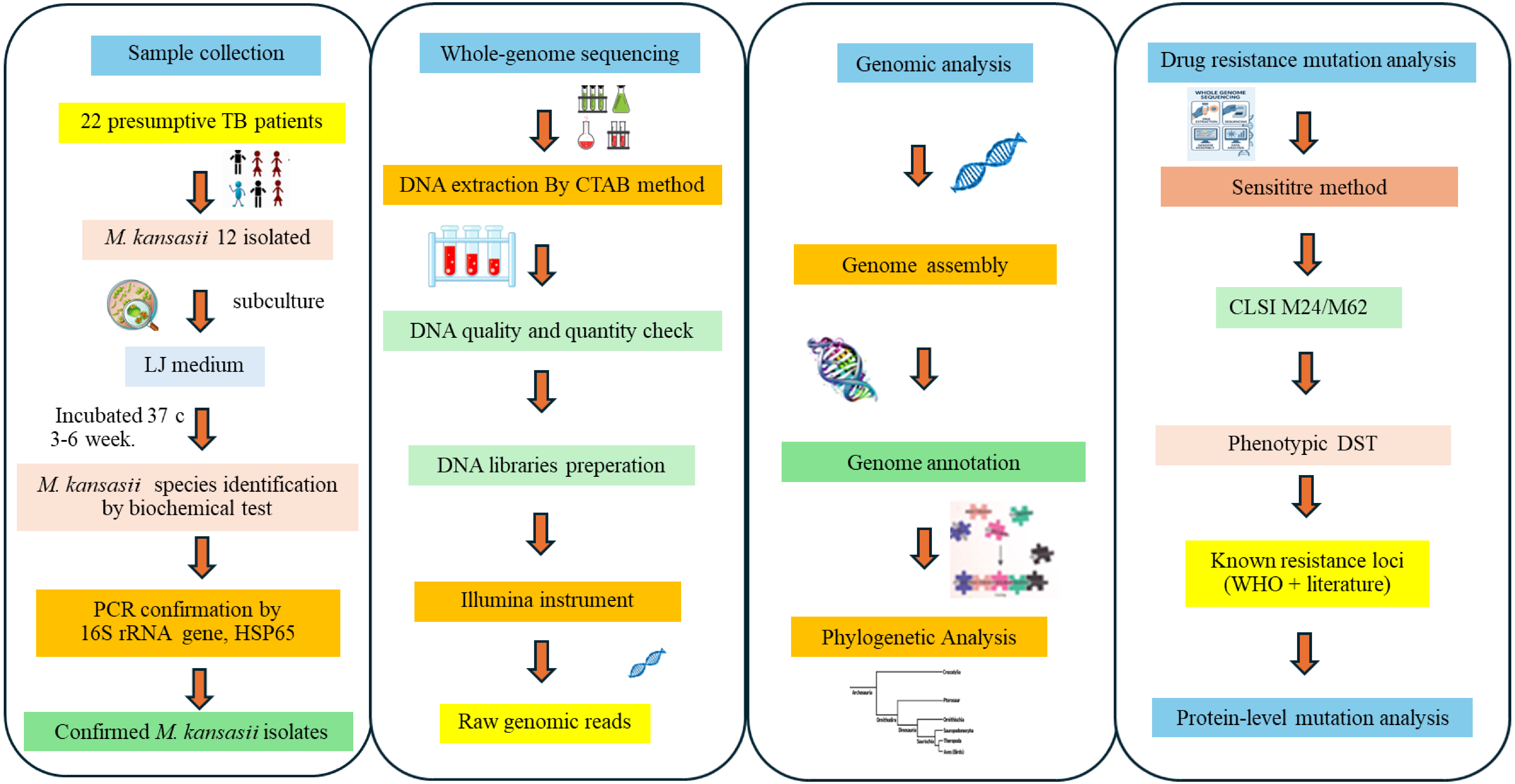
Objective: To examine the distribution of subtypes, based on whole-genome sequence analysis, and identify the genetic determinants of drug resistance for the isolates in India

1st Paper

Experimental design



Sample collection

22 presumptive TB patients

M. kansasii 12 isolated

subculture

LJ medium

Incubated 37 c
3-6 week.

M. kansasii species identification
by biochemical test

PCR confirmation by
16S rRNA gene, HSP65

Confirmed *M. kansasii* isolates

Whole-genome sequencing

DNA extraction By CTAB method

DNA quality and quantity check

DNA libraries preperation

Illumina instrument

Raw genomic reads

Genomic analysis

Genome assembly

Genome annotation

Phylogenetic Analysis

Drug resistance mutation analysis

Sensititre method

CLSI M24/M62

Phenotypic DST

Known resistance loci
(WHO + literature)

Protein-level mutation analysis

1st Paper

Results & Discussion

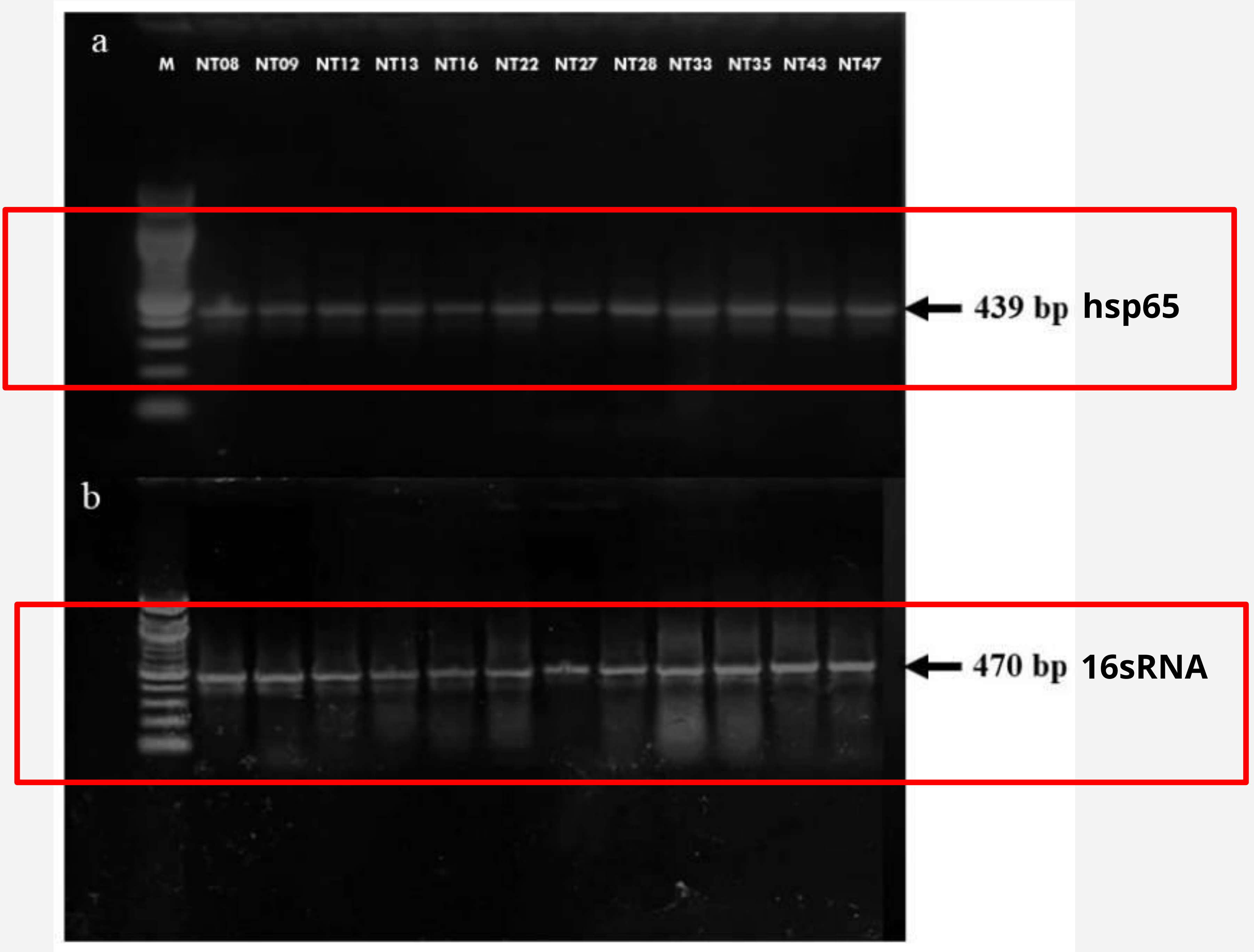
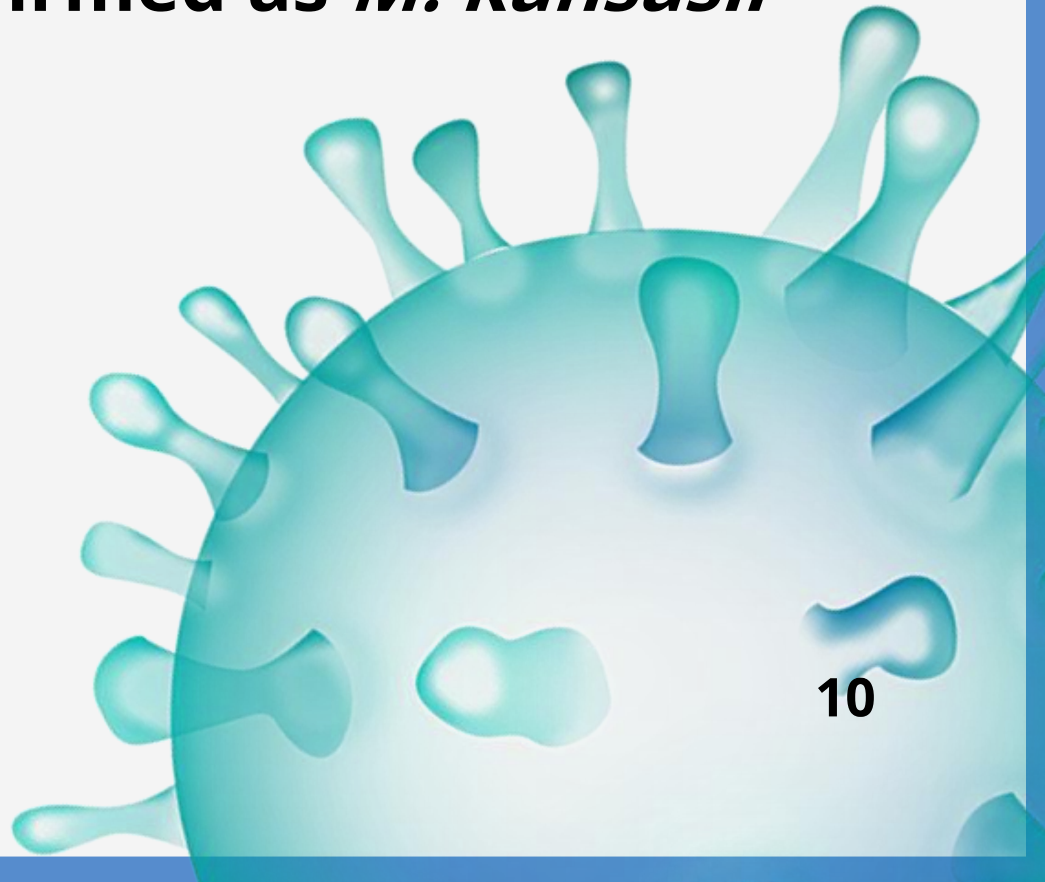


Figure 2. hsp65 PCR (a) and 16srRNA PCR (b) for the 12 isolates that were subjected to sequencing.

1. Identification by Molecular Methods

- 16S rRNA (470 bp) and *hsp65* (439 bp) successfully amplified in all isolates
- hsp65 PCR products analyzed by RFLP for species identification
- All 12 isolates confirmed as *M. kansasii*



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Results & Discussion

2. Subtype Classification

Figure 3. Phylogenetic tree based on 16srRNA, rpoB, hsp65, ITS, and tuf gene sequences

- **All 12 isolates were classified as subtype I**
- **This finding is consistent with reports from China, Europe, the United States, and Japan, where subtype I has been identified as a major cause of human infection**

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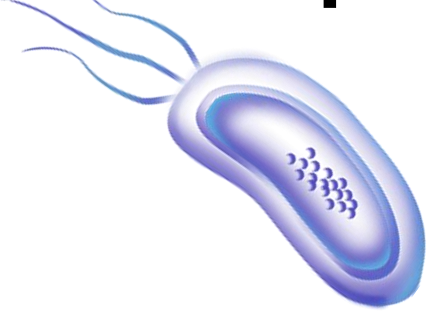

Results & Discussion

3. Association of Mutation with Drug Resistance

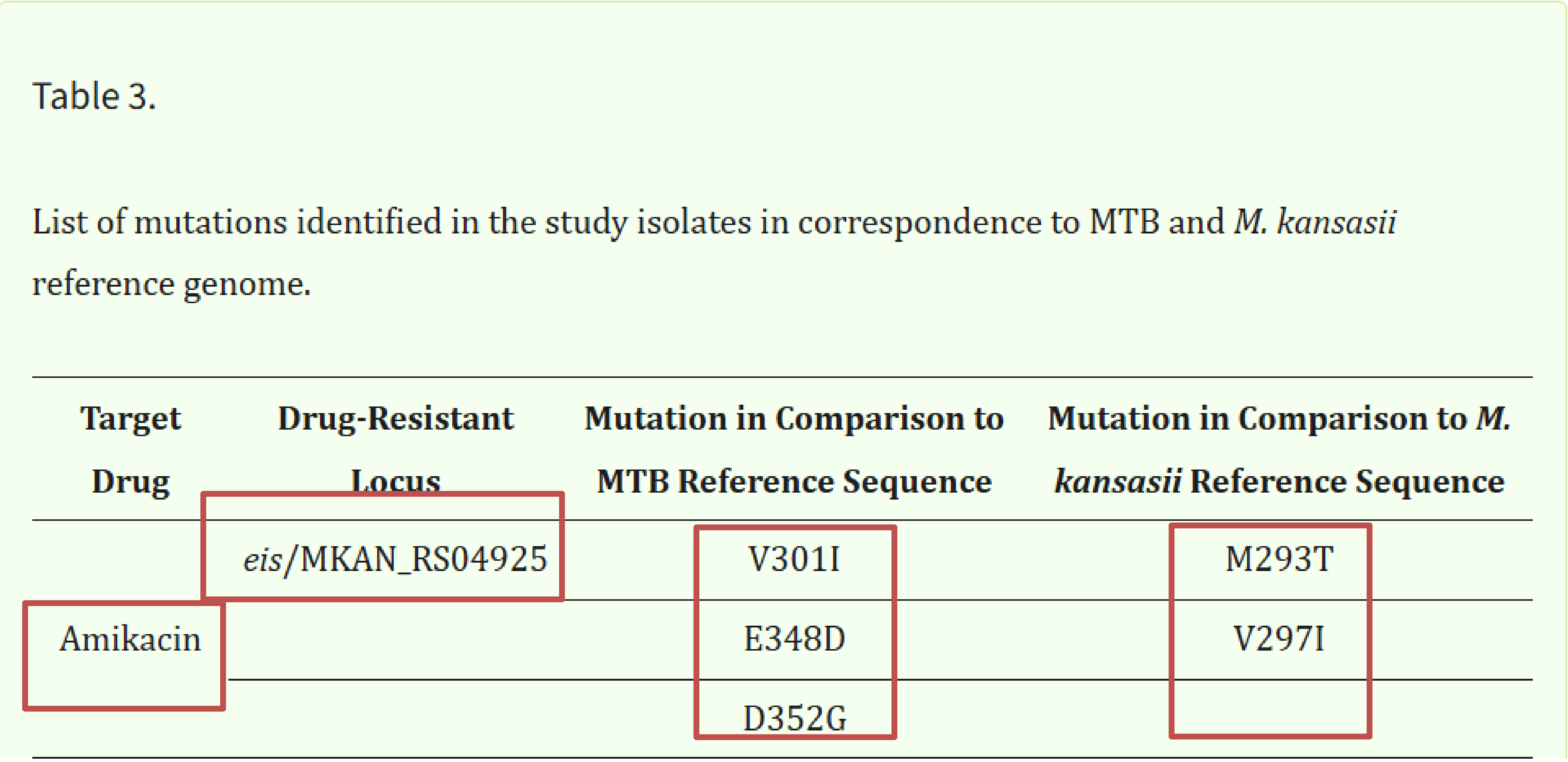

Table 3.

List of mutations identified in the study isolates in correspondence to MTB and *M. kansasii* reference genome.

Target Drug	Drug-Resistant Locus	Mutation in Comparison to MTB Reference Sequence	Mutation in Comparison to *M. kansasii* Reference Sequence
Amikacin	*eis*/MKAN_RS04925	V301I	M293T
		E348D	V297I
		D352G	

These mutations were not associated with amikacin resistance

3.1 Amikacin Resistance

- **Target gene : *eis* (*MKAN_RS04925*)**
- **No mutations found in 2 isolates with DST resistance to Amikacin**
- **Found 3 non-synonymous mutations in DST susceptible to Amikacin isolates**
- **Found 2 non-synonymous mutations When compared with the *M. kansasii* reference sequence**

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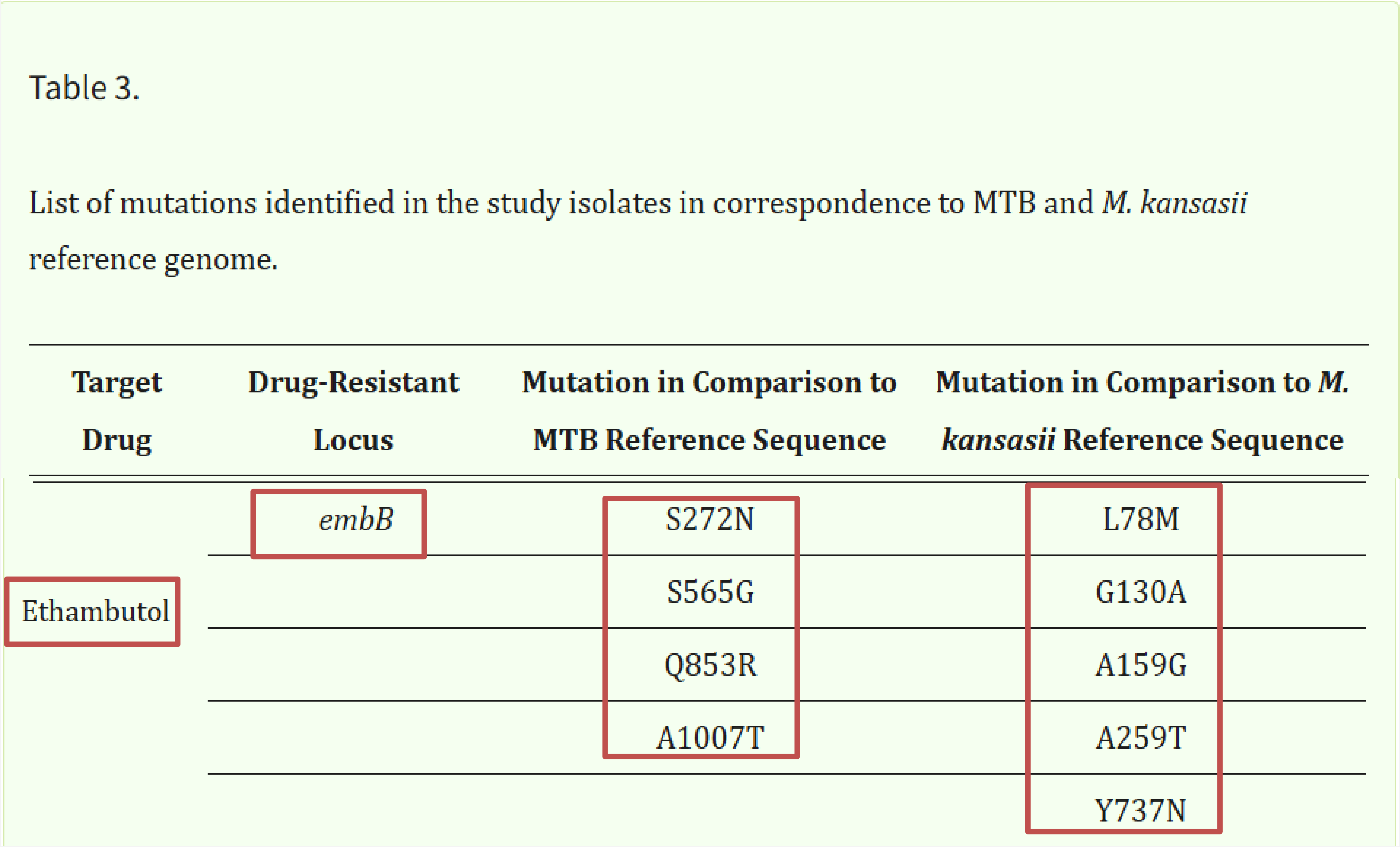
Results & Discussion

3. Association of Mutation with Drug Resistance



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List of mutations identified in the study isolates in correspondence to MTB and *M. kansasii* reference genome.

Target Drug	Drug-Resistant Locus	Mutation in Comparison to MTB Reference Sequence	Mutation in Comparison to *M. kansasii* Reference Sequence
Ethambutol	*embB*	S272N	L78M
		S565G	G130A
		Q853R	A159G
		A1007T	A259T
			Y737N

This interesting concurrence between phenotypic and genotypic results for some isolates implicates the need for further genetic studies exploring the association of these mutations with drug resistance

3.2 Ethambutol Resistance

- **Target gene : *embB***
- **Found 4 non-synonymous mutations of 10 isolates in *embB* vs. MTB reference**
- **5 of these isolates were phenotypically EMB-resistant**
- **1 isolate showed 5 non-synonymous *emb* mutations vs. *M. kansasii* reference and was EMB-resistant**
- **EMB-associated loci, their direct association with ethambutol resistance but in *M. kansasii* remains inconclusive.**

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Results & Discussion

3. Association of Mutation with Drug Resistance

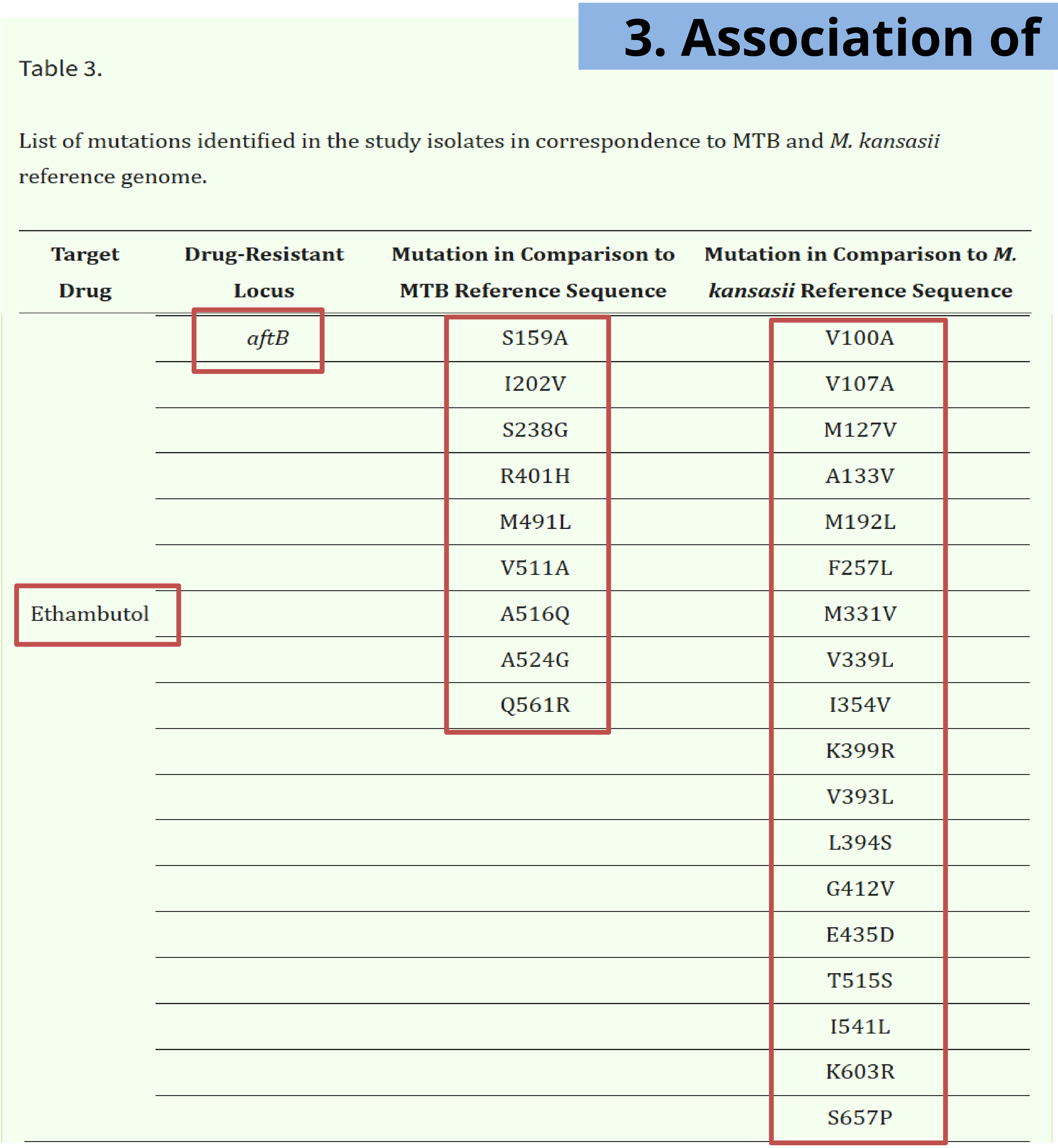


Table 3.

List of mutations identified in the study isolates in correspondence to MTB and *M. kansasii* reference genome.

Target Drug	Drug-Resistant Locus	Mutation in Comparison to MTB Reference Sequence	Mutation in Comparison to *M. kansasii* Reference Sequence
Ethambutol	*aftB*	S159A	V100A
		I202V	V107A
		S238G	M127V
		R401H	A133V
		M491L	M192L
		V511A	F257L
		A516Q	M331V
		A524G	V339L
		Q561R	I354V
			K399R
			V393L
			L394S
			G412V
			E435D
			T515S
			I541L
			K603R
			S657P

<u>3.2 Ethambutol Resistance</u>

- **Target gene : *aftB***
- **Nine mutations detected in three isolates vs. MTB reference**
- **Only one isolate showed phenotypic EMB resistance**
- **One isolate found 18 mutations vs. *M. kansasii* reference but phenotypic was EMB-susceptible**

Mutations in the *aftB* gene did not show a clear correlation with ethambutol resistance in *M. kansasii*

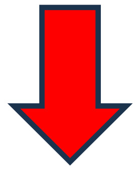
Results & Discussion

3. Association of Mutation with Drug Resistance

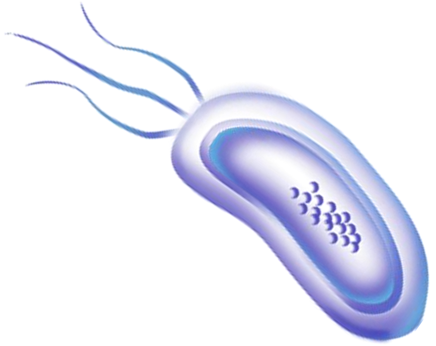
3.3 Rifampicin and Isoniazid Resistance

Target gene : ***rpoB and katG gene***

- **No mutations found in comparison with both MTB and *M. kansasii* reference genomes**



Other mechanism --- efflux pump, prolonged exposure----Resistant



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Results & Discussion

3. Association of Mutation with Drug Resistance

3.4 Clarithromycin Resistance

Target gene : *rrl gene*

No mutations were found when compared with *M. kansasii* and the MTB reference genome

One CLR-susceptible isolate found *rrl* mutation (A2089G) when compared with *M. kansasii* reference genome

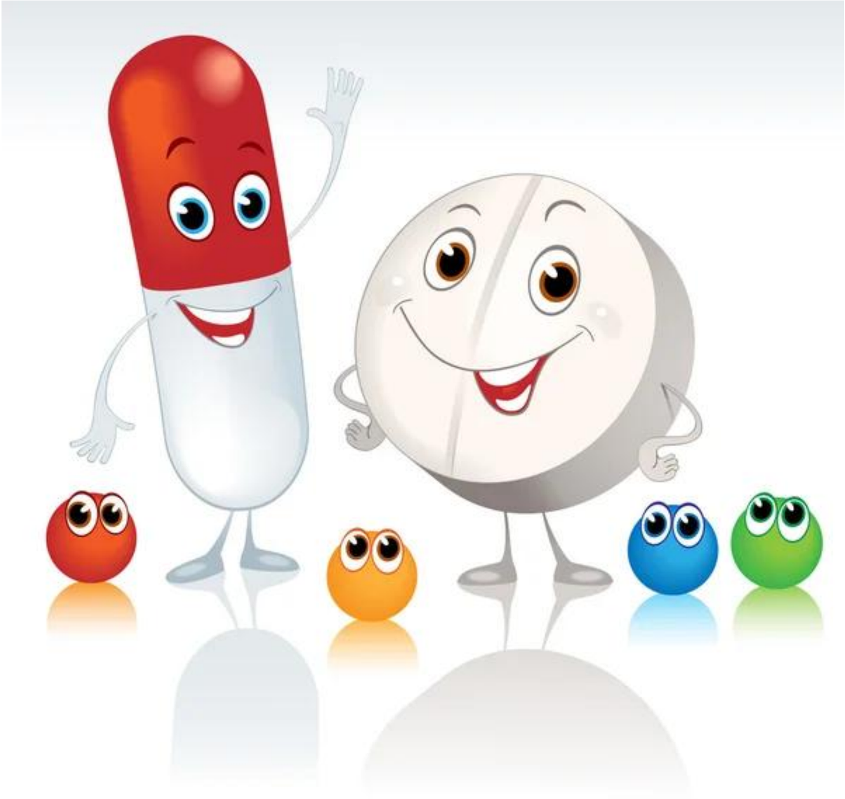
↓

Previous studies report both concordant and discordant patterns

Results & Discussion

3. Association of Mutation with Drug Resistance

3.5 Quinolone Resistance



Target gene : *gyrA and gyrB gene*

One isolates resistant to ciprofloxacin and one isolate resistant to moxifloxacin phenotypic DST.

No mutations were found when compared with *M. kansasii* and the MTB reference genome

Other mechanism of drug resistance in NTM

- **efflux pump**
- **porin channels**

Conclusions

- **First whole-genome analysis of *M. kansasii* from India**
- **All isolates confirmed as *M. kansasii* Subtype I**
- **Numerous mutations identified, but inconclusive**

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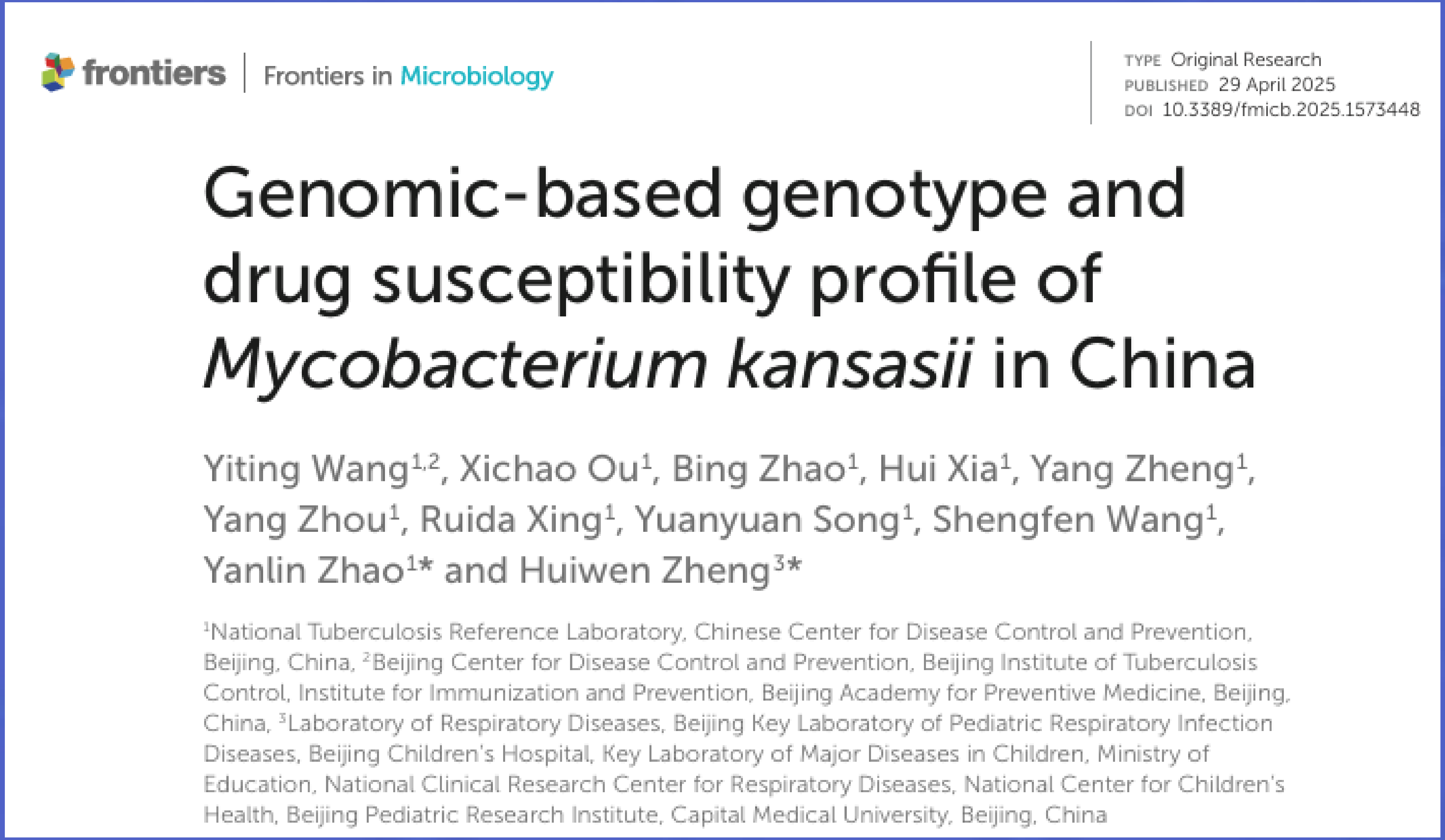

frontiers | Frontiers in Microbiology

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Genomic-based genotype and drug susceptibility profile of *Mycobacterium kansasii* in China

Yiting Wang[1,2], Xichao Ou[1], Bing Zhao[1], Hui Xia[1], Yang Zheng[1], Yang Zhou[1], Ruida Xing[1], Yuanyuan Song[1], Shengfen Wang[1], Yanlin Zhao[1]* and Huiwen Zheng[3]*

[1]National Tuberculosis Reference Laboratory, Chinese Center for Disease Control and Prevention, Beijing, China, [2]Beijing Center for Disease Control and Prevention, Beijing Institute of Tuberculosis Control, Institute for Immunization and Prevention, Beijing Academy for Preventive Medicine, Beijing, China, [3]Laboratory of Respiratory Diseases, Beijing Key Laboratory of Pediatric Respiratory Infection Diseases, Beijing Children's Hospital, Key Laboratory of Major Diseases in Children, Ministry of Education, National Clinical Research Center for Respiratory Diseases, National Center for Children's Health, Beijing Pediatric Research Institute, Capital Medical University, Beijing, China

Objective : To analyze subtypes, microbiological characteristics and antimicrobial susceptibility of *Mycobacterium kansasii* in China.

Experimental design

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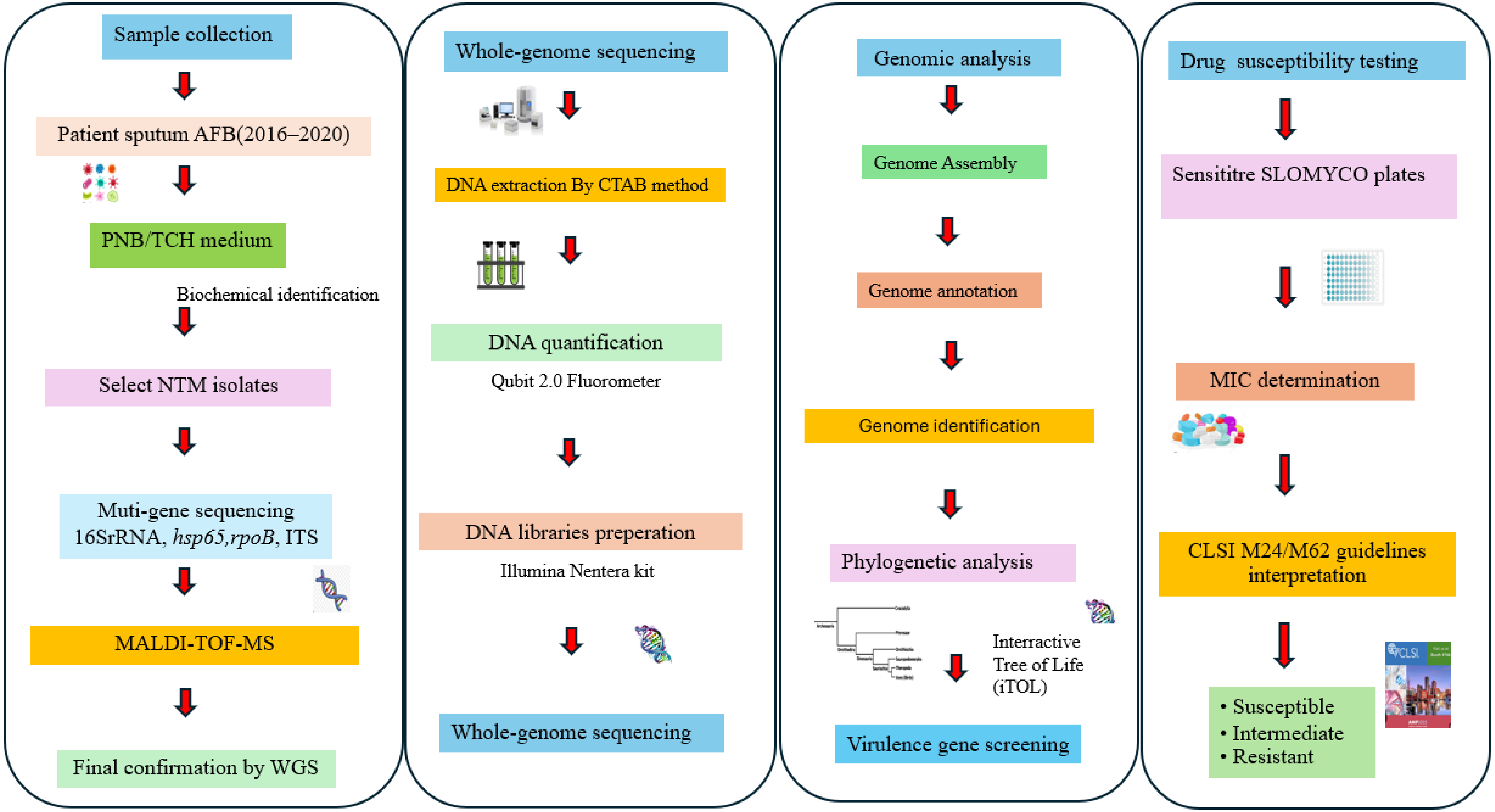


Sample collection

Patient sputum AFB(2016–2020)

PNB/TCH medium

Biochemical identification

Select NTM isolates

Muti-gene sequencing 16SrRNA, *hsp65*,*rpoB*, ITS

MALDI-TOF-MS

Final confirmation by WGS

Whole-genome sequencing

DNA extraction By CTAB method

DNA quantification

Qubit 2.0 Fluorometer

DNA libraries preperation

Illumina Nentera kit

Whole-genome sequencing

Genomic analysis

Genome Assembly

Genome annotation

Genome identification

Phylogenetic analysis

Interractive Tree of Life (iTOL)

Virulence gene screening

Drug susceptibility testing

Sensititre SLOMYCO plates

MIC determination

CLSI M24/M62 guidelines interpretation

- Susceptible
- Intermediate
- Resistant

Results & Discussion

2nd Paper

Identification and genotyping

- **ANI analysis (>95%) classified all 153 isolates as *M. kansasii***

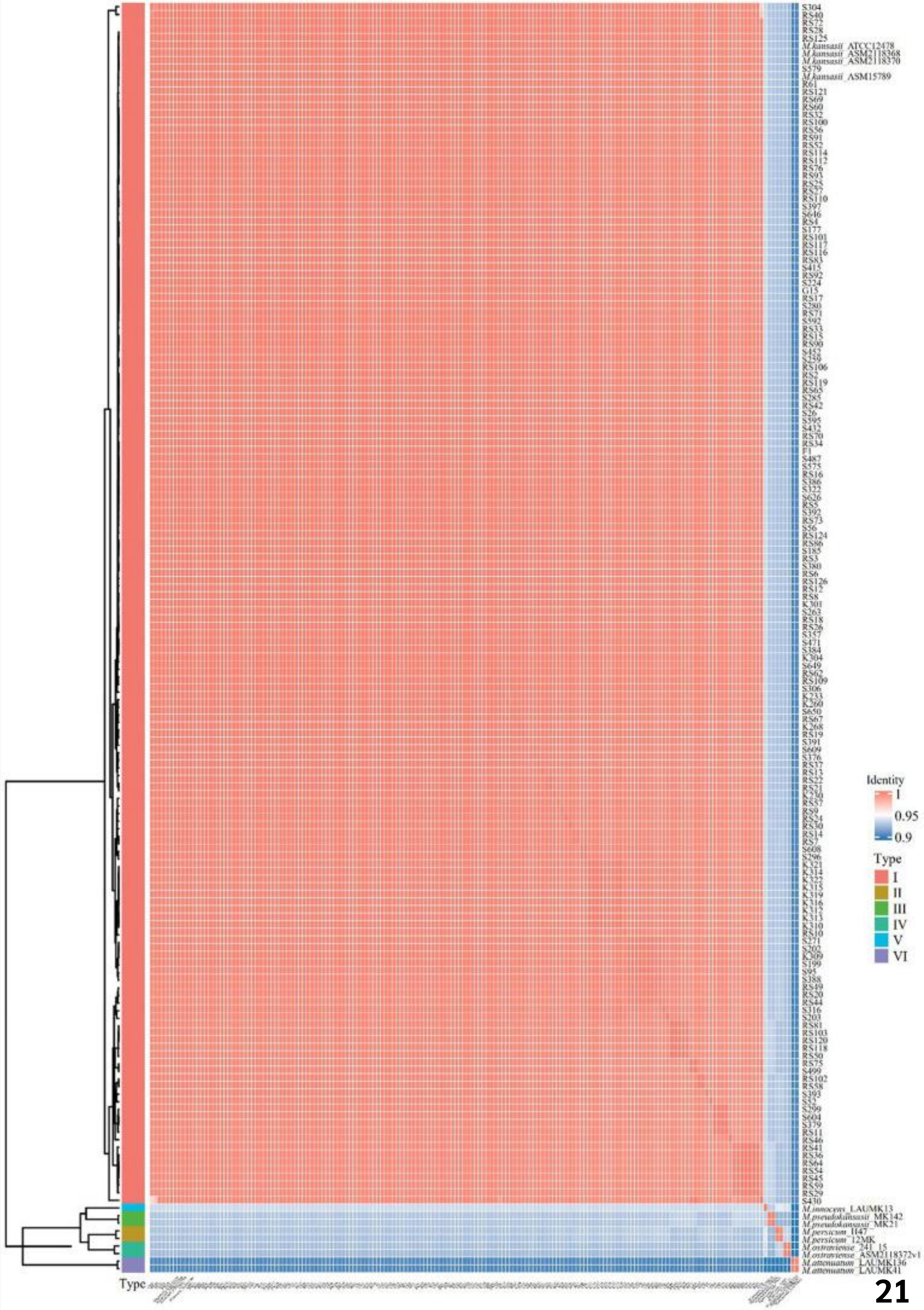


Figure 1. Pairwise comparisons of ANIs of *M. kansasii* subtypes I-VI. The heatmap displayed the Average Nucleotide Identity (ANI) values among 153 *M. kansasii* strains and the standard MKC strains. The color gradients in the heatmap represent different intensity levels of values. Red areas indicate higher ANI values, while blue areas signify lower ANI values. The bar chart on the left corresponds to different MKC species and conducts a clustering analysis of the samples

2nd Paper

Results & Discussion

Identification and genotyping

- WGS differentiated 153 isolates into 3 clusters and 141 genotypes
- 17 isolates were grouped into clusters (each containing 2–9 isolates)
- 136 isolates showed unique genotypic patterns
- These findings indicate high genetic diversity

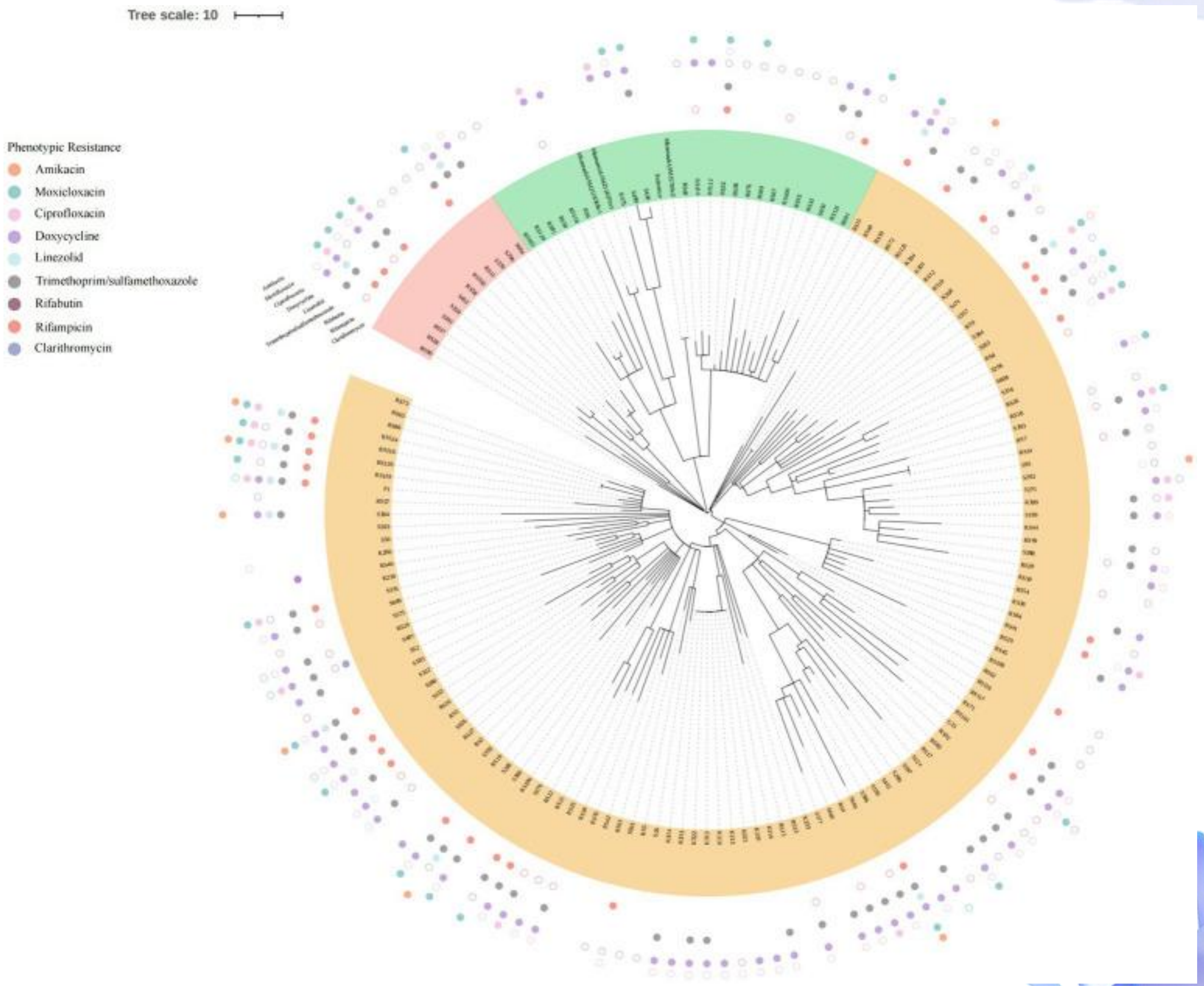


Figure 2. Phylogenetic tree based on core SNP. All 153MKC strains were divided into three clusters, with each cluster represented by a unique color. The outlying circles depicted the outcomes of the drug sensitivity tests. Distinct colors were employed to signify various drugs. Solid circles denoted drug resistance, while hollow circles represented intermedia

Results & Discussion

2nd Paper

Virulence factor-encoding gene

- ***EXS-1, EXS-3*, and *EXS-5* found in all 153 isolates**

- ***fbpA, fbpB, fbpC, hbhA, ideR, mbtH, mgtC,* and *phoP* were present in all *M. kansasii* subtype I strains**

- **These findings suggest that subtype I strains share a common set of virulence-associated genes potentially linked to their pathogenicity**

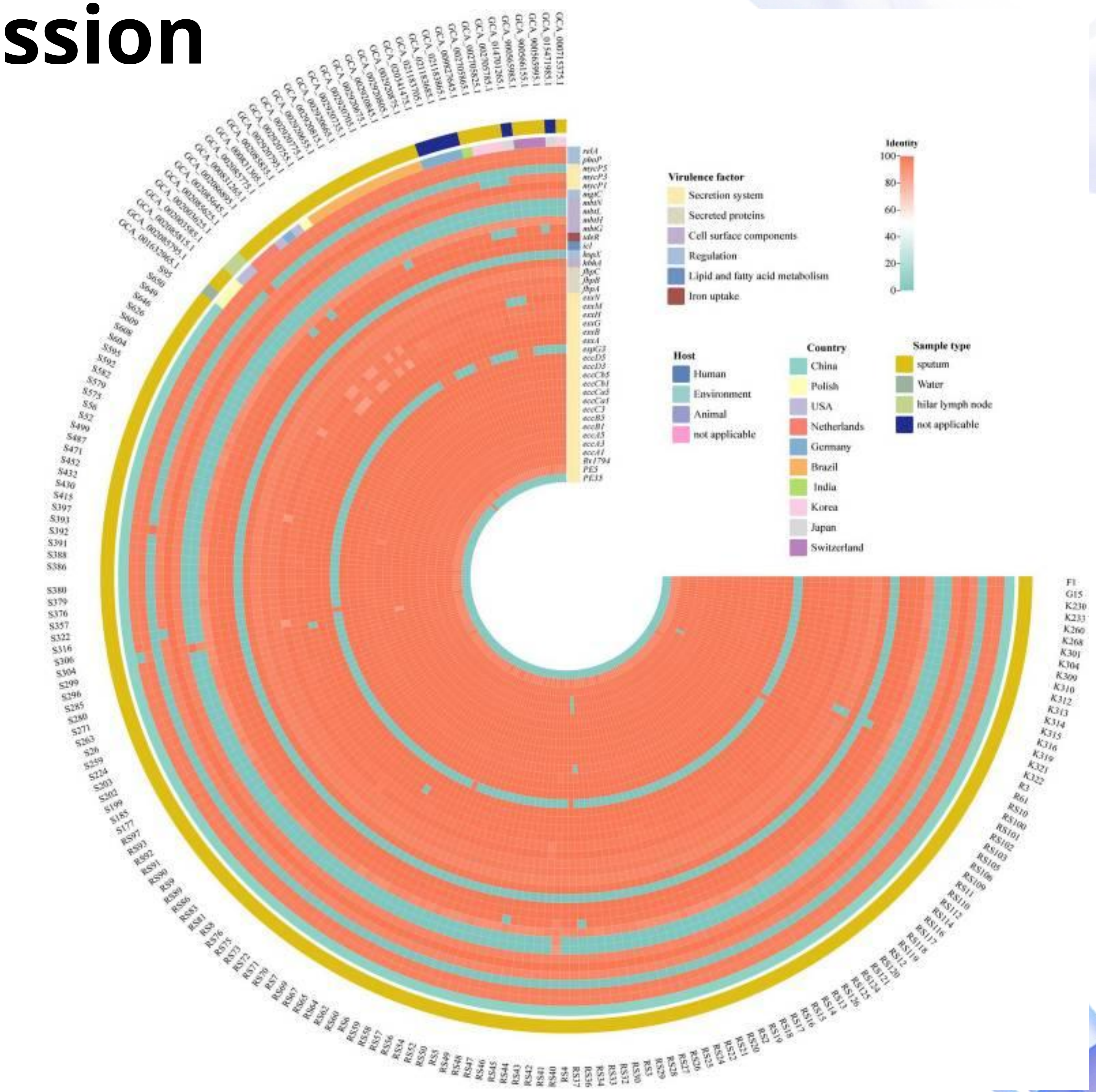


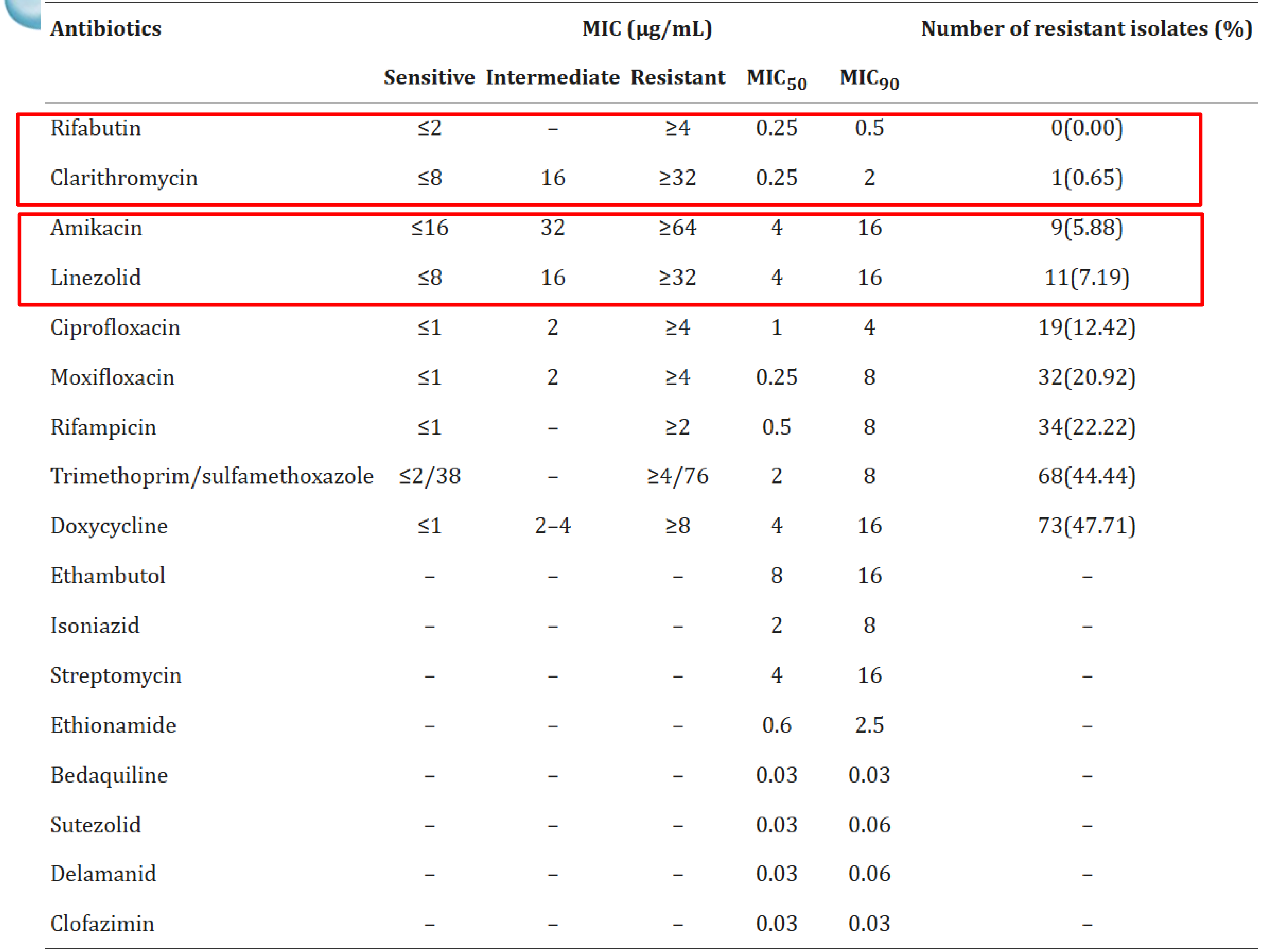
Figure 3 .Virulence factors in M. *kansasii* from different sources. The heatmap displayed the identity of multiple virulence genes. The horizontal axis is labeled with samples while the vertical axis enumerates individual virulence genes. A color—coded gradient is employed to depict the degree of identity among the genes. A score of 100 is represented by red, signifying a high level of sequence similarity, conversely, a light blue color corresponds to an identity score of 0, indicating a lack of significant similarity

2nd Paper

Results & Discussion

Drug susceptibility testing

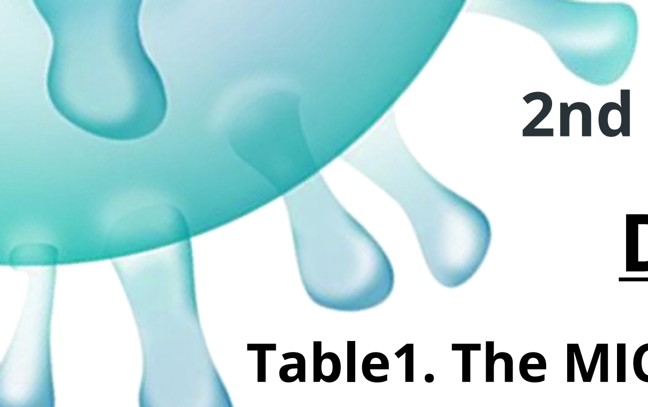
Table1. The MIC values and resistant rate of antibiotics for *M. kansasii*



Antibiotics	MIC (μg/mL)					Number of resistant isolates (%)
	Sensitive	Intermediate	Resistant	MIC_{50}	MIC_{90}	
Rifabutin	≤2	–	≥4	0.25	0.5	0(0.00)
Clarithromycin	≤8	16	≥32	0.25	2	1(0.65)
Amikacin	≤16	32	≥64	4	16	9(5.88)
Linezolid	≤8	16	≥32	4	16	11(7.19)
Ciprofloxacin	≤1	2	≥4	1	4	19(12.42)
Moxifloxacin	≤1	2	≥4	0.25	8	32(20.92)
Rifampicin	≤1	–	≥2	0.5	8	34(22.22)
Trimethoprim/sulfamethoxazole	≤2/38	–	≥4/76	2	8	68(44.44)
Doxycycline	≤1	2–4	≥8	4	16	73(47.71)
Ethambutol	–	–	–	8	16	–
Isoniazid	–	–	–	2	8	–
Streptomycin	–	–	–	4	16	–
Ethionamide	–	–	–	0.6	2.5	–
Bedaquiline	–	–	–	0.03	0.03	–
Sutezolid	–	–	–	0.03	0.06	–
Delamanid	–	–	–	0.03	0.06	–
Clofazimin	–	–	–	0.03	0.03	–

- **AST interpreted using CLSI breakpoint**
- **Rifabutin (RFB) and Clarithromycin (CLA) showed the highest activity**
- **Susceptibility: 100% and 99.35%**
- **Low resistance to Amikacin (AMK) (5.88%) and Linezolid (LZD) (7.19%)**

MIC50: the minimum concentration of an antimicrobial agent required to inhibit the growth of 50% of the organisms.
MIC90: the minimum concentration of an antimicrobial agent required to inhibit the growth of 90% of the organisms.

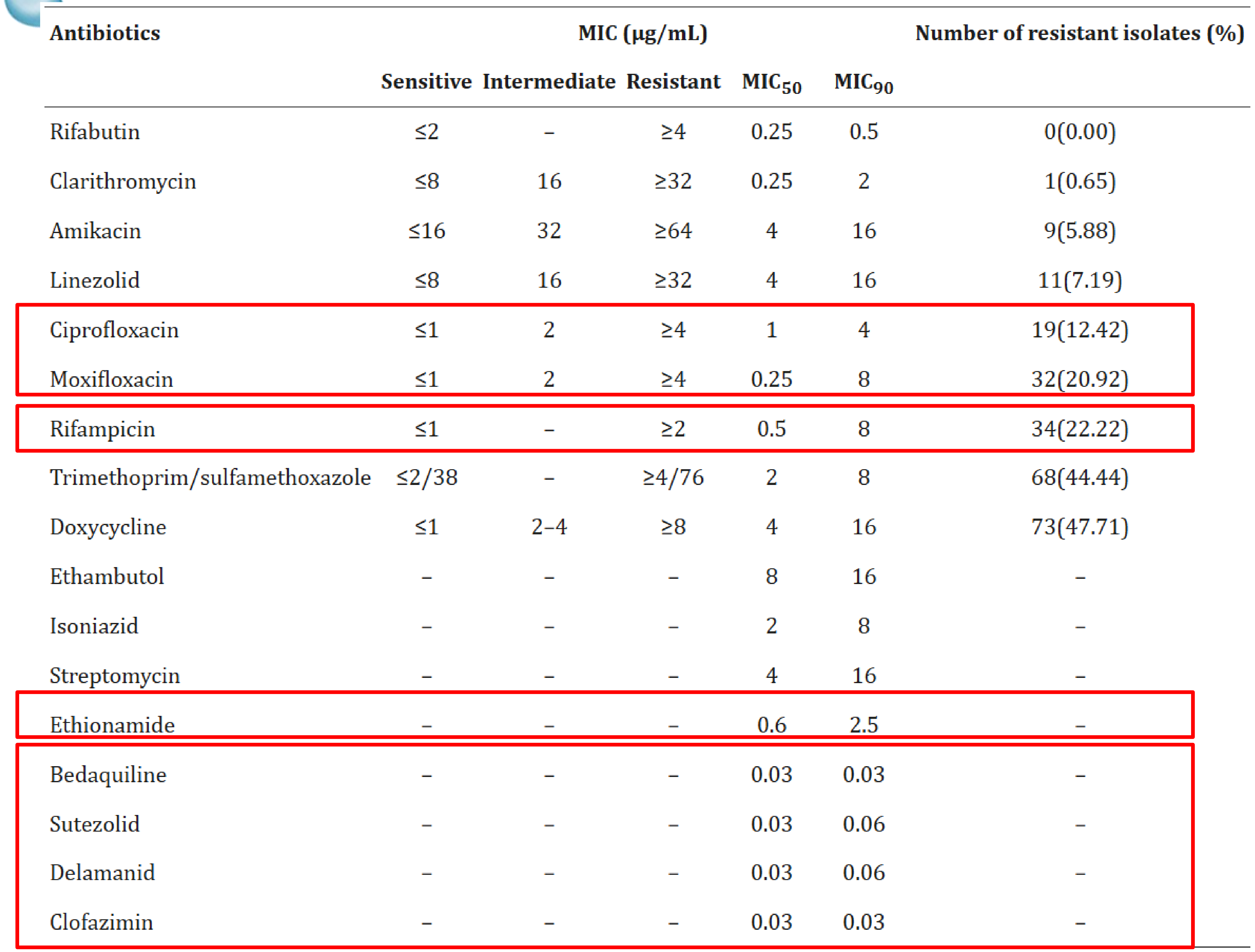


2nd Paper

Results & Discussion

Drug susceptibility testing

Table1. The MIC values and resistant rate of antibiotics for *M. kansasii*



Antibiotics	MIC (μg/mL)					Number of resistant isolates (%)
	Sensitive	Intermediate	Resistant	MIC_{50}	MIC_{90}	
Rifabutin	≤2	–	≥4	0.25	0.5	0(0.00)
Clarithromycin	≤8	16	≥32	0.25	2	1(0.65)
Amikacin	≤16	32	≥64	4	16	9(5.88)
Linezolid	≤8	16	≥32	4	16	11(7.19)
Ciprofloxacin	≤1	2	≥4	1	4	19(12.42)
Moxifloxacin	≤1	2	≥4	0.25	8	32(20.92)
Rifampicin	≤1	–	≥2	0.5	8	34(22.22)
Trimethoprim/sulfamethoxazole	≤2/38	–	≥4/76	2	8	68(44.44)
Doxycycline	≤1	2–4	≥8	4	16	73(47.71)
Ethambutol	–	–	–	8	16	–
Isoniazid	–	–	–	2	8	–
Streptomycin	–	–	–	4	16	–
Ethionamide	–	–	–	0.6	2.5	–
Bedaquiline	–	–	–	0.03	0.03	–
Sutezolid	–	–	–	0.03	0.06	–
Delamanid	–	–	–	0.03	0.06	–
Clofazimin	–	–	–	0.03	0.03	–

- **AST interpreted using CLSI breakpoint**
- **Higher resistance to fluoroquinolones**
  - CIP: 12.42%, MXF: 20.92%
- **Rifampicin (RIF) resistance: 22.22%**
- **Ethionamide (ETO) showed moderate activity (MIC50 : 0.6 μg/mL)**
- **Newer agents (BDQ, DLM, CFZ, SZD) showed very low MIC50/MIC90 values**

MIC50: the minimum concentration of an antimicrobial agent required to inhibit the growth of 50% of the organisms
MIC90: the minimum concentration of an antimicrobial agent required to inhibit the growth of 90% of the organisms

2nd Paper

Results & Discussion

Drug susceptibility testing

- **MIC distribution of antimicrobial agents against *M. kansasii***
- **Most isolates showed low MICs for Rifabutin (RFB) and Clarithromycin (CLA)**
- **Higher resistance to fluoroquinolones and Rifampicin (RIF)**
- **Newer agents (BDQ, DLM, CFZ, SZD) showed very low MIC50/MIC90 values**

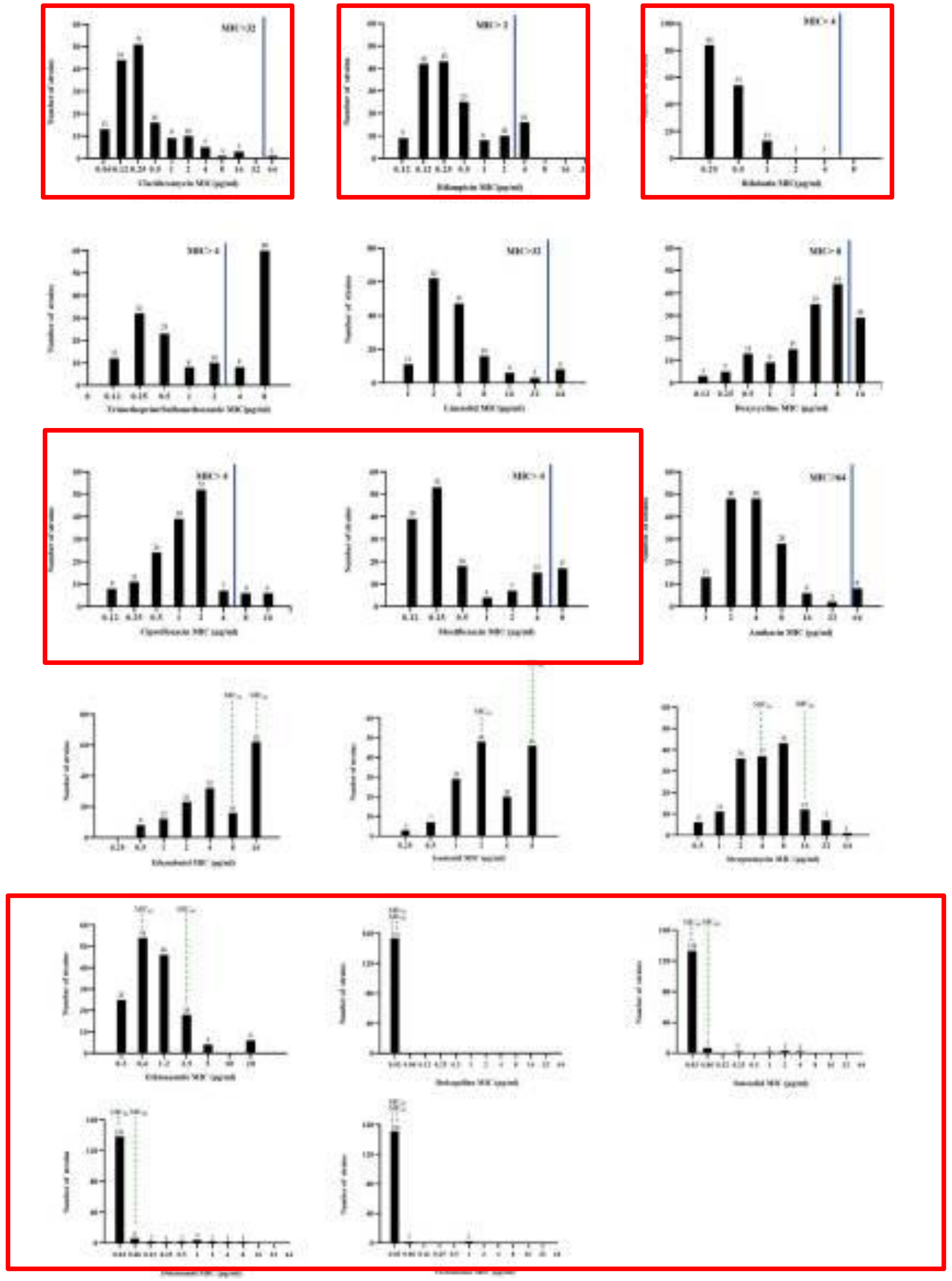


Figure 4. MIC distributions of *M. kansasii* clinical isolates. The solid blue line indicates the CLSI-recommended threshold for determining drug susceptibility, above which a resistant strain is considered. Drugs lacking critical values of MIC50 and MIC90 are denoted by dashed lines. MIC50: the minimum concentration of an antimicrobial agent required to inhibit the growth of 50% of the organisms. MIC90: the minimum concentration of an antimicrobial agent required to inhibit the growth of 90% of the organisms.

2nd Paper

Results & Discussion

Drug susceptibility testing

Standard regimen: RIF, INH, EMB

RIF resistance rate: 22.22%

- **Similar to Shanghai, lower than Beijing**
- **Higher than Poland and Brazil (fully susceptible to RIF)**

Indicates geographical variation in resistance patterns

Rifabutin (RFB): 100% susceptibility

- **Strong candidate as RIF alternative**

AMK, LZD, and SZD showed excellent in vitro activity

Clarithromycin (CLA): 99.35% susceptible

- **Resistance may increase with macrolide overuse**

BDQ, DLM, and CFZ demonstrated

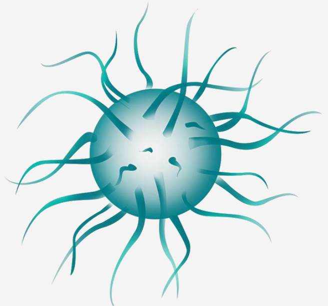
- **Potential alternative therapies for *M. kansasii***

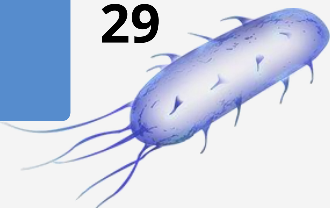
Conclusions

- *M. kansasii* type I is the predominant genotype in China.
- Rifabutin (RFB) and Clarithromycin (CLR) showed strong activity against *M. kansasii* strains.
- New drugs Bedaquiline (BDQ), Delamanid (DLM), Sutezolid (SZD), and Clofazimine (CFZ) show potential as effective treatments for *M. kansasii* infection

Criticisms

	Strong points	Weak points
1st Paper **Rajendran et al., 2023**	• **Frist report from India on WGS of *M. kansasii*** • **Integration of genomic data with phenotypic DST**	• **Very small sample (12 isolates)** • **The phenotypic or genotypic drug-resistance patterns could not be correlated with treatment outcome**
2nd Paper **Wang et al., 2025**	• **Large sample (153 isolates)** • **Integrated genomic, virulence, and phenotype DST data**	• **Clinical information and treatment outcomes were unavailable** • **Environmental MKC isolates were not included** • **Exclusively from China, limit the generalizability of the findings to other geographic regions**





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THANK YOU
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