

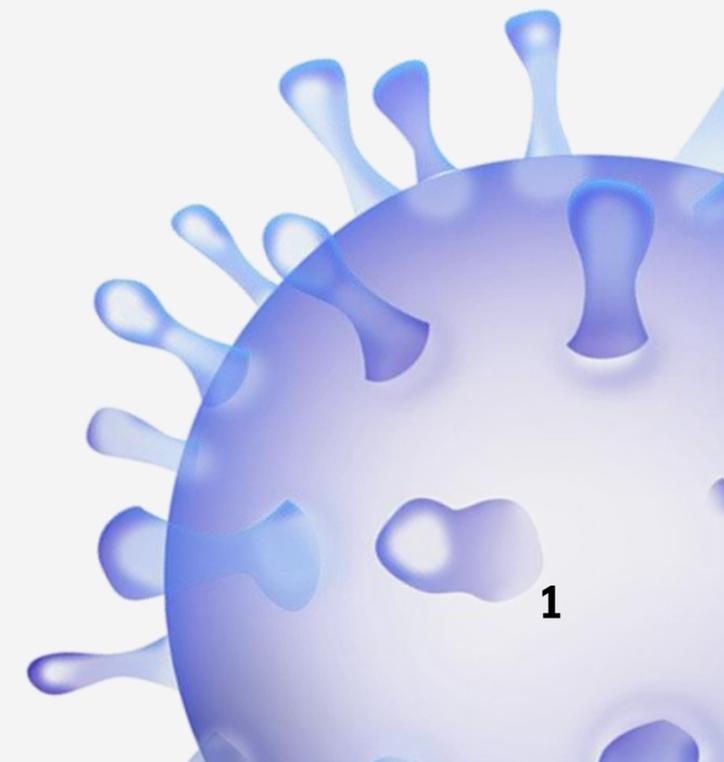
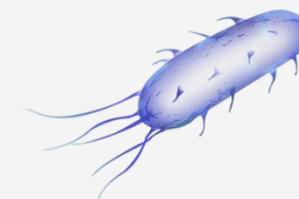
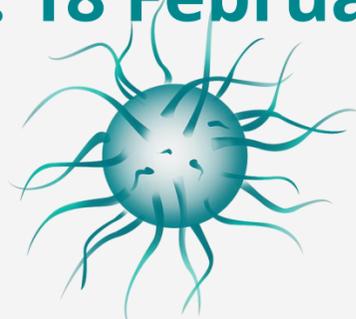
GENOMIC CHARACTERIZATION AND DRUG SUSCEPTIBILITY PROFILES OF *MYCOBACTERIUM KANSASII* : INSIGHTS FROM WHOLE-GENOME SEQUENCING STUDIES IN ASIA

By : Weawwadee Sribunrueang

ID : 685070044-6 1st year M.Sc. student

Advisor : Asst. Prof. Dr. Auttawit Sirichoat

Date: 18 February 2026



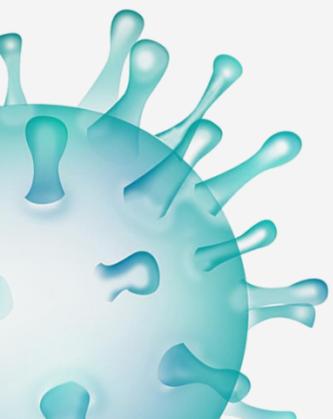
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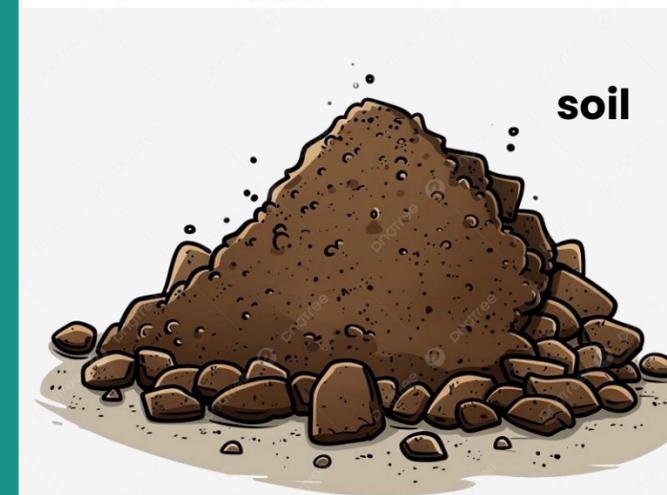
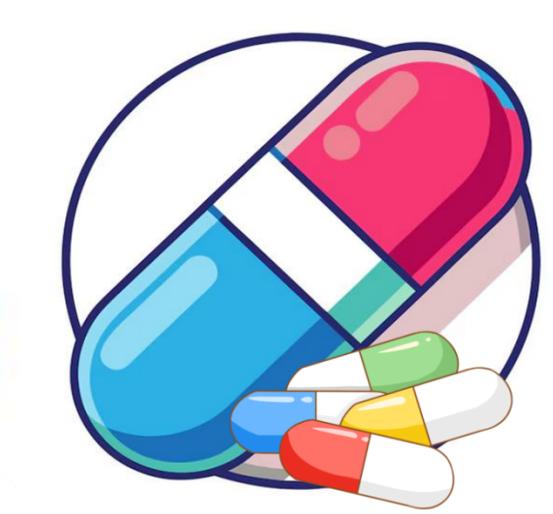
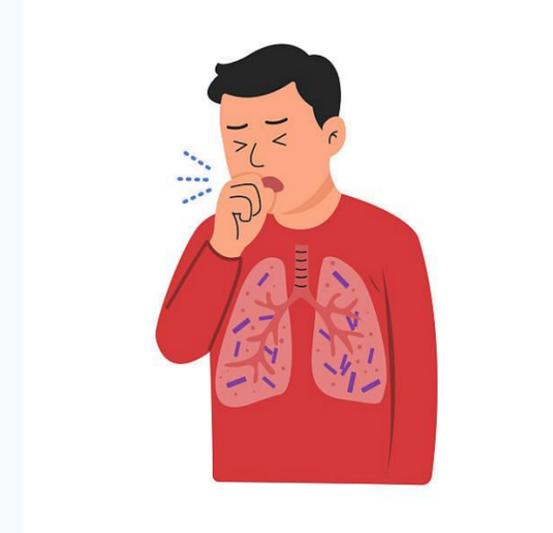
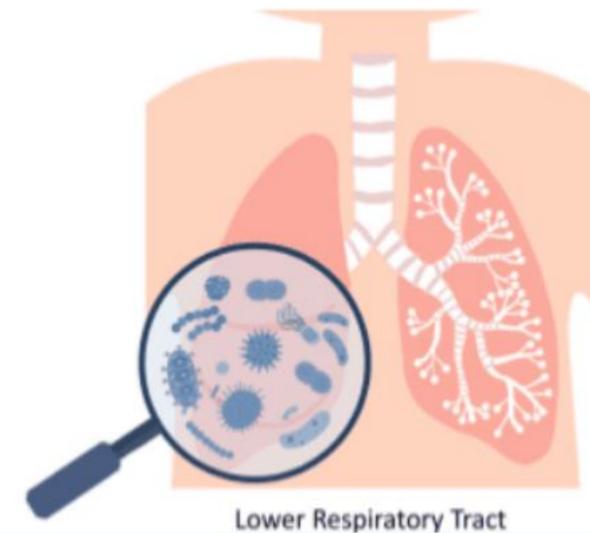


Introduction

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)

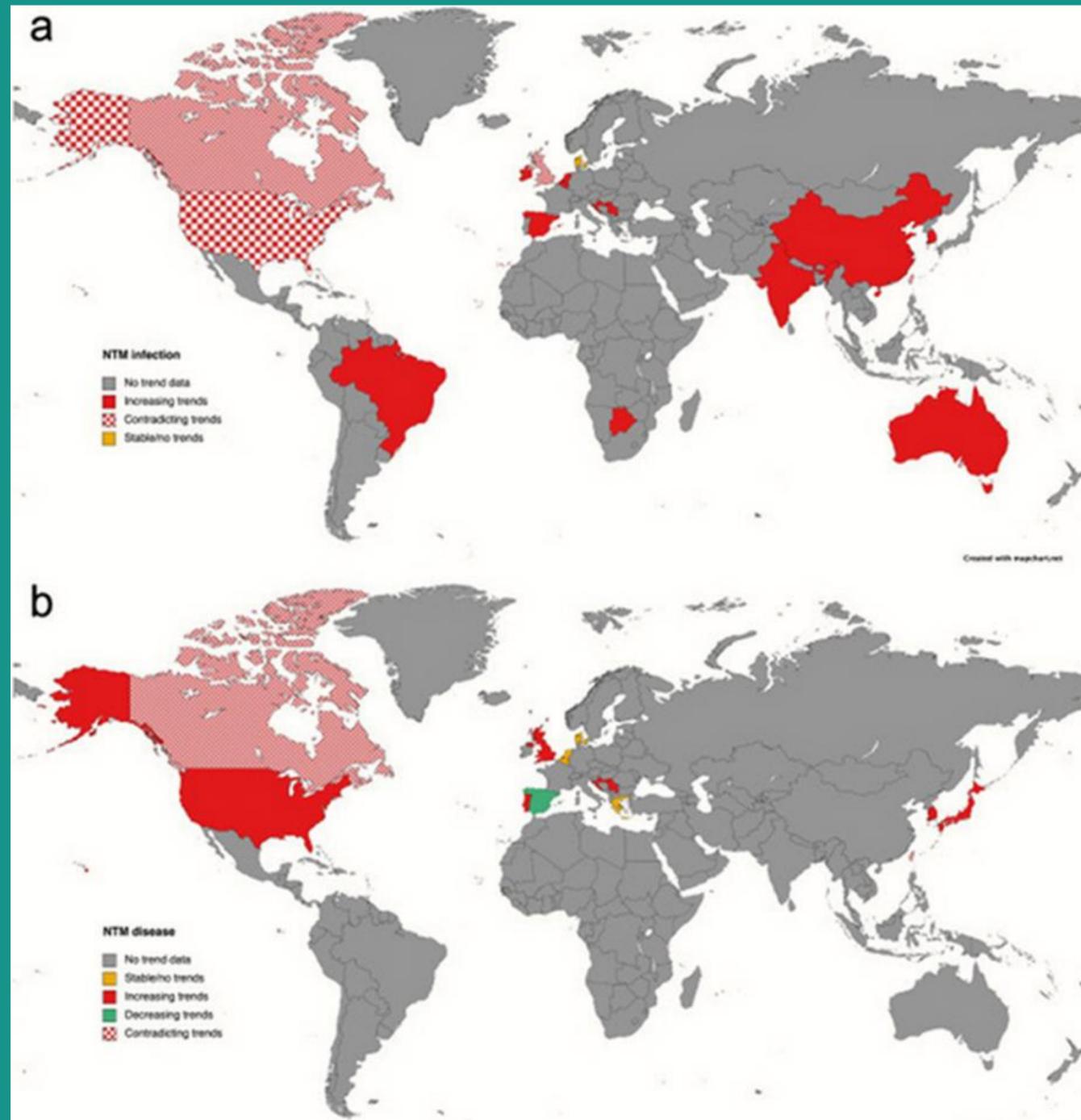


Slow growing mycobacterium (SGM) (≥ 7 days)	Rapid growing mycobacterium (RGM) (< 7 days)	
Group 1 Photochromogenic <ul style="list-style-type: none">• <i>M. kansasii</i>• <i>M. simiae</i>	Group 4 <ul style="list-style-type: none">• <i>M. abscessus</i> complex• <i>M. chelonae</i>• <i>M. fortuitum</i> complex• <i>M. peregrinum</i>	
• <i>M. marinum</i>		
Group 2 Photochromogenic <ul style="list-style-type: none">• <i>M. scrofulaceum</i>• <i>M. szulgai</i>	<ul style="list-style-type: none">• <i>M. smegmatis</i>• <i>M. vaccae</i>	
• <i>M. gordonae</i>		
Group 3 Non-chromogenic <ul style="list-style-type: none">• <i>M. avium</i> complex• <i>M. xenopi</i>• <i>M. mageritense</i>• <i>M. haemophilum</i>• <i>M. genavense</i>		
• <i>M. ulcerans</i>	<ul style="list-style-type: none">• <i>M. tuberculosis</i> complex• <i>M. leprae</i>	
• <i>M. terrae</i> complex		
True human pathogens	Opportunistic human pathogens	Saprophytes

(RUNYON, 1959)

Introduction

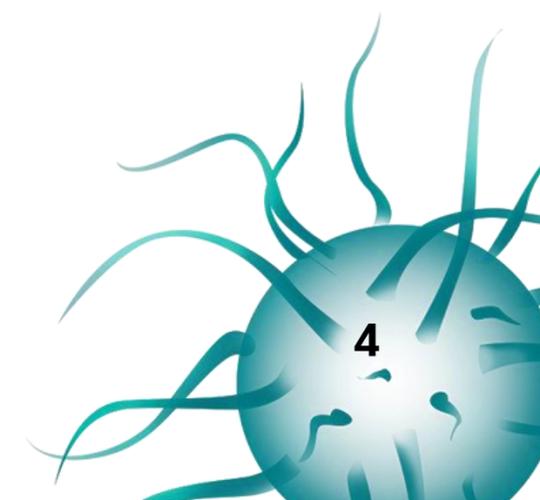
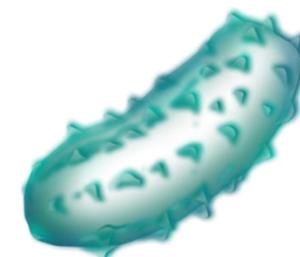
Trend of NTM in global



(Dahl et al. 2022)

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

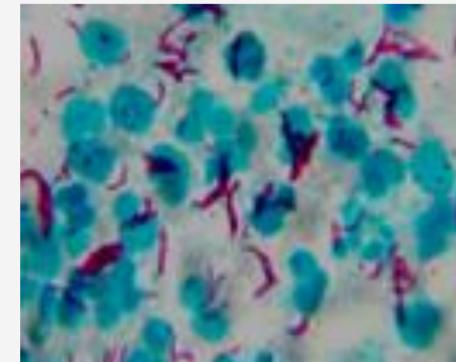
(Dahl et al.2022)



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>



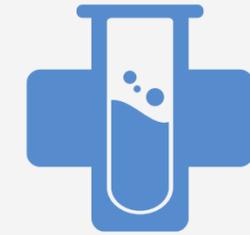
M.kansasii causing severe pneumonia in a patient with leukemia

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 Rajendran¹, Chandrasekaran Padmapriyadarsini¹, Naveenkumar Nagarajan¹, Roja Samyuktha¹, Vadivu Govindaraju¹, Radhika Golla¹, Shanmugavel Ashokkumar¹, Sivakumar Shanmugam¹

Affiliations + expand

PMID: 37887765 PMCID: PMC10610326 DOI: 10.3390/pathogens12101249

2nd Paper

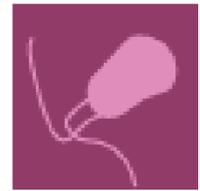
> [Front Microbiol](#). 2025 Apr 29;16:1573448. doi: 10.3389/fmicb.2025.1573448. eCollection 2025.

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

Yiting Wang^{1,2}, Xichao Ou¹, Bing Zhao¹, Hui Xia¹, Yang Zheng¹, Yang Zhou¹, Ruida Xing¹, Yuanyuan Song¹, Shengfen Wang¹, Yanlin Zhao¹, Huiwen Zheng³

Affiliations + expand

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



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 *

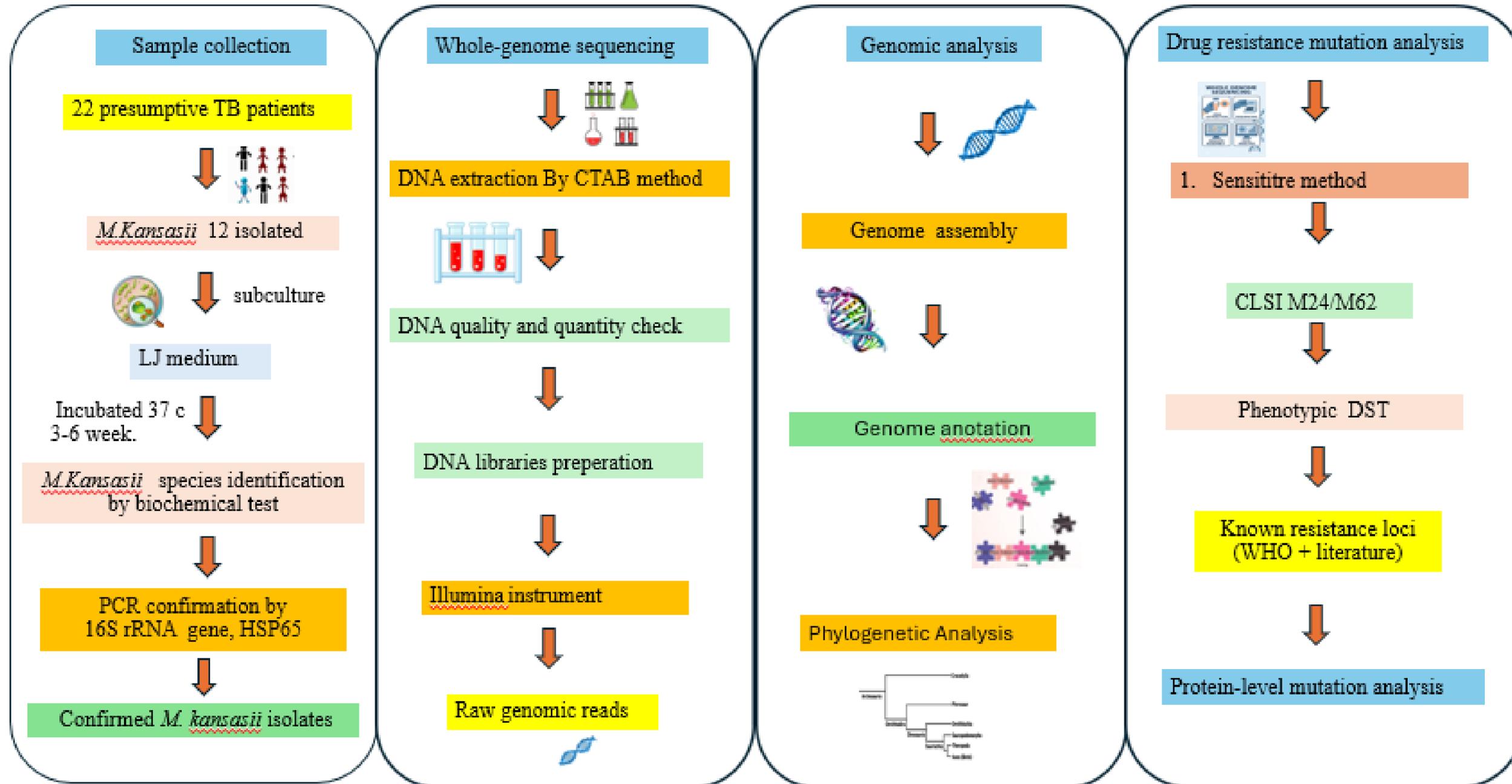
ICMR—National Institute for Research in Tuberculosis, Chennai 600031, India; priya.raj@icmr.gov.in (P.R.); padmapriyadarsi.nic@icmr.gov.in (C.P.); vasanthccmb@gmail.com (N.N.); rojasamyuktha312@gmail.com (R.S.); vadivu0219@gmail.com (V.G.); sashokkumarbiotech@gmail.com (S.A.)

* Correspondence: shanmugam.sk@icmr.gov.in

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

Experimental design

1st Paper



1st Paper

Result

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 isolates confirmed as *M. kansasii*

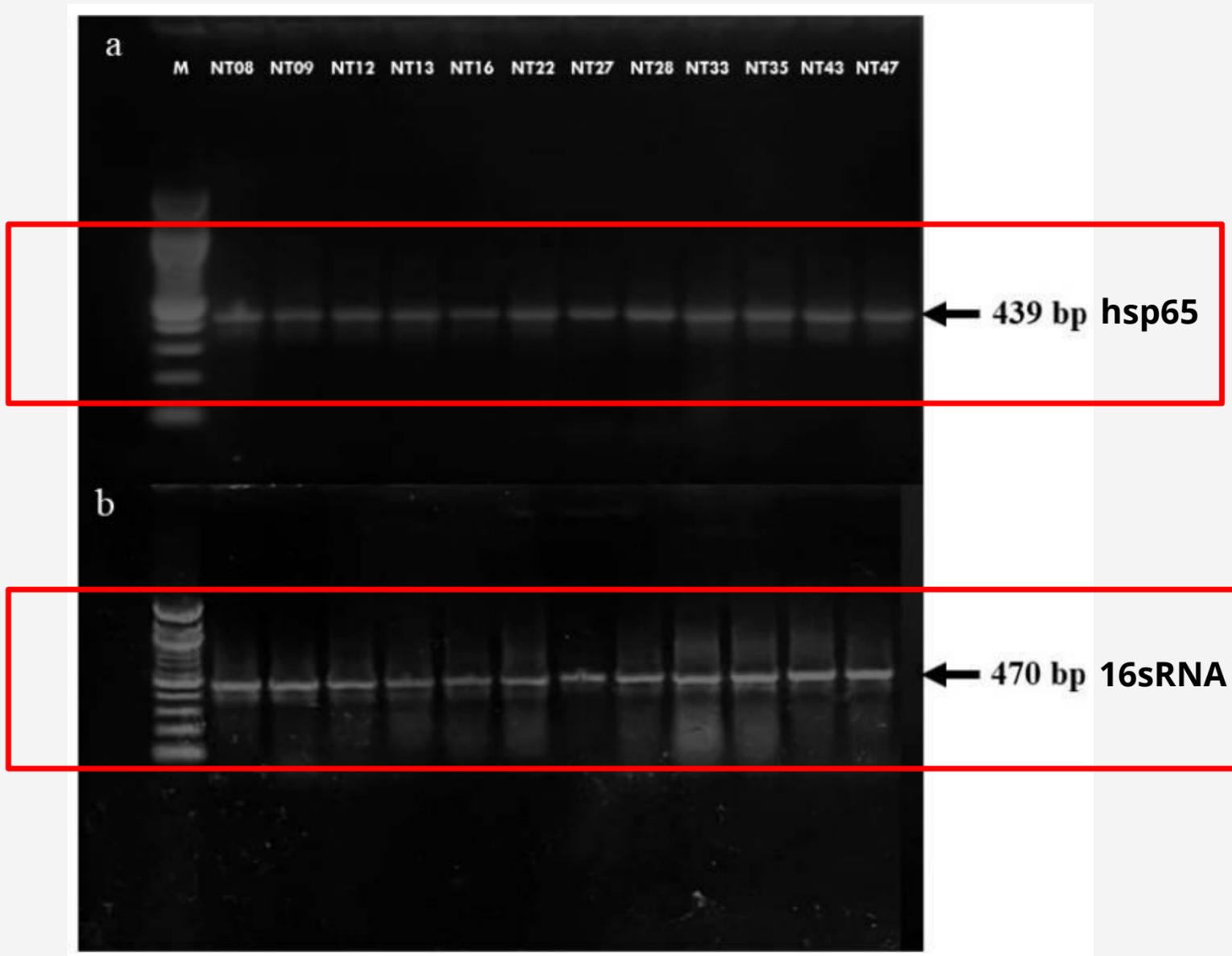
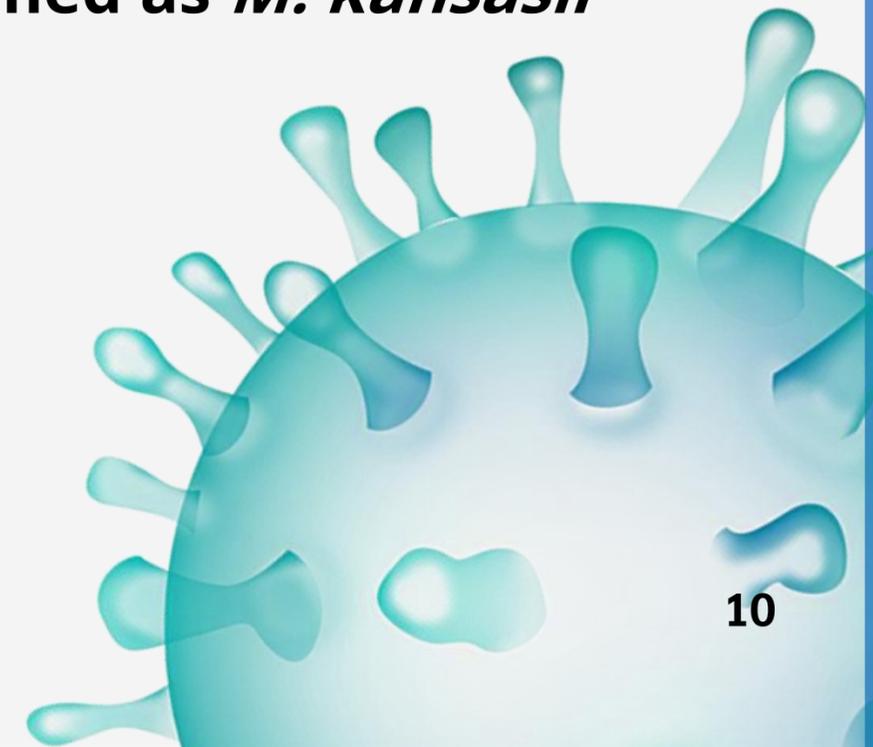


Figure 2. HSP65 PCR (a) and 16srRNA PCR (b) for the 12 isolates that were subjected to sequencing.



1st Paper

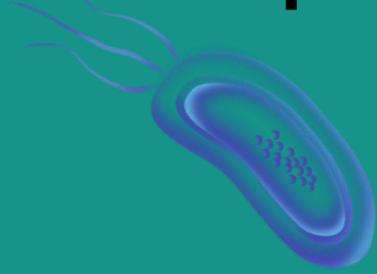
Result

2. Subtype Classification



- 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.

Figure 3. Phylogenetic tree based on 16srRNA, rpoB, hsp65, ITS, and tuf gene sequences constructed using neighbour-joining method (* former classification).



Result

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 <i>M. kansasii</i> Reference Sequence
Amikacin	<i>eis</i> /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 DS resistance to Amikacin**
- **Found 3 non-synonymous mutations in D susceptible to Amikacin isolates.**
- **Found 2 non-synonymous mutations Who compared with the *M. kansasii* reference sequence**

3. Association of Mutation with Drug Resistance



3.2 EMB Resistance

- Target gene : *embB* and *embCA*
- Found 4 non-synonymous mutations in *embB* were detected in 10 isolates 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 in *M. kansasii* remains inconclusive.

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 <i>M. kansasii</i> Reference Sequence
Ethambutol	<i>embB</i>	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. 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 <i>M. kansasii</i> Reference Sequence
Ethambutol	<i>aftB</i>	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

3.2 EMB Resistance

- 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*.

Result

3. Association of Mutation with Drug Resistance

3.2 EMB Resistance

embB and *embCA* genes are associated with EMB resistance in MTB

Mutations were found in both EMB-resistant and EMB-susceptible isolates, their specificity for EMB resistance in *M. kansasii* was not warranted.

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.

Another study by Bakula et al., reported M306I, G406P, and M423I amino acid substitutions, which were associated with EMB resistance in MTB but not yet reported in NTM

Result

3. Association of Mutation with Drug Resistance

3.3 RIF and INH Resistance



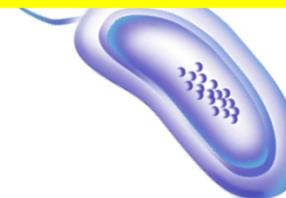
Target gene : *rpoB* and *katG* gene

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

Klein et al., have reported that mutations in the rifampin-resistant isolates (n = 5) appeared to be associated with high-level resistance (256 mg/mL)

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

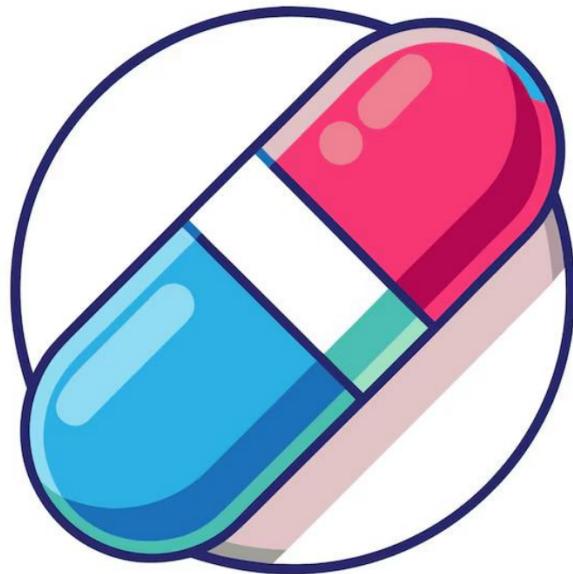
Further genotypic studies are also needed to confirm the correlation between the mutations and high level of RIF resistance to demonstrate its clinical significance.



Result

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

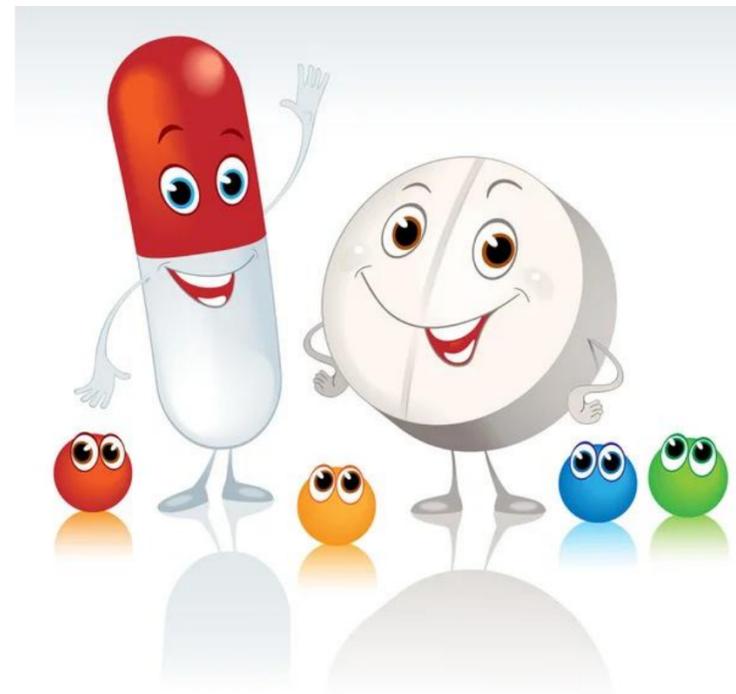
Previous studies report both concordant and discordant patterns

- A similar observation of a single but different *rrl* mutation (A2266C) was previously reported by Bakula et al
- study from China reported 9 CLR-resistant isolates had mutations at position 2058 and 2059 and discordance patterns where 16 CLR-resistant isolates were not found to have mutations in the *rrl* gene

Result

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**

The 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 limited interpretability**

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

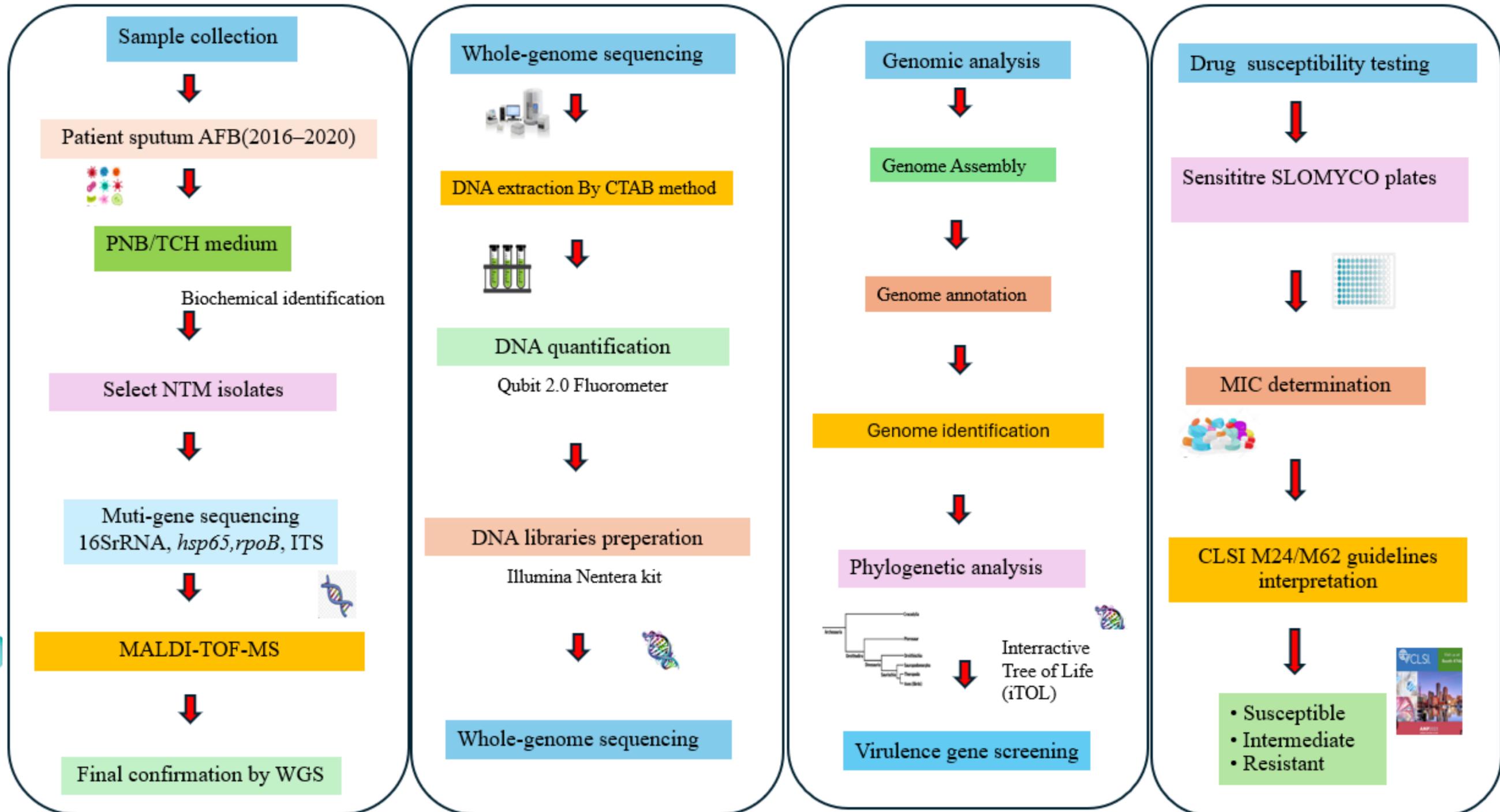
Yiting Wang^{1,2}, Xichao Ou¹, Bing Zhao¹, Hui Xia¹, Yang Zheng¹, Yang Zhou¹, Ruida Xing¹, Yuanyuan Song¹, Shengfen Wang¹, Yanlin Zhao^{1*} and Huiwen Zheng^{3*}

¹National Tuberculosis Reference Laboratory, Chinese Center for Disease Control and Prevention, Beijing, China, ²Beijing Center for Disease Control and Prevention, Beijing Institute of Tuberculosis Control, Institute for Immunization and Prevention, Beijing Academy for Preventive Medicine, Beijing, China, ³Laboratory 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

2nd Paper



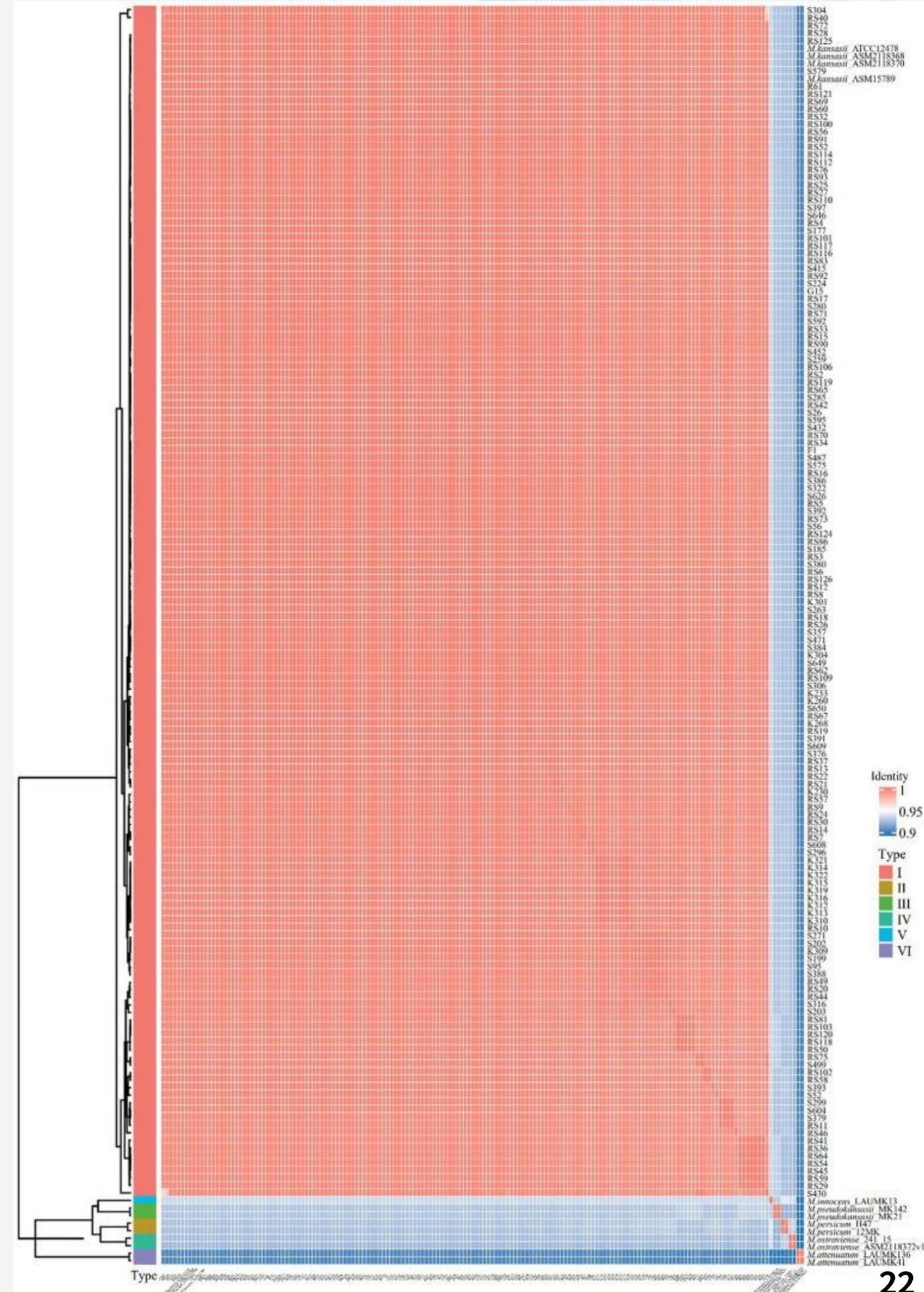
Result

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.



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
- The clustering rate of *M. kansasii* was 11.1% (17/153)
- These findings indicate high genetic diversity
- Differences from previous studies likely reflect the higher resolution of WGS

Tree scale: 10

Phenotypic Resistance

- Amikacin
- Moxifloxacin
- Ciprofloxacin
- Doxycycline
- Linezolid
- Trimethoprim/sulfamethoxazole
- Rifabutin
- Rifampicin
- Clarithromycin

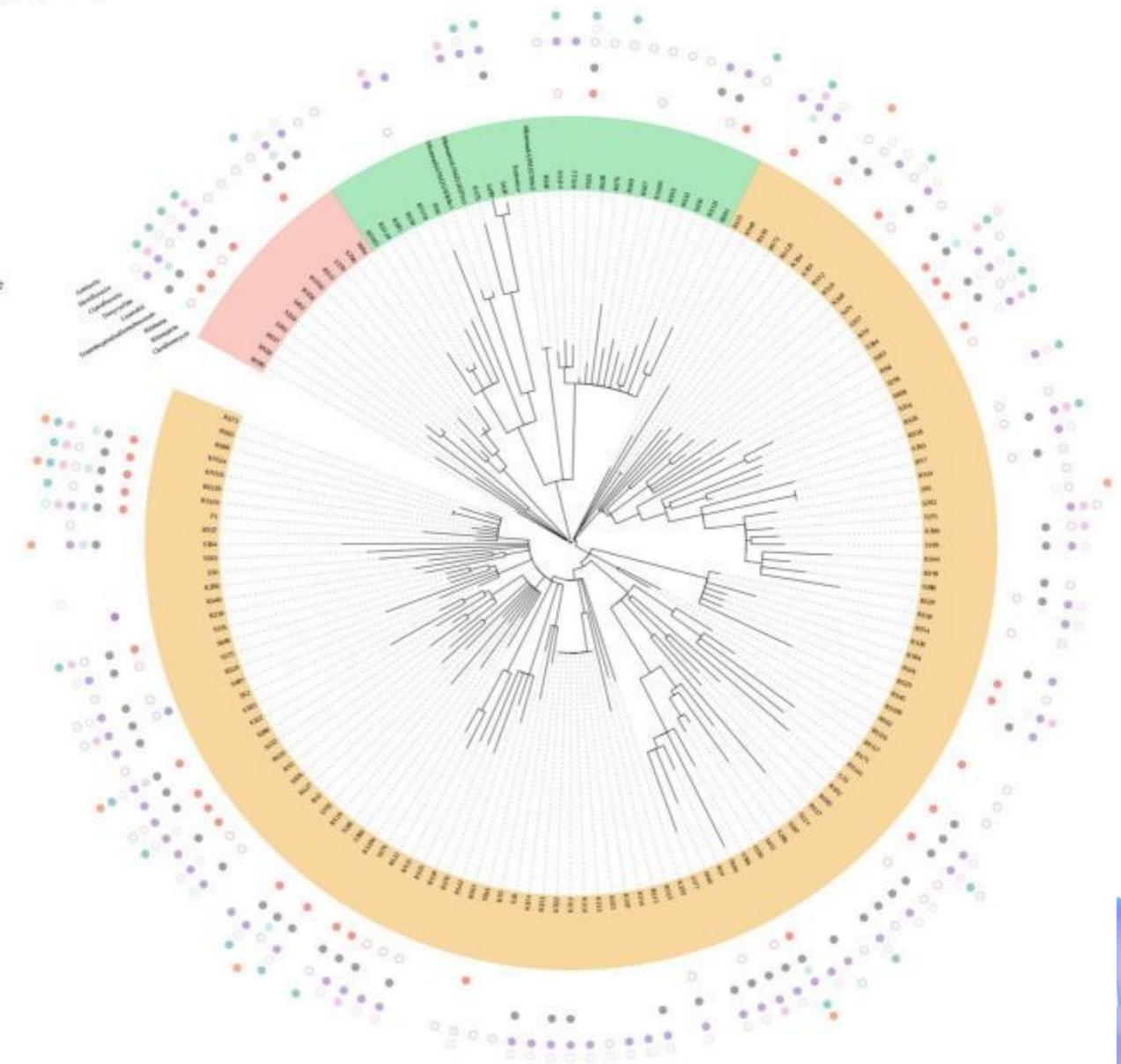


Figure 2. Phylogenetic tree based on core SNP. All 153MKC strains were divided into three clusters, with each cluster represented by a unique color. The outlying circles depicted the outcomes of the drug sensitivity tests. Distinct colors were employed to signify various drugs. Solid circles denoted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

Result

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
- In contrast, previous studies from China reported the absence of *fbpC* and a much lower prevalence of *esxG* (ESX-3 substrate)
- These differences suggest that *M. kansasii* strains circulating in different geographic areas

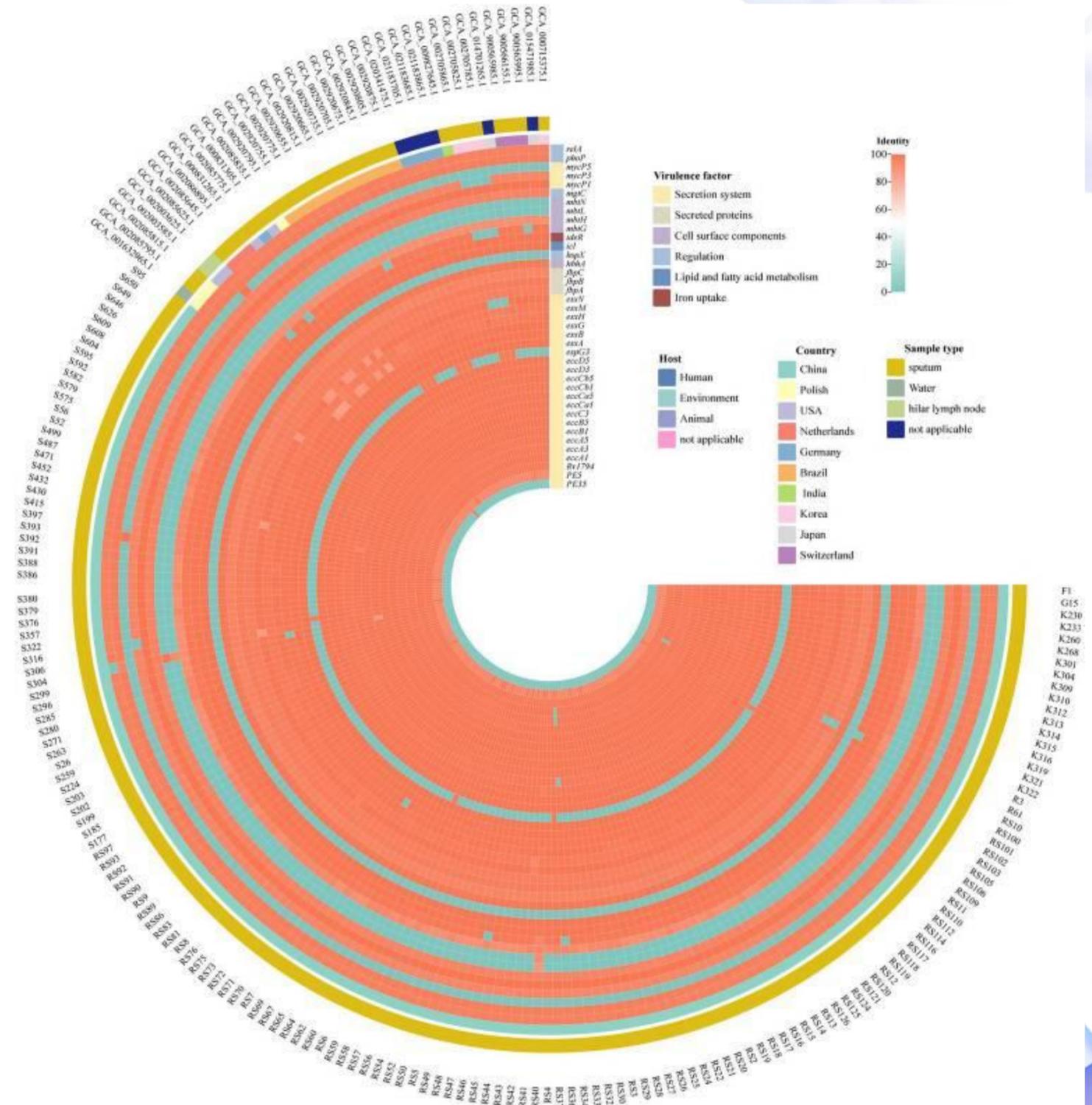


Figure 3. Virulence factors in *M. kansasii* from different sources. The heatmap displayed the identity of multiple virulence genes. The horizontal axis is labeled with samples while the vertical axis enumerates individual virulence genes. A color—coded gradient is employed to depict the degree of identity 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. 24

Drug susceptibility testing

Table 1. The MIC values and resistant rate of antibiotics for *M. kansasii*

Antibiotics	MIC ($\mu\text{g/mL}$)			MIC ₅₀		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	$\leq 2/38$	-	$\geq 4/76$	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 AMK (5.88%) and 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.

Drug susceptibility testing**Table 1. The MIC values and resistant rate of antibiotics for *M. kansasii***

Antibiotics	MIC ($\mu\text{g/mL}$)			MIC ₅₀		MIC ₉₀	Number of resistant isolates (%)
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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	$\leq 2/38$	-	$\geq 4/76$	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%
- 45.45% of MXF-resistant isolates also resistant to CIP
- Rifampicin (RIF) resistance: 22.22%
- Ethionamide (ETO) showed moderate activity (MIC₅₀: 0.6 $\mu\text{g/mL}$)
- 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.

Drug susceptibility testing

Result

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

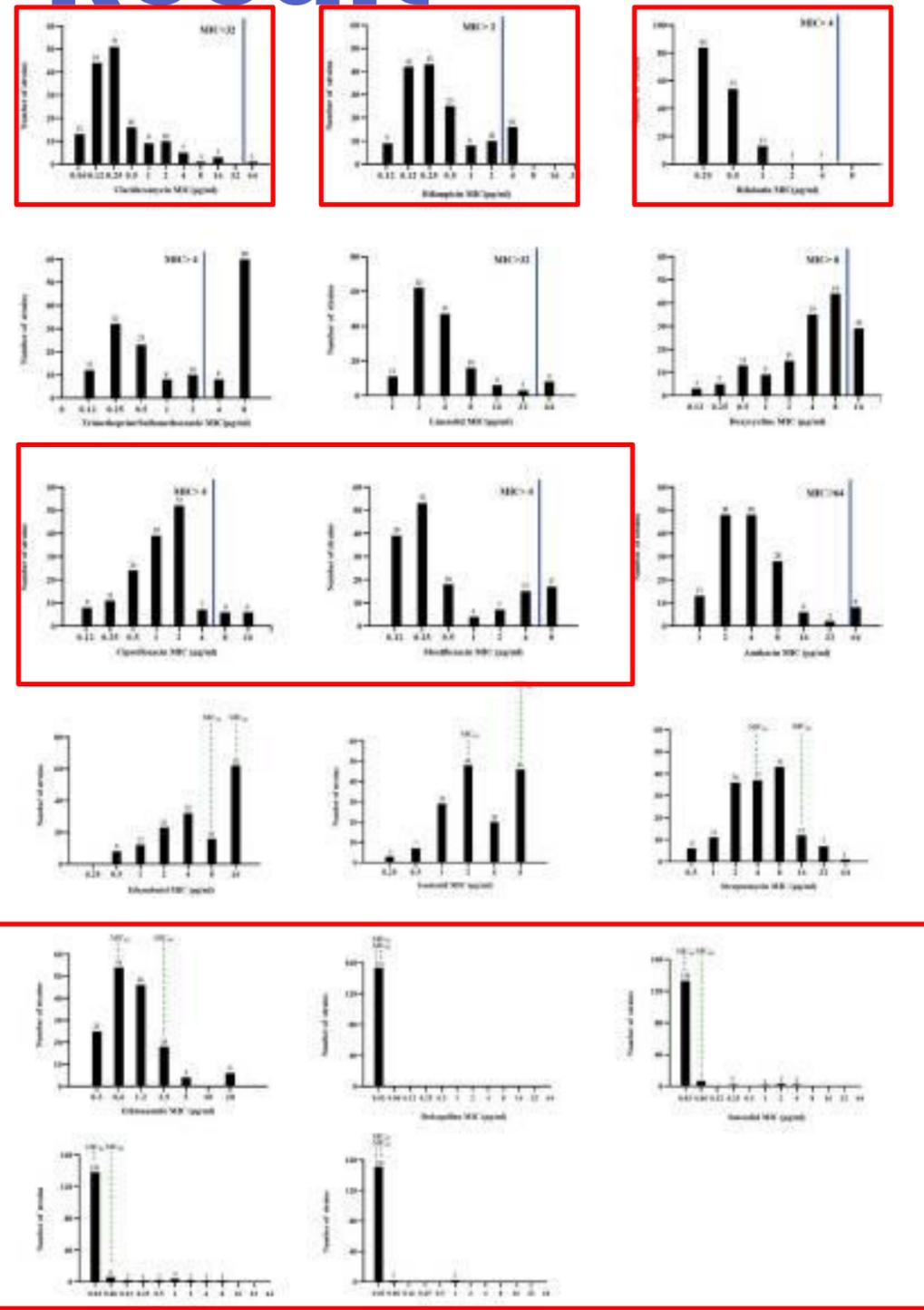


Figure 4. MIC distributions of *M. kansasii* clinical isolates. The solid blue line indicates the CLSI-recommended threshold for determining drug susceptibility, above which a resistant strain is considered. Drugs lacking critical values of MIC50 and MIC90 are denoted by dashed lines. MIC50: the minimum 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.

Drug susceptibility testing

Standard regimen: RIF, INH, EMB

- INH and EMB have limited prognostic value

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 consistent activity

- Potential alternative therapies for *M. kansasii*

Result

2nd Paper

Association between drug resistance profiles and genotypes

Table2 Drug resistance profiles among different *M. kansasii* genotypes

Drug		No. susceptible (%)	No. resistant (%)	χ^2	p-value
Clarithromycin	Cluster1	40(100.00)	0(0.00)	0.80	0.67
	Cluster2	27(96.43)	0(0.00)		
	Cluster3	84(98.82)	1(1.18)		
Rifampicin	Cluster1	32(80.00)	8(20.00)	0.73	0.70
	Cluster2	23(82.14)	5(17.86)		
	Cluster3	64(75.29)	21(24.71)		
Rifabutin	Cluster1	40(100.00)	0(0.00)	0.81	0.67
	Cluster2	28(100.00)	0(0.00)		
	Cluster3	84(98.82)	1(1.18)		
Trimethoprim/ sulfamethoxazole	Cluster1	27(67.50)	13(32.5)	3.17	0.21
	Cluster2	16(57.14)	12(42.86)		
	Cluster3	43(50.59)	42(49.41)		
Linezolid	Cluster1	37(92.50)	3(7.50)	2.24	0.33
	Cluster2	25(89.29)	0(0.00)		
	Cluster3	75(88.24)	7(8.24)		
Doxycycline	Cluster1	4(10.00)	18(45.00)	1.49	0.48
	Cluster2	5(17.86)	13(46.43)		
	Cluster3	20(23.53)	43(50.59)		
Ciprofloxacin	Cluster1	27(0.68)	5(12.50)	0.69	0.71
	Cluster2	12(42.86)	4(14.29)		
	Cluster3	44(51.76)	9(10.59)		
Moxifloxacin	Cluster1	28(70.00)	12(30.00)	3.41	0.18
	Cluster2	19(67.86)	7(25.00)		
	Cluster3	67(78.82)	13(15.29)		
Amikacin	Cluster1	39(97.50)	1(2.50)	2.00	0.37
	Cluster2	27(96.43)	1(3.57)		
	Cluster3	77(90.59)	7(8.24)		

P < 0.05 was defined to be statistically significant.

- No statistically significant association between clusters and resistance profiles
- However, Cluster 3 showed numerically higher resistance rates for several drugs, including CLA, RIF, RFB, TMP/SMX, LZD, DOX, and AMK, compared with Clusters 1 and 2.)
- In contrast, fluoroquinolone resistance (CIP and MXF) was lower in Cluster 3 than in Clusters 1 and 2.

Conclusions

- *M. kansasii* type I is the predominant genotype in China.
- Rifabutin (RFB) and Clarithromycin (CLR) showed strong activity against *M. kansasii* strains.
- Three clusters of *M. kansasii* were identified based on core genome SNP analysis.
- New drugs Bedaquiline (BDQ), Delamanid (DLM), Sutezolid (SZD), and Clofazimine (CFZ) show potential as effective treatments for *M. kansasii* infection

Criticism

Strong points

Weak points

1st Paper

Rajendran et al., 2023

- First report from India on WGS of *M. kansasii*
- Integration of genomic data with phenotypic DST

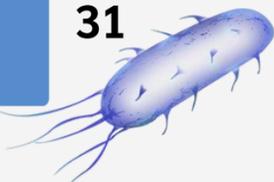
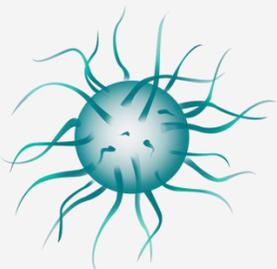
- Very small sample (12 isolates)
- The phenotypic or genotypic drug-resistance patterns could not be correlated with treatment outcome.

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.



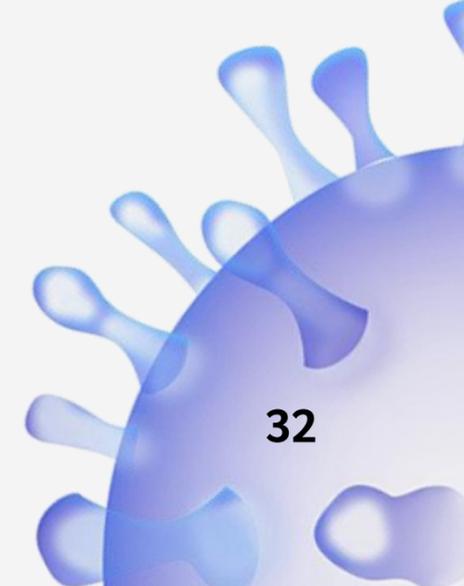
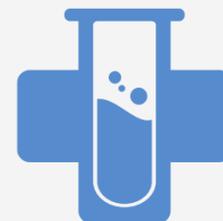
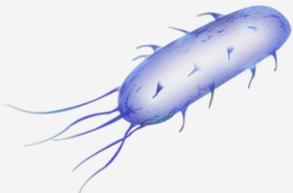


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**THANK YOU
FOR YOUR KIND ATTENTION**

