Molecular diagnosis of drug-resistant Mycobacterium tuberculosis

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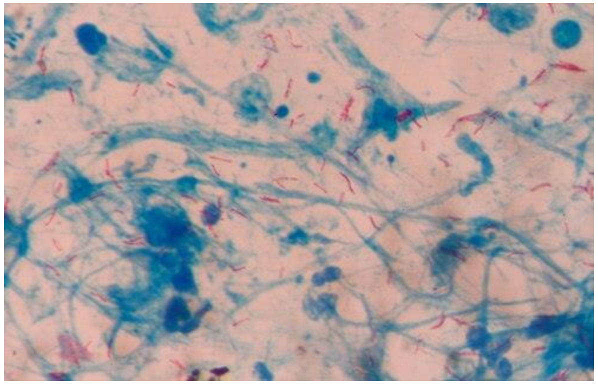
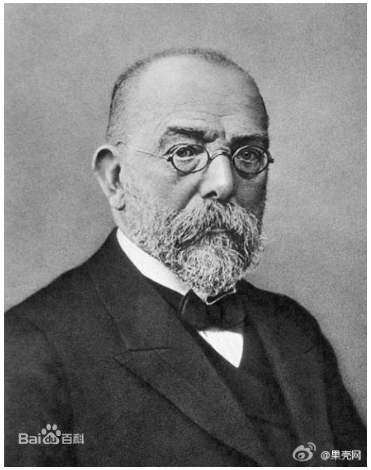
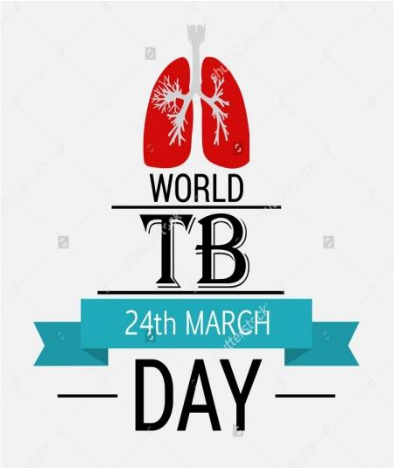
Tuberculosis (TB)---more than 10,000 years history

- **TB is a potentially serious infectious disease that mainly affects the lungs, which is caused by Mycobacterium tuberculosis (Mtb)**



- **Robert Koch firstly discovered the Mtb using acid-fast stain in 1882**

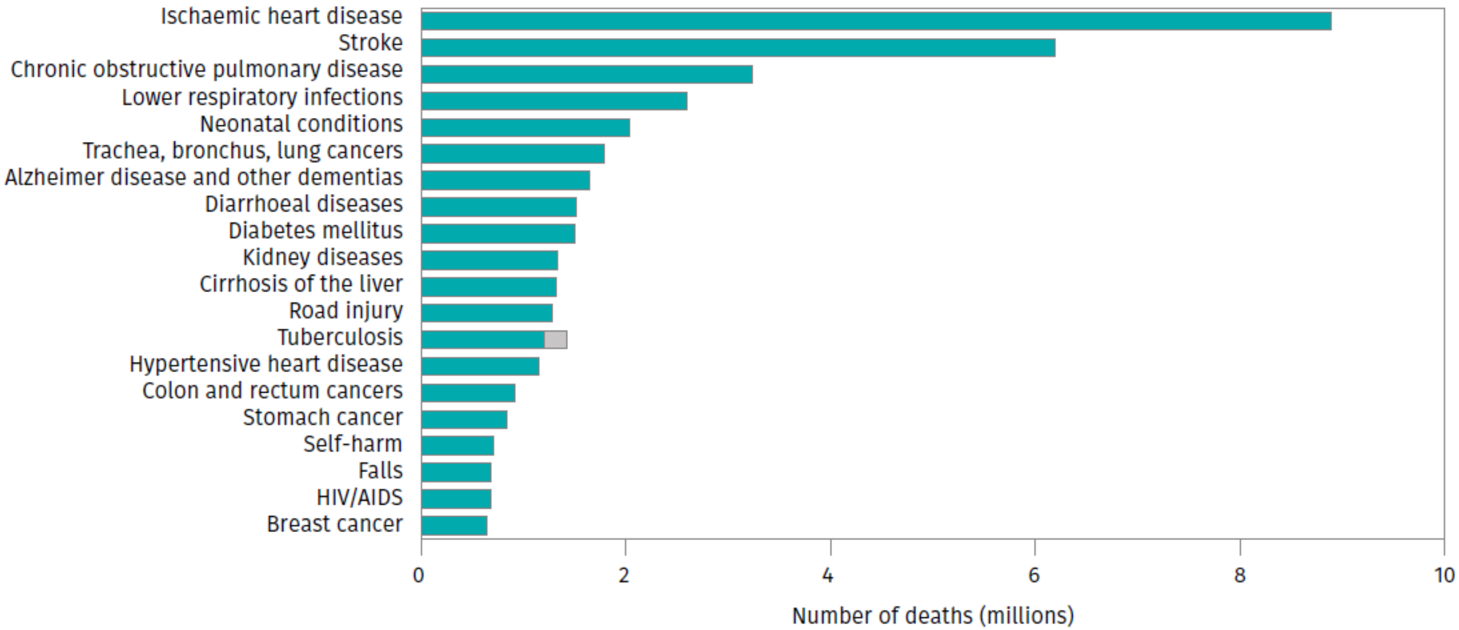




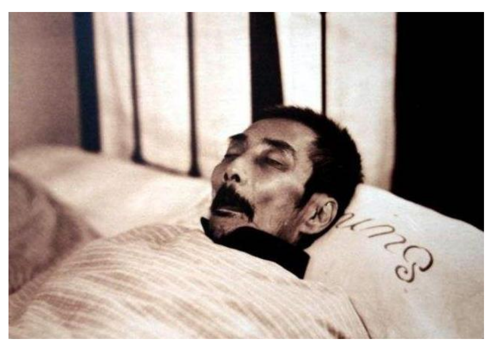
TB is the world's top infectious killer

Top causes of death worldwide in 2019[a,b]

Deaths from TB among HIV-positive people are shown in grey.



Daiyu Lin



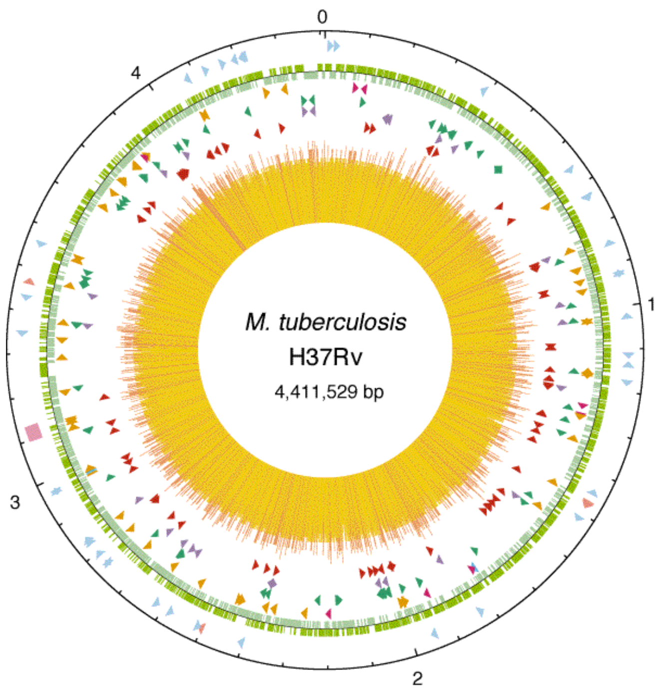
Xun Lu

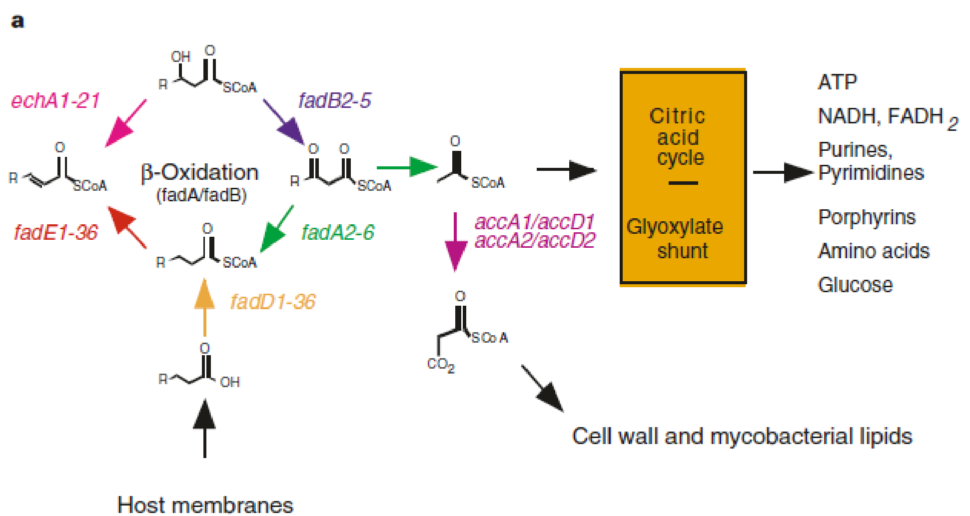
Huiyin Lin

Mtb genome

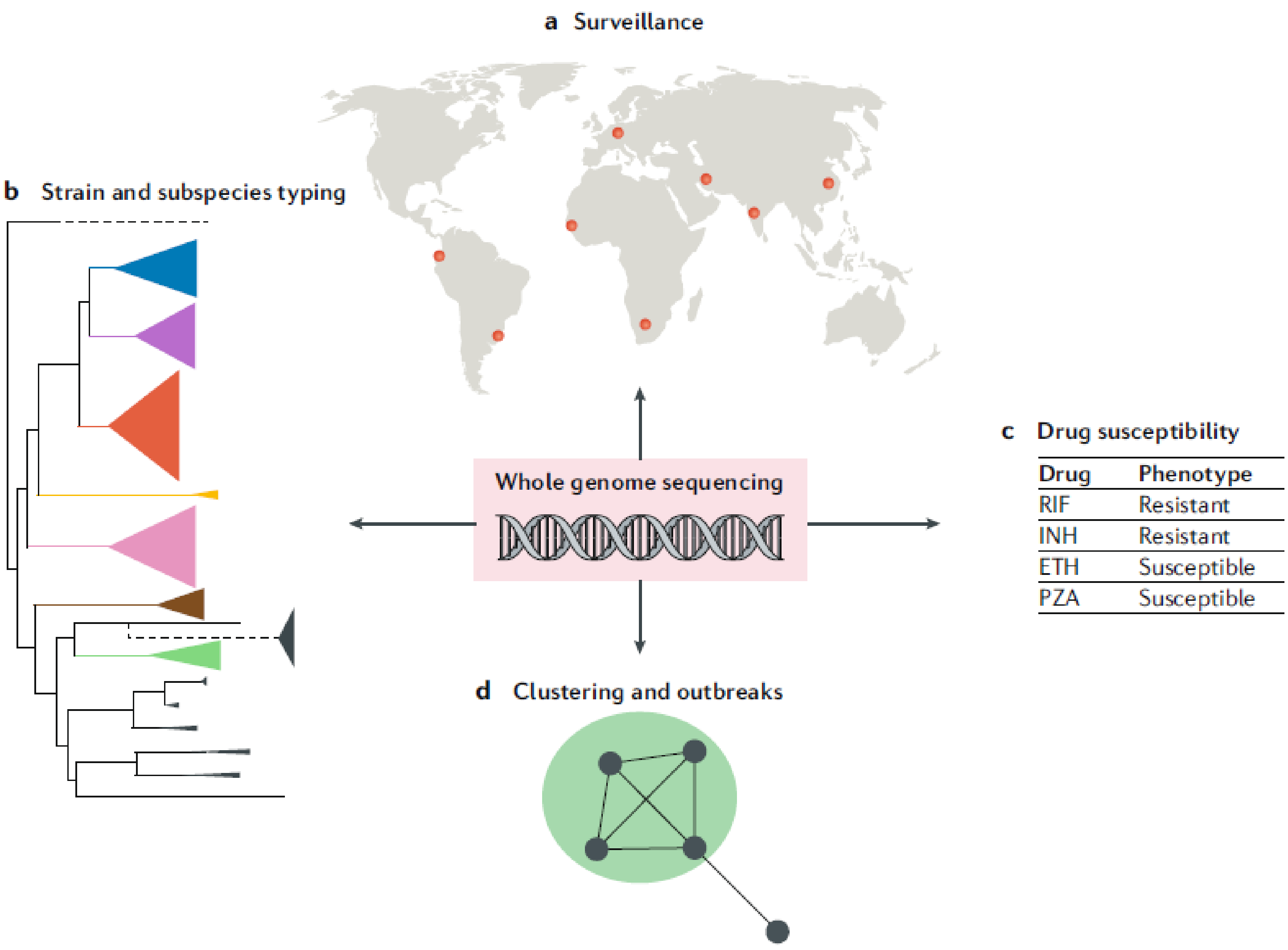
➢Mtb has circular chromosomes of about **4,200,000** nucleotides long, the G+C content is about **65%,** the genome contains about 4000 genes

➢Genes that code for **lipid metabolism** are a very important part of the bacterial genome, and **8%** of the genome is involved in this activity



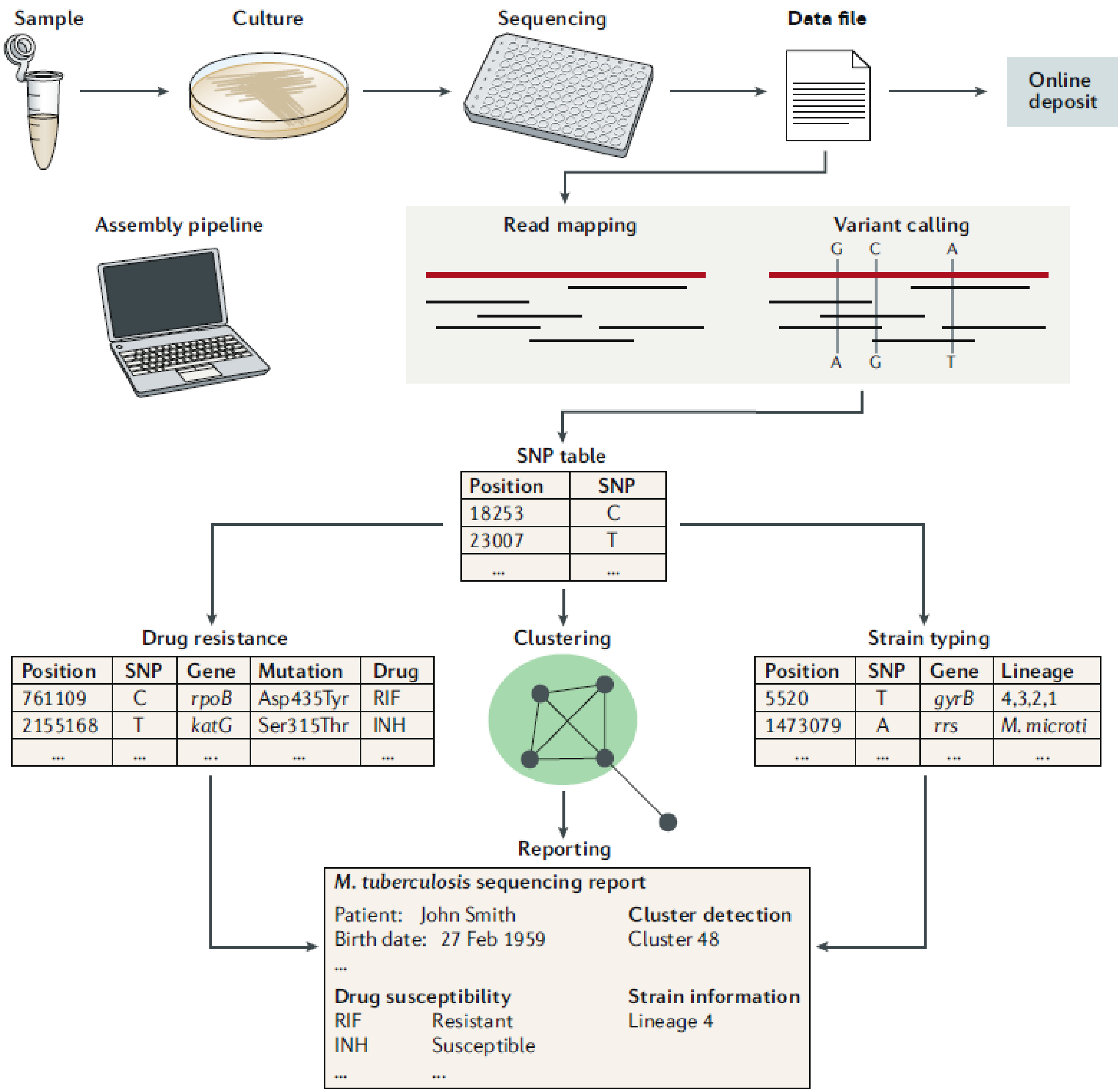


The primary applications for whole genome Sequencing (WGS) of Mtb



Meehan CJ, et al. *Nat Rev Microbiol*. 2019

Standard workflow for WGS of *Mtb complex isolates*



Case report

➢2020-08-06： BALF mNGS

病原微生物宏基因检测结果
1-有效检出细菌
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2-有效检出分枝杆菌
结核分枝杆菌复合群(Mycobacterium tuberculosis complex)
3-有效检出真菌
--
4-有效检出病毒
--
5-有效检出其他病原微生物
--

2. 病原微生物列表

2.1 检出细菌列表

类型	属	种	检出序列数（百分比）	置信度
未发现				

2.2 检出分枝杆菌列表

类型	属	种	检出序列数（百分比）	置信度
B:G+	分枝杆菌属	结核分枝杆菌复合群 *Mycobacterium tuberculosis complex*	3 (99%)	99%

2.3 检出支原体/衣原体列表

类型	属	种	检出序列数（百分比）	置信度
未发现				

2.4 检出真菌列表

类型	属	种	检出序列数（百分比）	置信度
未发现				

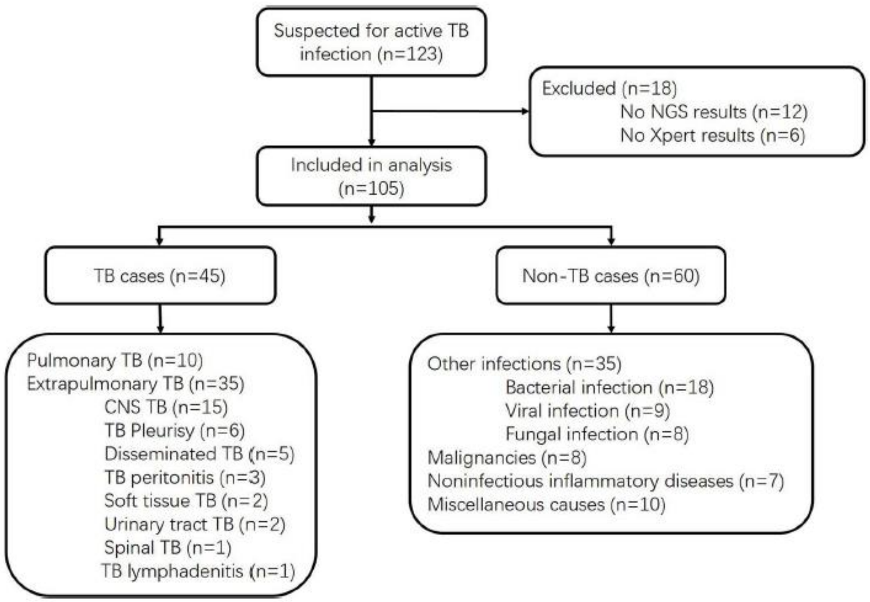
2.5 检出DNA病毒列表

类型	属	种	检出序列数（百分比）	置信度
未发现				

2.6 检出其他病原微生物列表

类型	属	种	检出序列数（百分比）	置信度
未发现				

Diagnosis efficacy of Mtb via metagenomic NGS of direct clinical samples



	Sensitivity (95% CI; *n/N*)	Specificity (95% CI; *n/N*)
All samples		
Conventional	29% (0.16–0.44; 13/45)*	100% (0.94–1; 60/60)
Xpert	42% (0.28–0.58; 19/45)	100% (0.94–1; 60/60)
mNGS	44% (0.30–0.60; 20/45)	98% (0.91–1; 59/60)
Xpert and mNSG	60% (0.44–0.74; 27/45)*	98% (0.91–1; 59/60)
Pulmonary samples		
Conventional	38% (0.14–0.68; 5/13)	100% (0.77–1; 14/14)
Xpert	62% (0.32–0.86; 8/13)	100% (0.77–1; 14/14)
mNGS	62% (0.32–0.86; 8/13)	100% (0.77–1; 14/14)
Xpert and mNSG	69% (0.39–0.91; 9/13)	100% (0.77–1; 14/14)
CSF samples		
Conventional	19% (0.04–0.46; 3/16)	100% (0.89–1; 33/33)
Xpert	19% (0.04–0.46; 3/16)	100% (0.89–1; 33/33)
mNGS	44% (0.2–0.7; 7/16)	97% (0.84–99; 32/33)
Xpert and mNSG	56% (0.30–0.80; 9/16)	97% (0.84–99; 32/33)
Other extrapulmonary samples		
Conventional	31% (0.11–0.59; 5/16)	100% (0.75–1; 13/13)
Xpert	50% (0.25–0.75; 8/16)	100% (0.75–1; 13/13)
mNGS	31% (0.11–0.59; 5/16)	100% (0.75–1; 13/13)
Xpert and mNSG	56% (0.30–0.80; 9/16)	100% (0.75–1; 13/13)

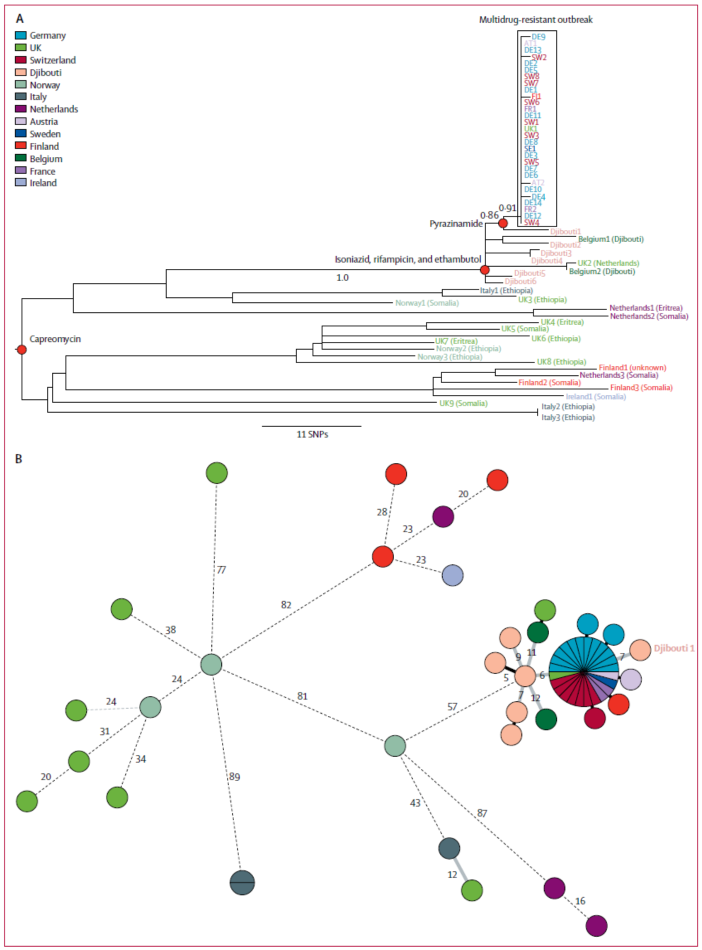
**The combined sensitivity of Xpert and mNGS reached statistical significance compared to conventional methods (McNemar-test $P < 0.001$).*

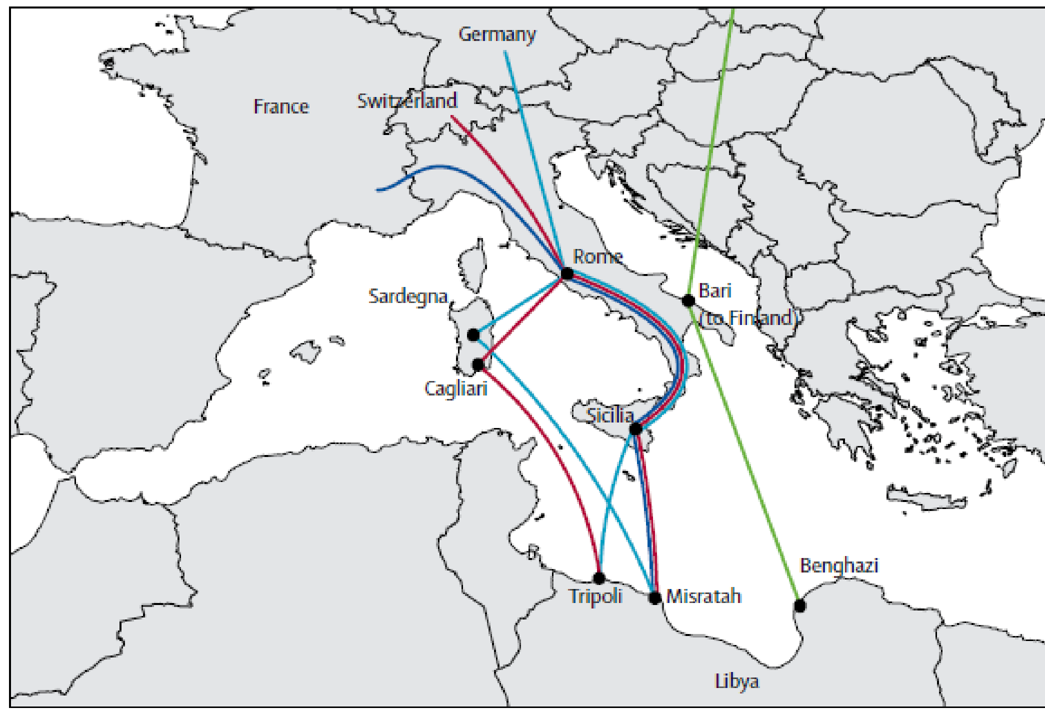
Zhou X, et al. *Front Cell Infect Microbiol*. 2019

Outbreak investigation and genetic diversity

THE LANCET
Infectious Diseases

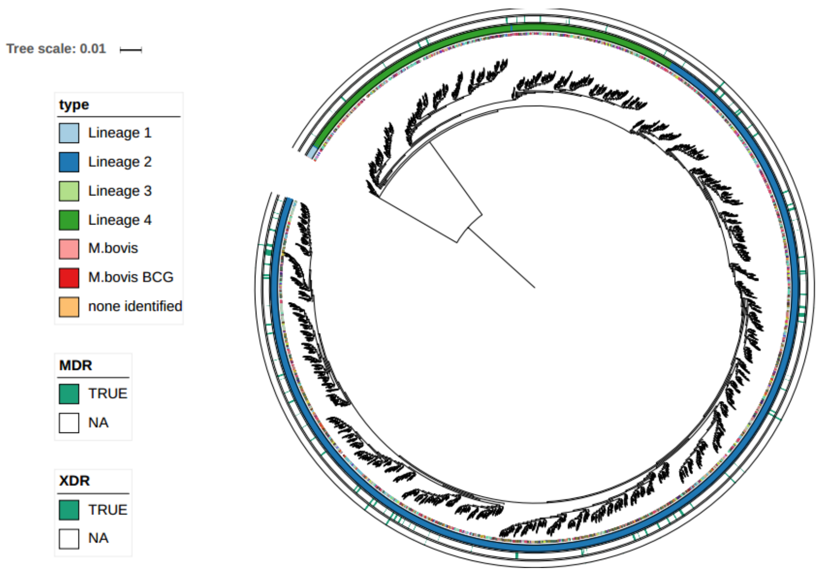
A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study





A national survey of clinical Mtb complex isolates

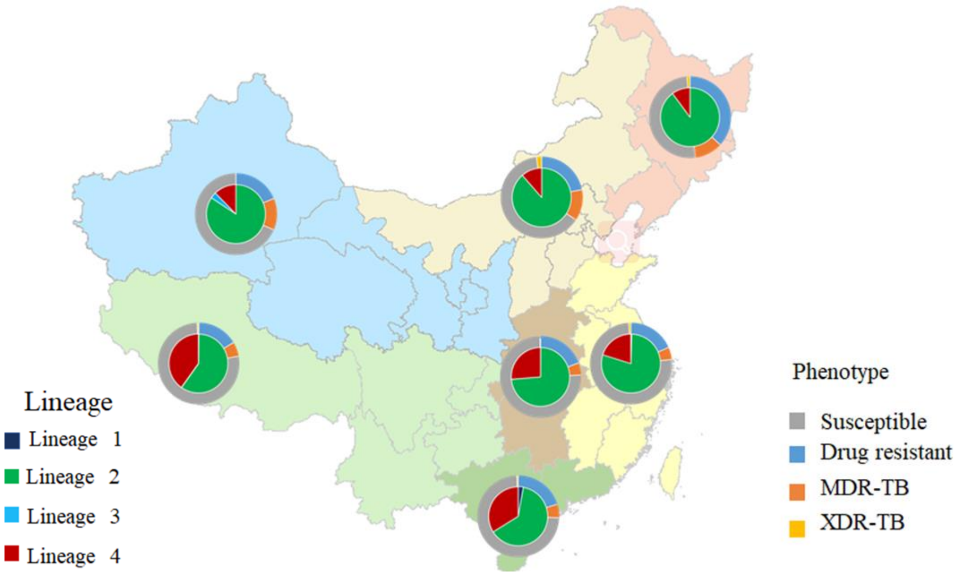
Sites of Surveillance

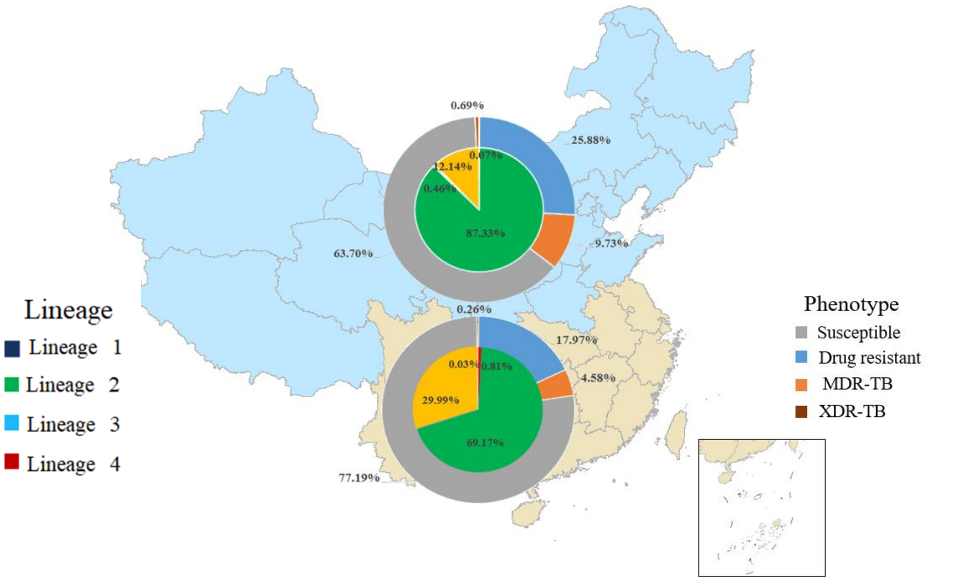


Liu D, et al. Clin Microbiol Infect. 2021

Mtb lineage distribution in China

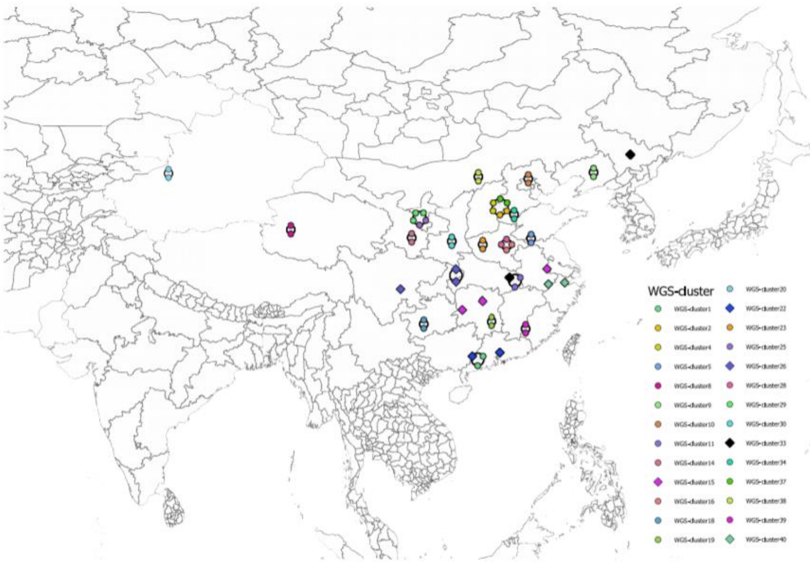
- **Lineage 2 is the main strain in China**
- **The higher percentage of lineage 4 was observed in Southern China**
- **The rate of MDR isabout 5-10%**



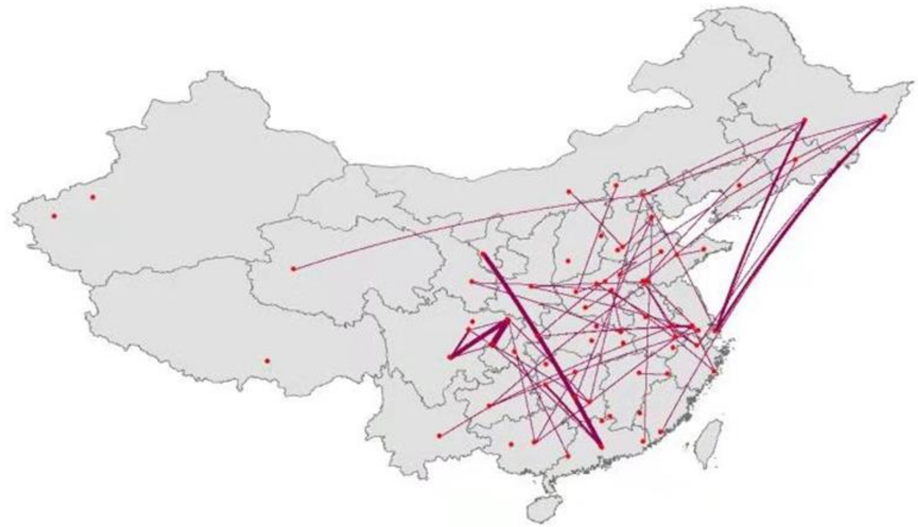


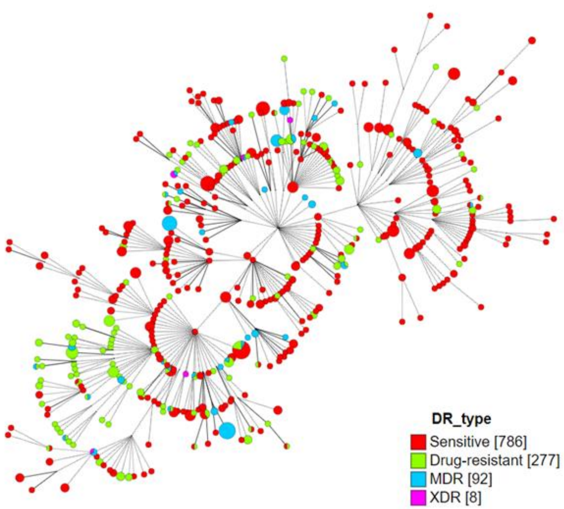
MDR Mtb clustering from 2007 to 2013

2007



2013





Clusters within provinces (rate)	Clusters across provinces (rate)	Total
265 (53.5%)	230 (46.5%)	495 (100%)

Predicting susceptibility and drug resistance in Mtb

The NEW ENGLAND JOURNAL of MEDICINE

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

Table 1. Numbers of Isolates According to Country of Sample Origin and Drug-Resistance Profile.

Country	Period Isolated	Enriched for Resistance	Susceptible to All Four Drugs	Susceptible to Three Drugs*	Isoniazid-Resistant, Rifampin-Susceptible	Isoniazid-Susceptible, Rifampin-Resistant	Isoniazid-Resistant, Rifampin-Resistant	Other Pattern	Total
Australia	2006–2016	Yes	0	0	4	0	38	0	42
Belgium	2007–2015	Yes	121	0	2	0	97	14	234
Canada	2003–2014	Yes	11	1,118	164	14	24	12	1,343
China	2009–2012	Yes	0	44	0	0	236	0	280
Germany	1998–2015	No	248	0	9	1	13	2	273
Italy	2008–2016	Yes and no†	82	1	9	0	132	2	226
Netherlands	1993–2016	Yes and no†	420	42	24	1	149	31	667
Pakistan	2014–2015	Yes	47	5	11	6	345	1	415
Peru	1997–2009	Yes	24	12	49	18	199	13	315
Russia	2008–2010	Yes	282	0	116	15	407	22	842
Serbia	2008–2014	Yes	0	0	0	0	105	0	105
South Africa	2012–2014	Yes	593	11	37	69	151	130	991
Spain	2013–2015	Yes	45	3	5	2	8	1	64
eSwatini‡	2009–2010	Yes	2	130	14	4	116	7	273
Thailand	1998–2013	Yes	0	53	7	4	188	0	252
United Kingdom	2009–2017	Yes and no†	3,036	82	167	6	442	154	3,887
Total			4,911	1,501	618	140	2,650	389	10,209

* Isolates in this category were missing results for pyrazinamide.
† More than one collection was derived from Italy, the Netherlands, and the United Kingdom, some of which were enriched and some of which were not enriched for resistance. Details are provided in the Supplementary Appendix.
‡ Until recently, eSwatini was known as Swaziland.

Predicting susceptibility and drug resistance in Mtb

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Table 2. Prediction of Phenotypes of Resistance or Susceptibility to Individual Drugs.*

Analysis and Drug	Resistant Phenotype					Susceptible Phenotype					Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Sensitivity, All†	Specificity, All†	NGP	RP
	R	S	U	F	Total	R	S	U	F	Total								
	number of isolates										*percent*							
WGS, all isolates																		
Isoniazid	3067	90	93	44	3294	65	6313	215	117	6710	97.1 (96.5–97.7)	99.0 (98.7–99.2)	97.9 (97.4–98.4)	98.6 (98.3–98.9)	93.1	94.1	4.7	32.9
Rifampin	2743	69	7	84	2903	85	6763	232	147	7227	97.5 (96.9–98.1)	98.8 (98.5–99.0)	97.0 (96.3–97.6)	99.0 (98.7–99.2)	94.5	93.6	4.6	28.7
Ethambutol	1410	81	94	55	1640	468	6835	781	70	8154	94.6 (93.3–95.7)	93.6 (93.0–94.1)	75.1 (73.0–77.0)	98.8 (98.5–99.1)	86.0	83.8	10.2	16.7
Pyrazinamide	863	82	117	77	1139	204	6146	197	108	6655	91.3 (89.3–93.0)	96.8 (96.3–97.2)	80.9 (78.4–83.2)	98.7 (98.4–99.0)	75.8	92.4	6.4	14.6

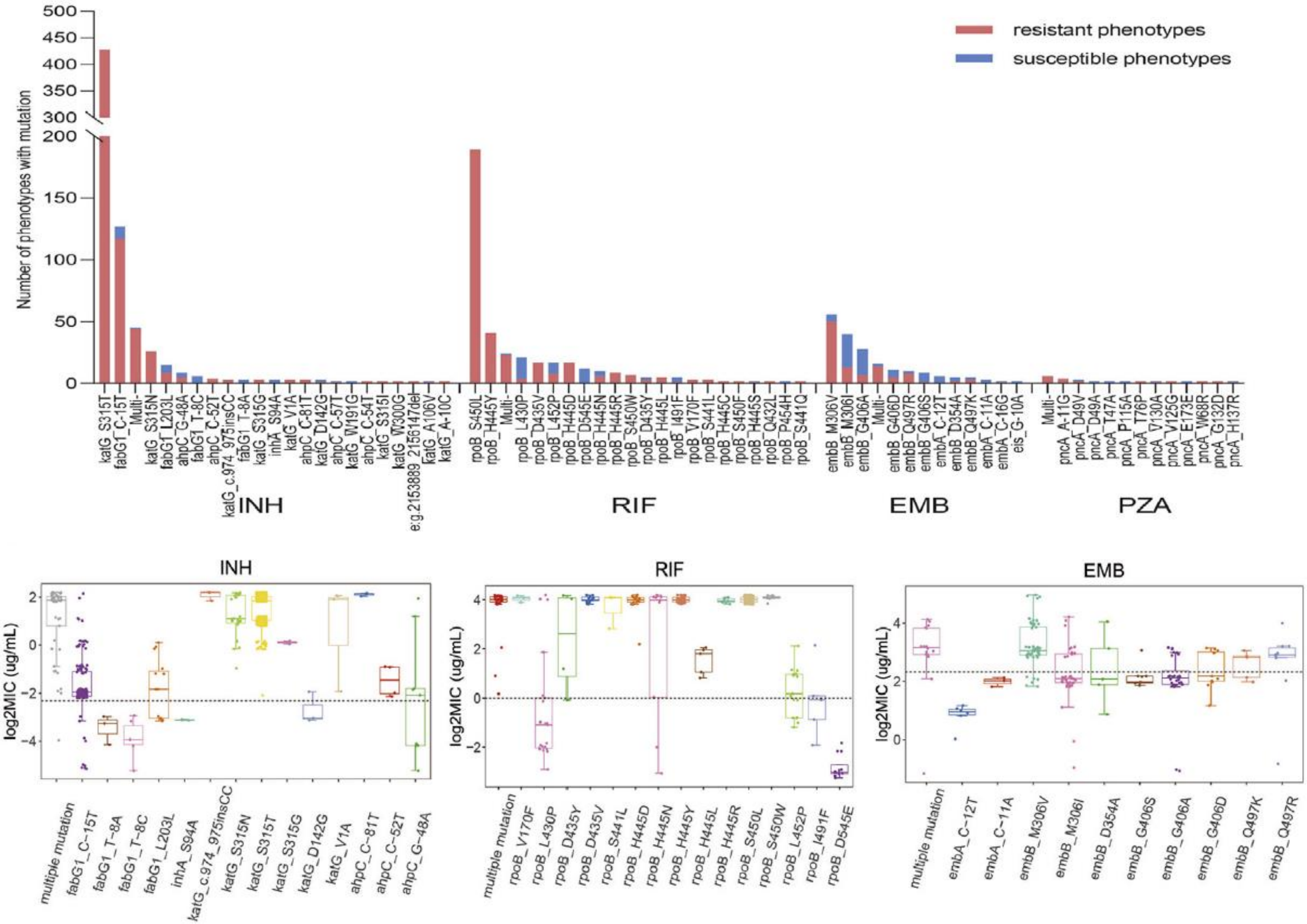
Prediction of phenotypes of resistance to ten drugs and multidrug resistance

Drug	Resistant phenotype			Susceptible phenotype			Sensitivity (95% CI)	Specificity (95% CI)
	R	S	Total	R	S	Total		
Isoniazid	668	41	709	44	4127	4171	94.22 (92.50–95.94)	98.95 (98.64–99.26)
Rifampicin	350	12	362	52	4466	4518	96.69 (94.84–98.53)	98.85 (98.54–99.16)
Ethambutol	105	8	113	90	4677	4767	92.92 (88.19–97.65)	98.11 (97.73–98.50)
Pyrazinamide	49	46	97	19	2230	2249	50.52 (40.57–60.47)	99.16 (98.78–99.53)
Moxifloxacin	182	32	214	44	4622	4666	85.05 (80.27–89.83)	99.06 (98.78–99.34)
Rifabutin	313	13	326	44	4510	4554	96.01 (93.89–98.13)	99.03 (98.75–99.31)
Amikacin	27	3	30	3	4847	4850	90.00 (79.26–100.00)	99.94 (99.87–100.00)
Kanamycin	35	2	37	4	4839	4843	94.59 (87.30–100.00)	99.92 (99.88–100.00)
Streptomycin	550	80	630	38	4212	4250	87.30 (84.70–89.90)	99.11 (98.82–99.39)
Ethionamide	75	8	83	100	4697	4797	90.36 (84.01–96.71)	97.91 (96.36–98.34)
Multidrug resistance	291	15	306	28	4546	4574	95.10 (92.68–97.52)	99.39 (99.16–99.61)

Abbreviations: R, resistant; S, susceptible.

Liu D, et al. Clin Microbiol Infect. 2021

Distribution and log_2^{MIC} of different mutations of first-line drugs



Liu D, et al. Clin Microbiol Infect. 2021

Profiling lung microbiota using Metagenomic NGS

◆ **Key point: Characterizing the Lung microbiota profile and investigating its association with development of pulmonary TB**

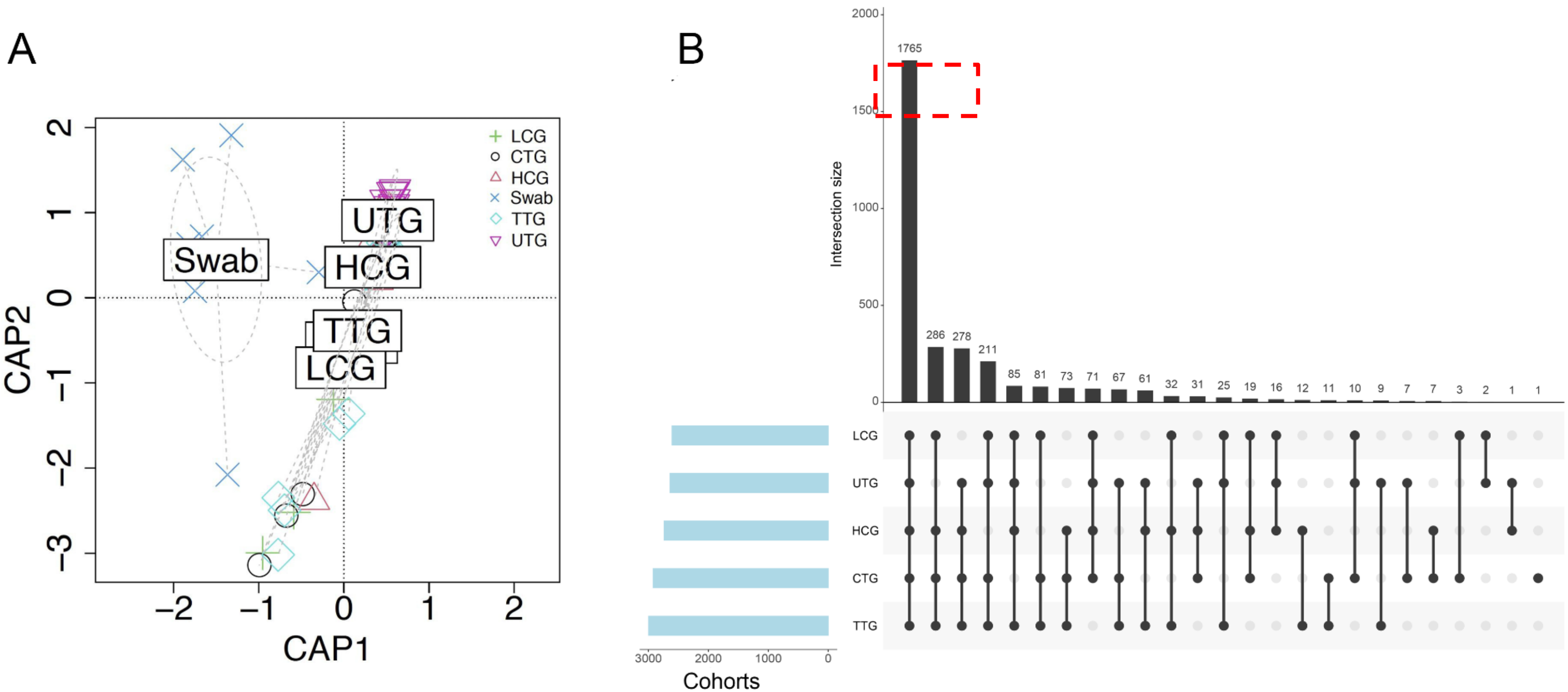
Classification	Abbreviation	No.	Definition	Samples
Healthy controls	HCG	8	negative chest radiological signs and IFN-γ release assay (IGRA)	Throat swab & BALF
Untreated pulmonary TB	UTG	12	not taking any antibiotics	BALF
Treated pulmonary TB	TTG	15	receiving more than two weeks of standard anti-TB treatment	BALF
Cured pulmonary TB	CTG	11	bacterial negative conversion and finished long-term anti-TB treatment	BALF
Lung cancer	LCG	7	confirmed by cellular or tissue pathology diagnoses	BALF

⇩

metagenomic next-generation sequencing

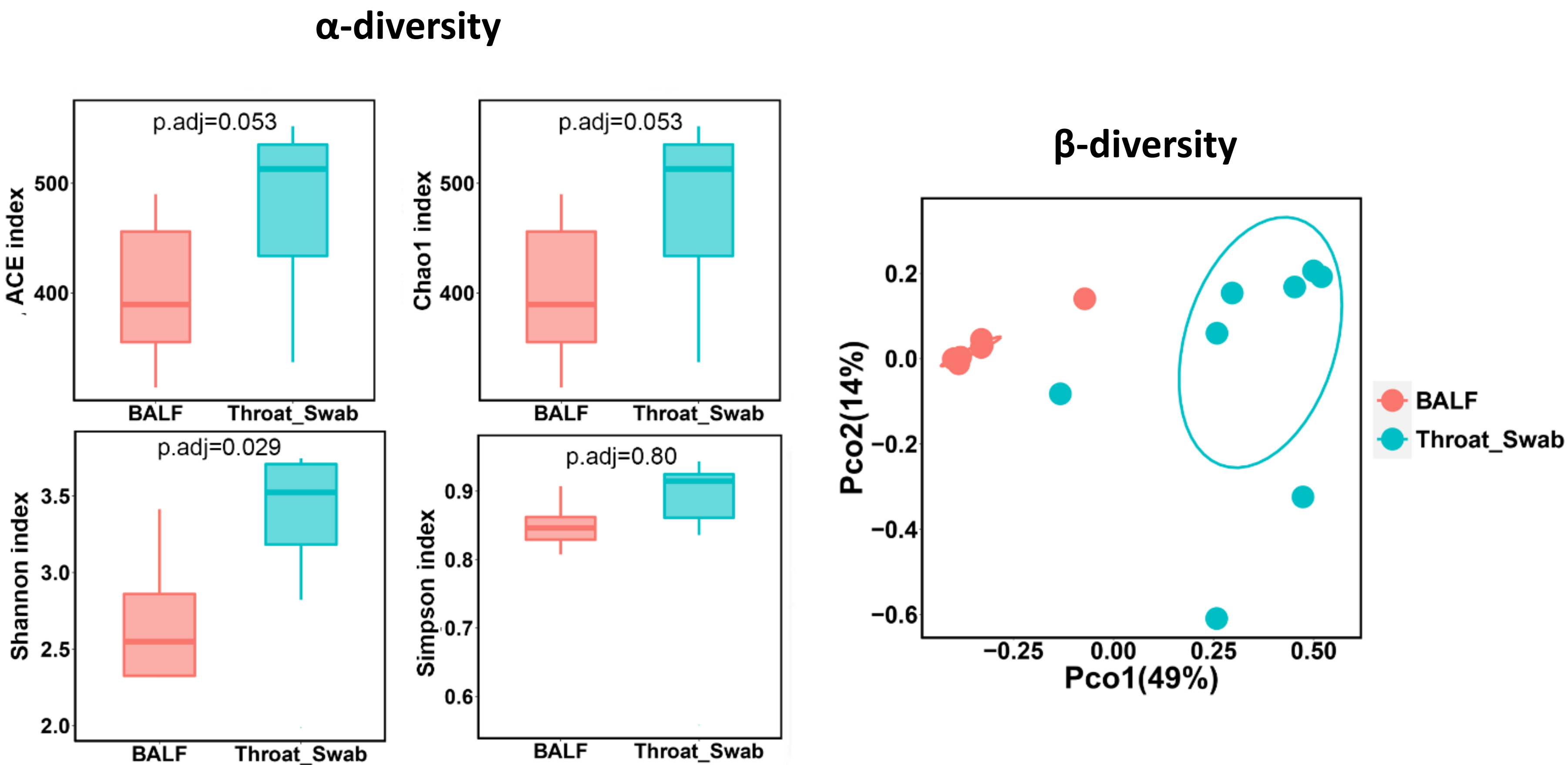
Xiao G, et al. Microbiol Spectr，2022

Overview of the microbiota sequencing data in all groups



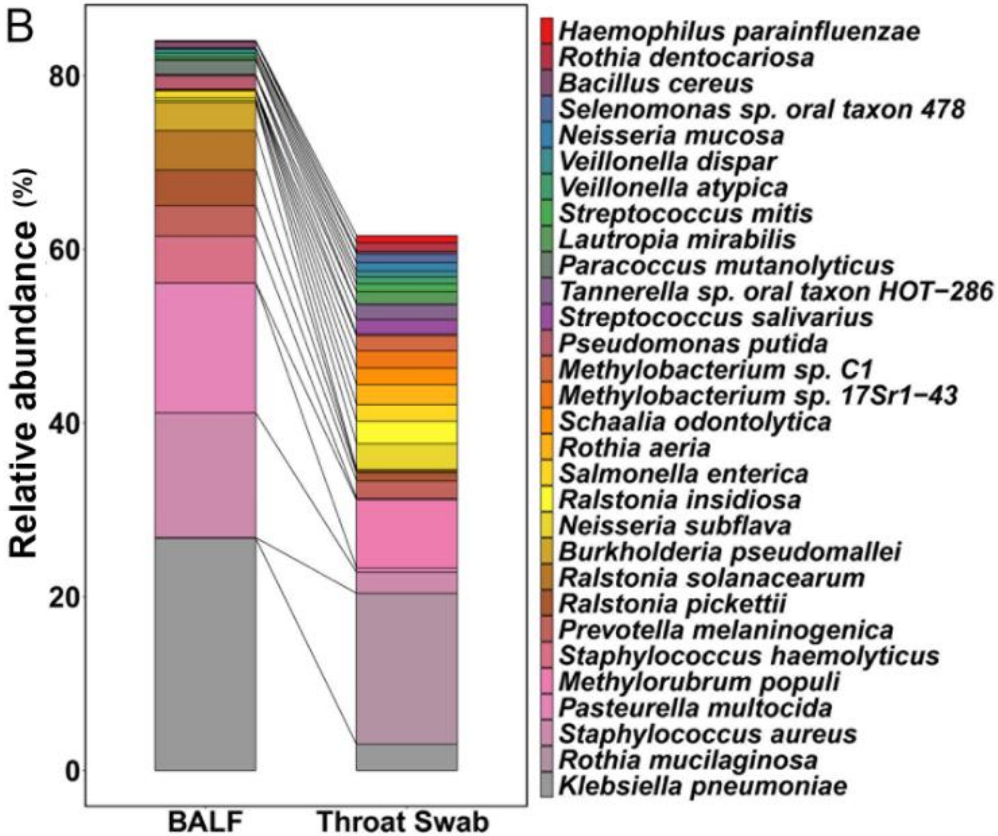
➤ **Totally 1765 species were contained in BALF samples from all the groups**

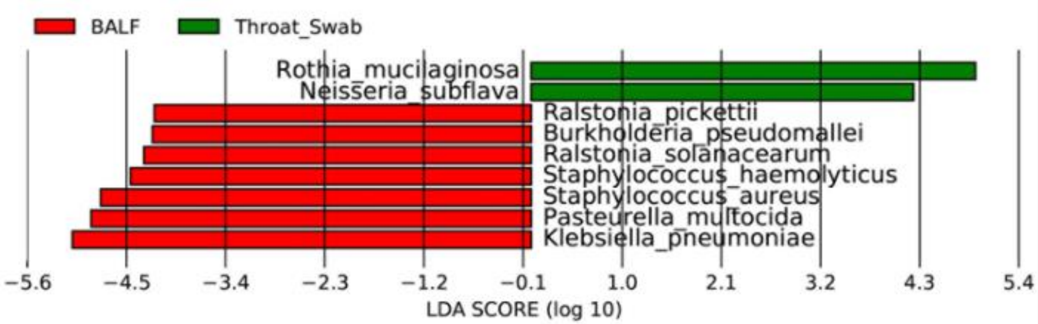
Microbiota profiles differ significantly between throat swabs and BALF samples in HC



- A divergent composition of the microbiota between the upper and lower respiratory tract

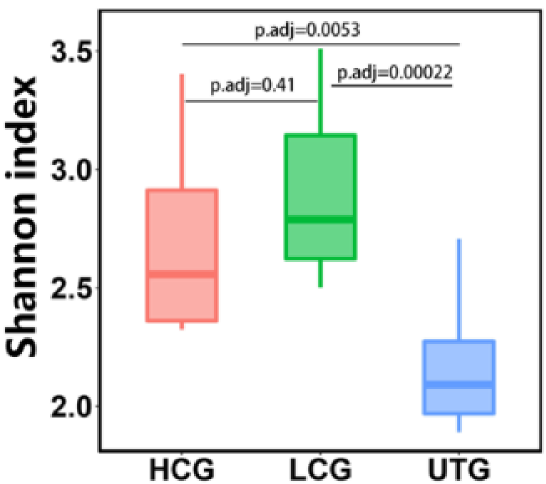
Microbiota profiles differ significantly between throat swabs and BALF samples in HC

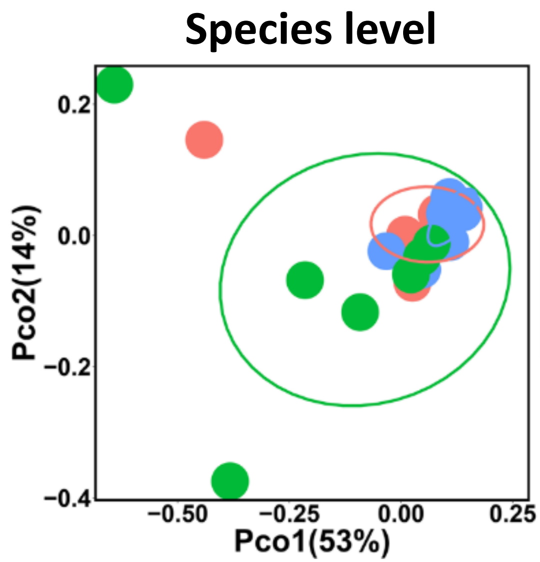


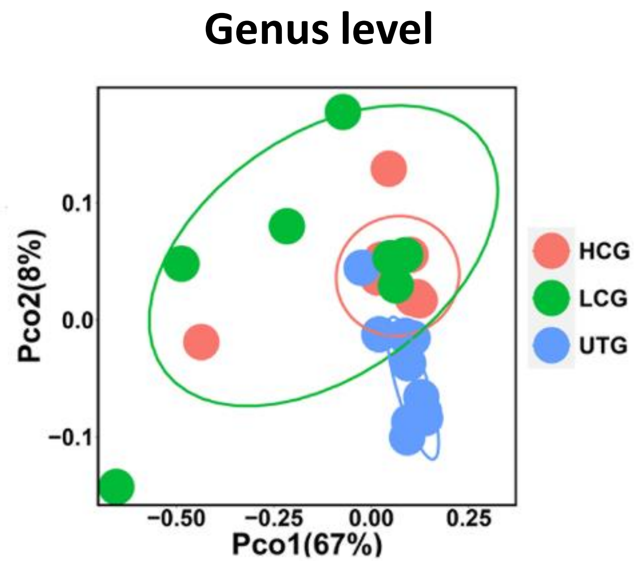


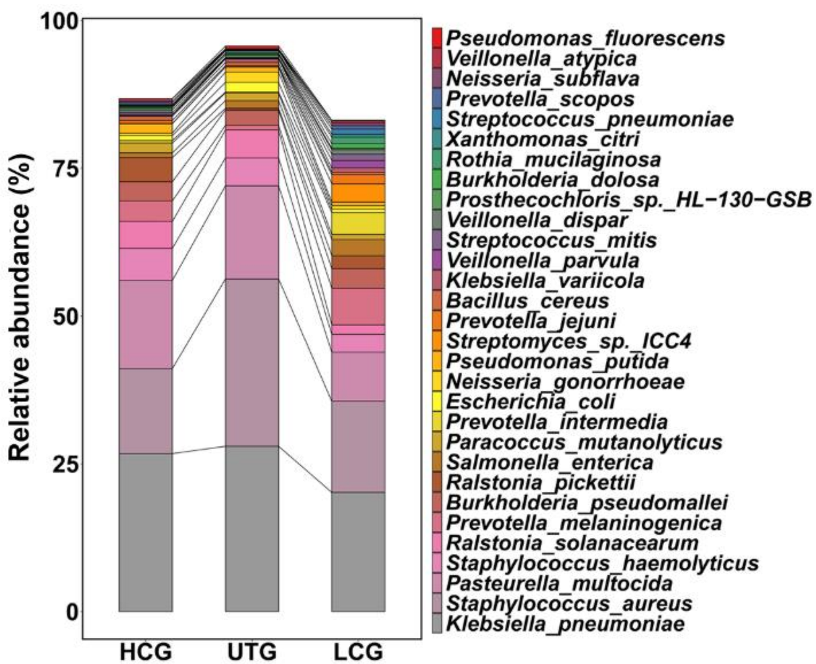
- **The top 30 species in throat swabs and BALF accounted for 61.58% and 84.03% of the total bacteria**
- **Top 3 in BALF:** ***Klebsiella pneumoniae, Staphylococcus_aureus, Pasteurella_multocida***
- **Top 1 in throat swab:** ***Rothia mucilaginosa***

The pulmonary TB displays an unique lung microbiota profile



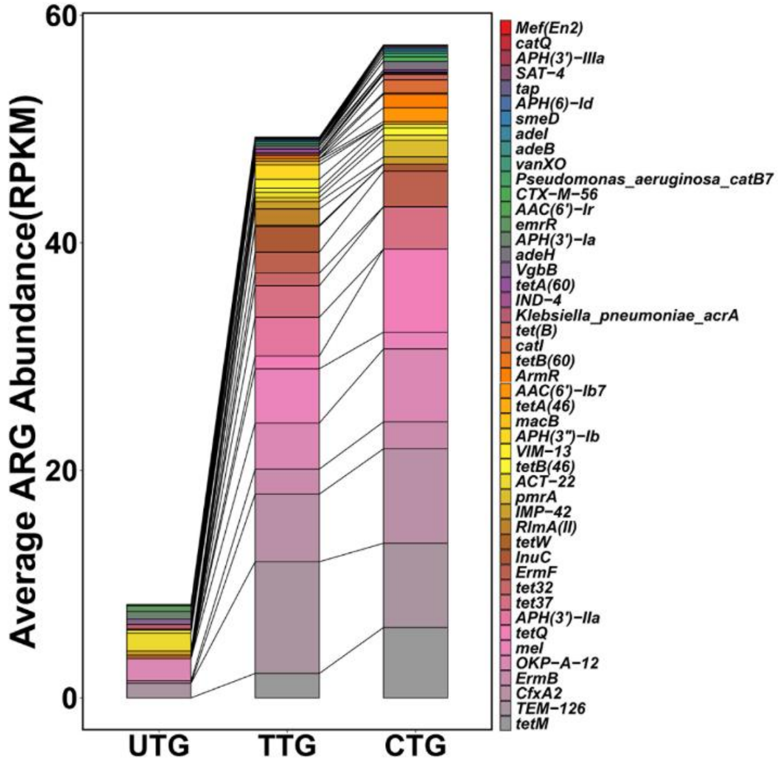






- ***K. pneumoniae, S. aureus*** **and** ***P. multocida*** **were the three most predominant species within the three groups, accounting for 56.04%, 72.06% and 43.89% of the total bacteria**

Anti-TB treatment increases the diversity and abundance of ARGs

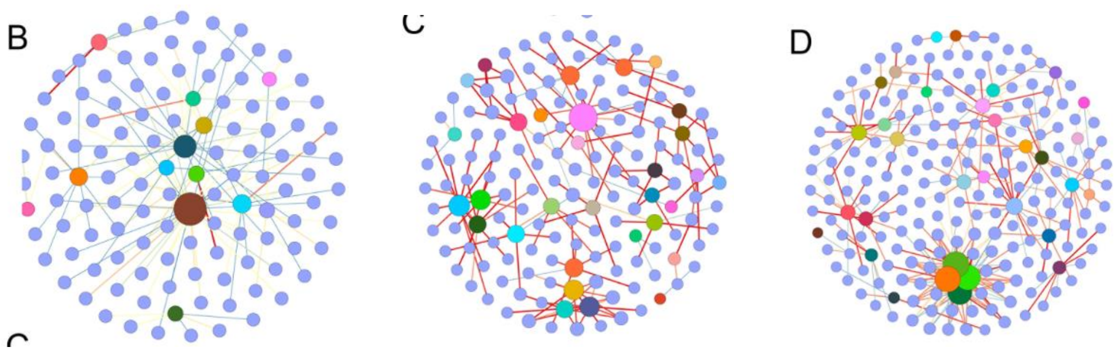


➢ The most common **antibiotic resistance genes (ARG)** types

✓tetracycline resistance genes (*tetM, tetQ, tet32, tet37, tetW*)

✓β-lactam resistance genes (*TEM-126, CfxA2, OKP-A-12, IMP-42, ACT-22, VIM-13*)

✓macrolide-lincosamide-streptogramin B (*MLSB)* resistance genes (*ErmB, mel, ErmF*)

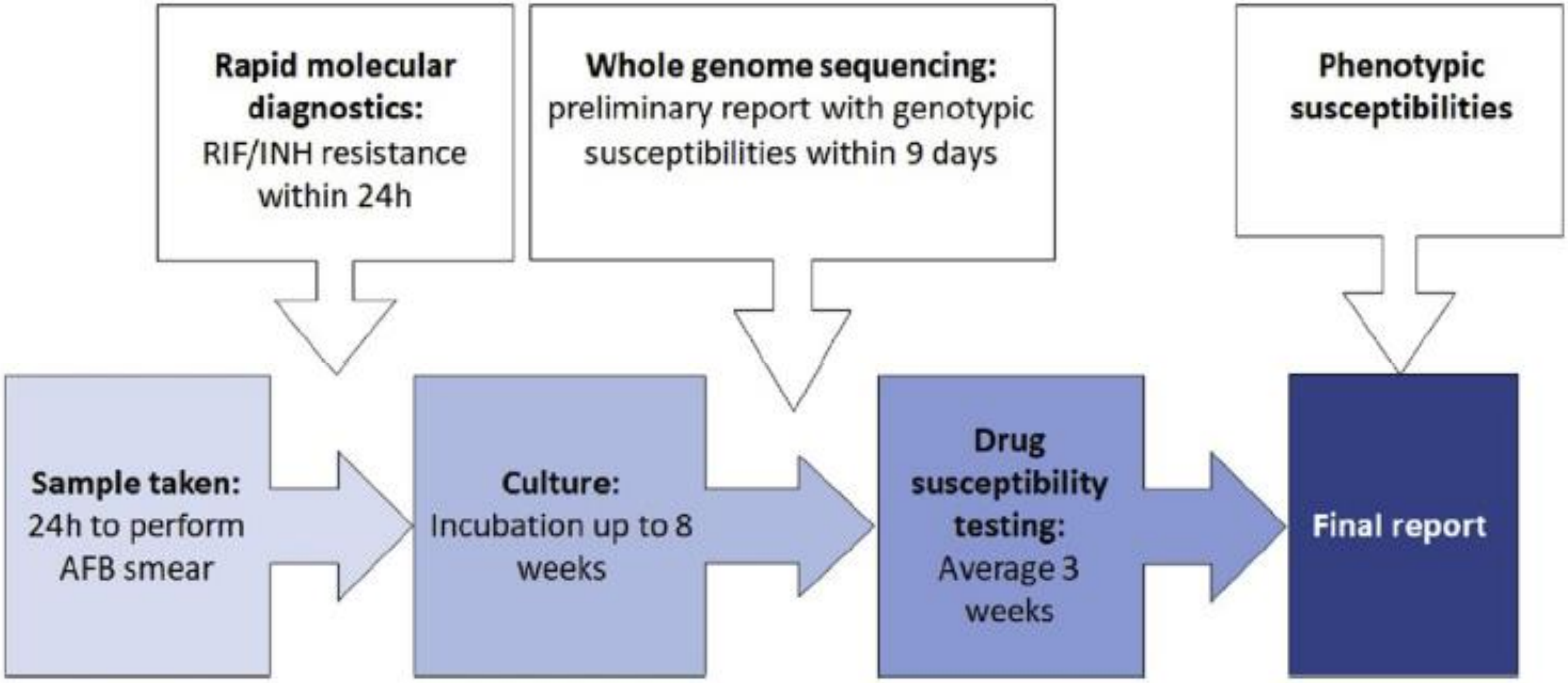


UTG TTG CTG

➢co-occurrence network

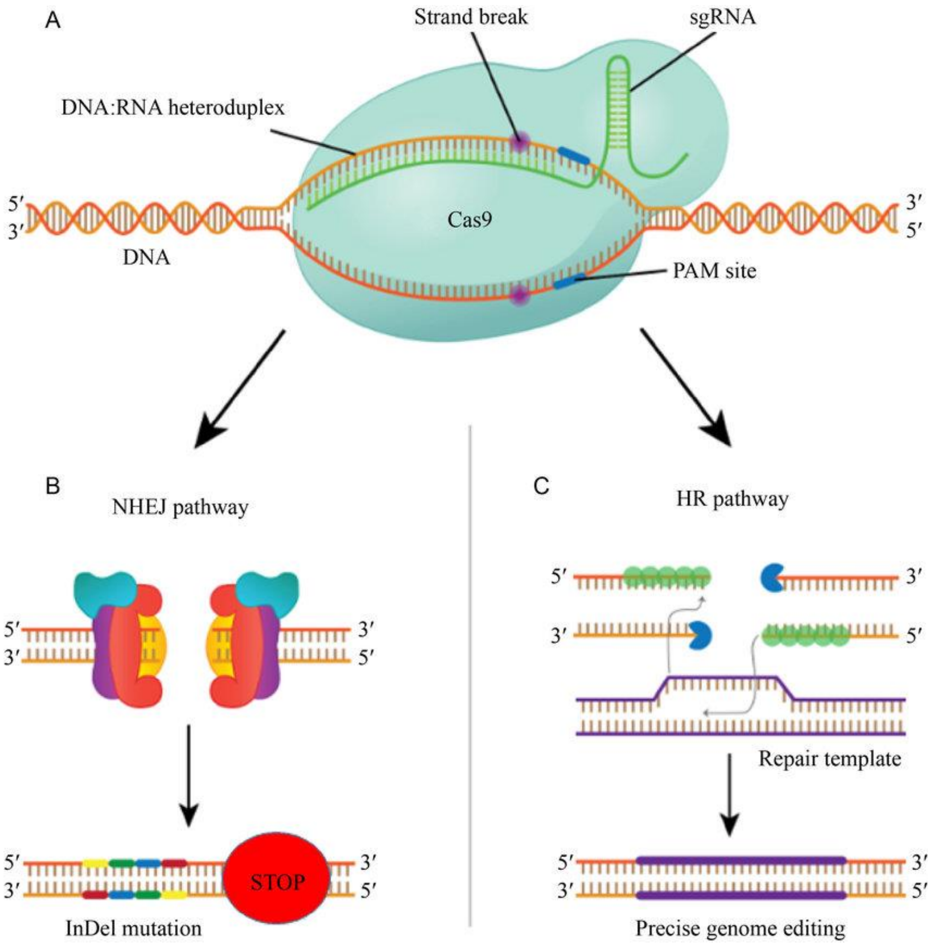
Xiao G, et al. Microbiol Spectr，2022

Processing of mycobacterial samples and WGS



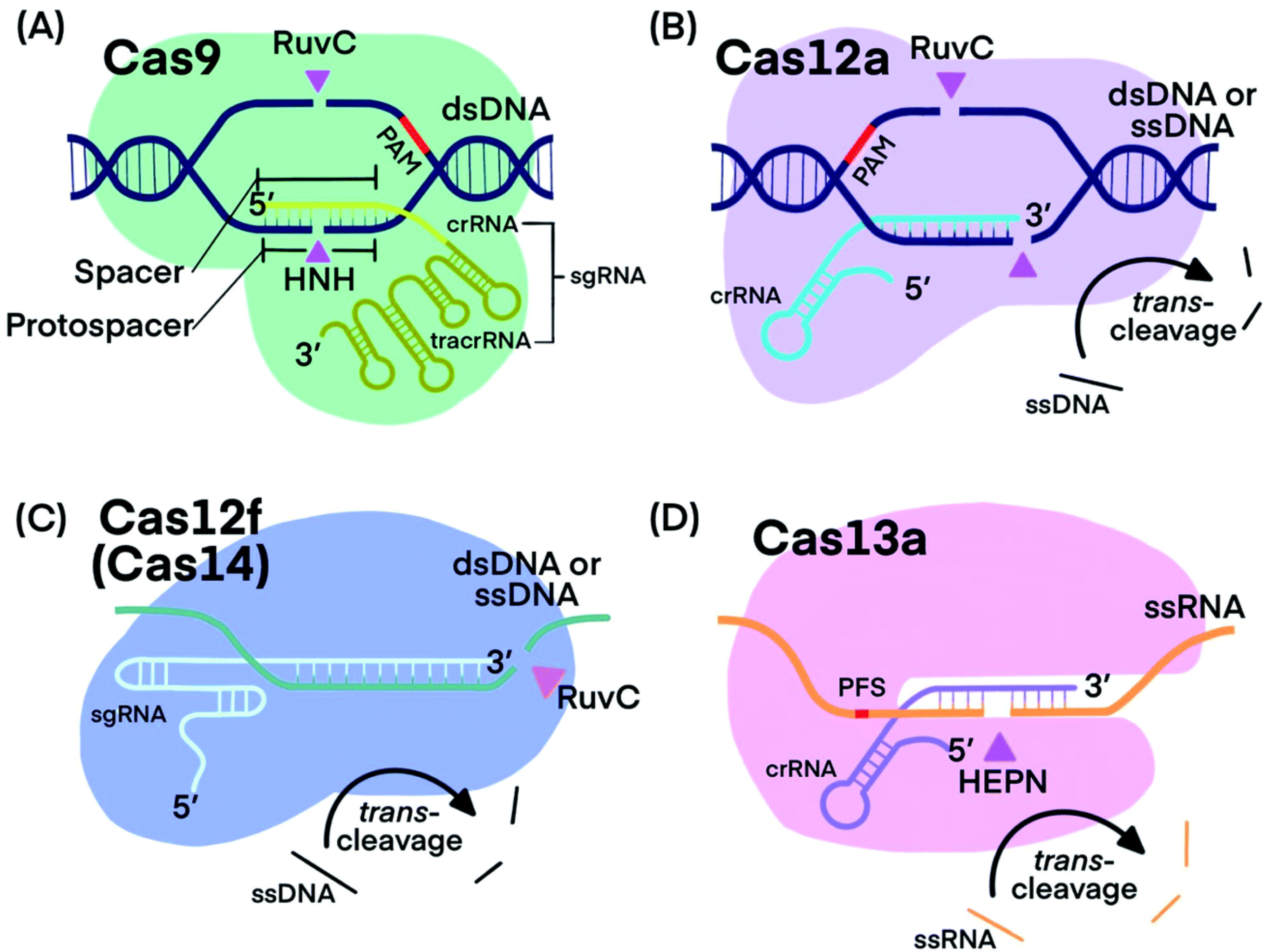
CRISPR system---powerful tool for pathogen diagnosis

The Nobel Prize In Chemistry 2020

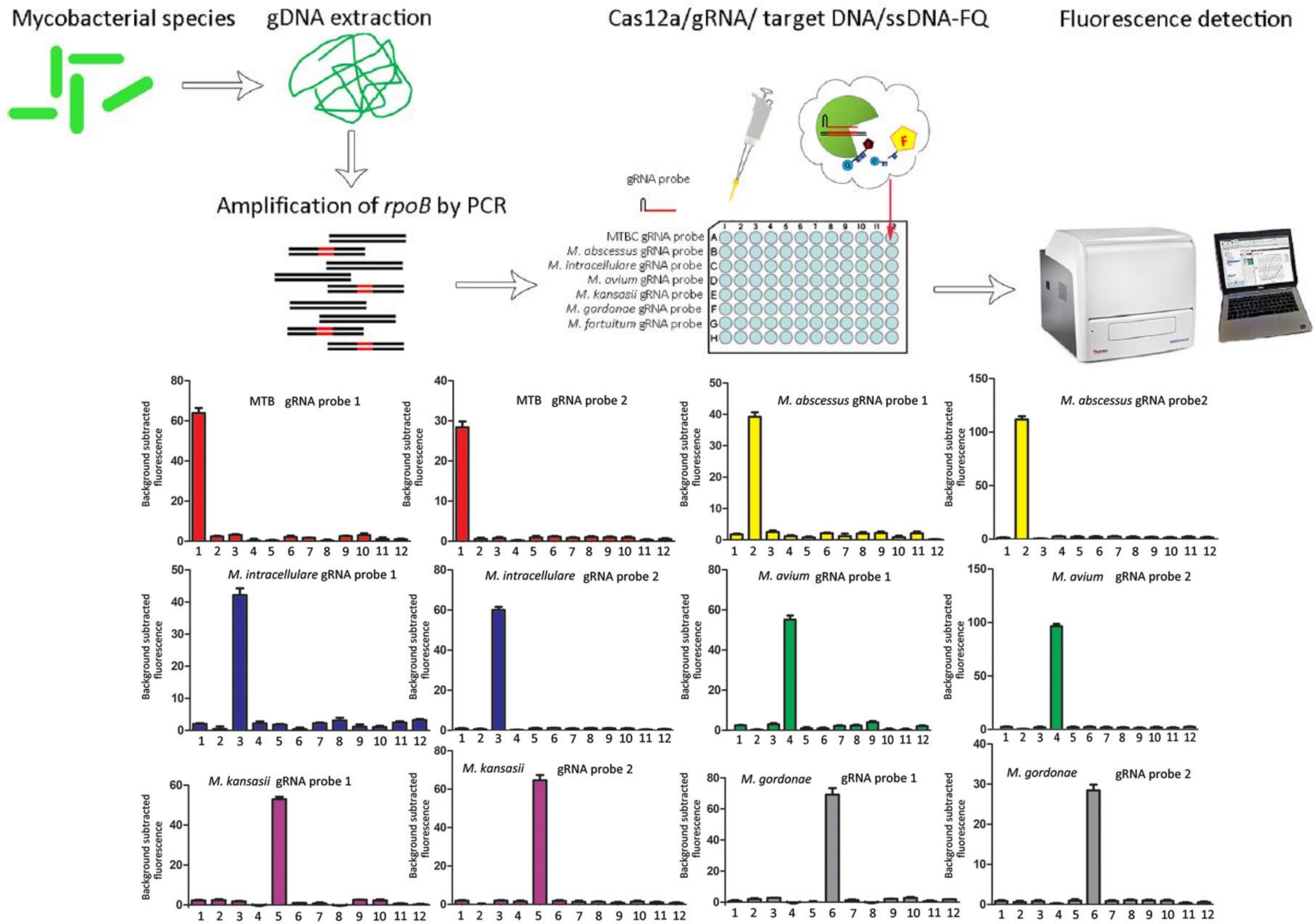


- ➢**The CRISPR/Cas9 system that is used to cut DNA has changed biology forever**

The Cas proteins behind the CRISPR diagnostics



Cas12a/gRNA-based Platform for Identification of Mycobacterium Species



Xiao G, et al. J Clin Microbiol. 2021

Cas12a/gRNA-based Platform for Identification of Mycobacterium Species

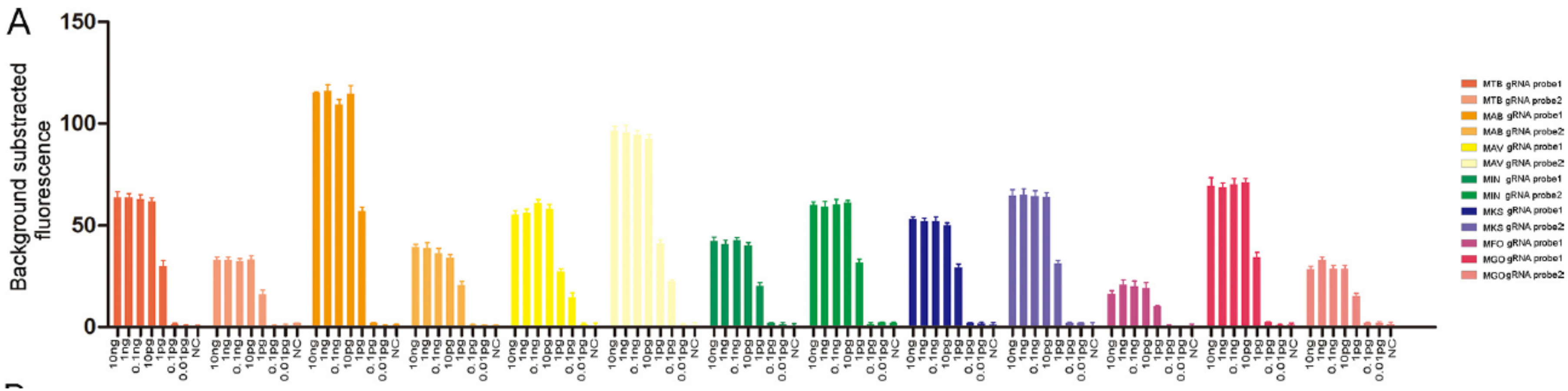
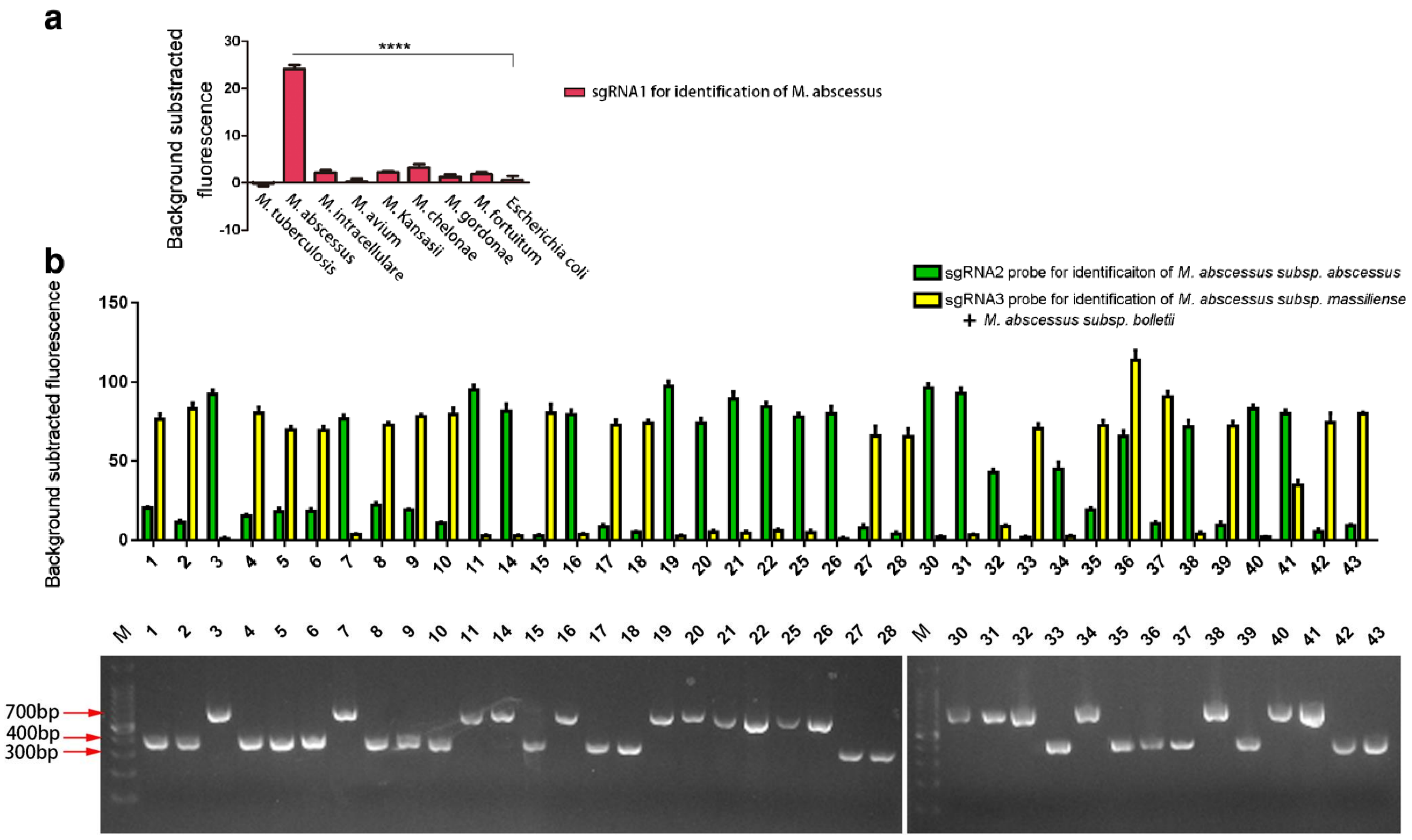


TABLE 1 Identification of clinical isolates using FnCas12a/gRNA-based platform

Clinical organism	No. of strains	No. correctly identified (%)	No. misidentified	gRNA probe name
M. tuberculosis	10	10 (100)	0	MTB gRNA probe1
M. tuberculosis	10	10 (100)	0	MTB gRNA probe2
M. abscessus	15	15 (100)	0	MAB gRNA probe1
M. abscessus	15	15 (100)	0	MAB gRNA probe2
M. intracellulare	15	15 (100)	0	MIN gRNA probe1
M. intracellulare	15	14 (93.33)	1	MIN gRNA probe2
M. avium	10	10 (100)	0	MAV gRNA probe1
M. avium	10	10 (100)	0	MAV gRNA probe2
M. gordonae	10	10 (100)	0	MGO gRNA probe1
M. gordonae	10	10 (100)	0	MGO gRNA probe2
M. kansasii	7	7 (100)	0	MKA gRNA probe1
M. kansasii	7	7 (100)	0	MKA gRNA probe2
M. fortuitum	6	6 (100)	0	MFO gRNA probe

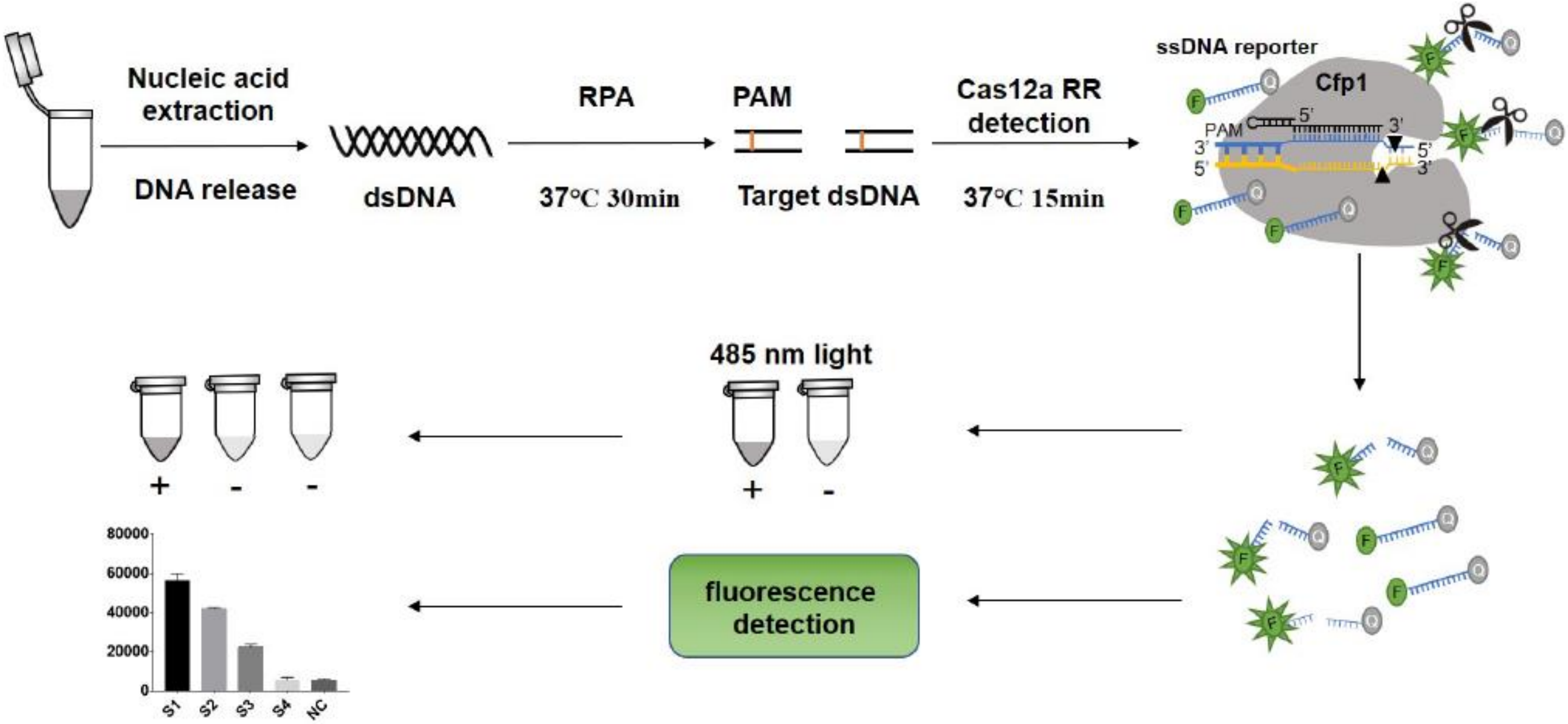
Xiao G, et al. J Clin Microbiol. 2021

Identification of Mycobacterium abscessus subspecies using the Cas12a/gRNA



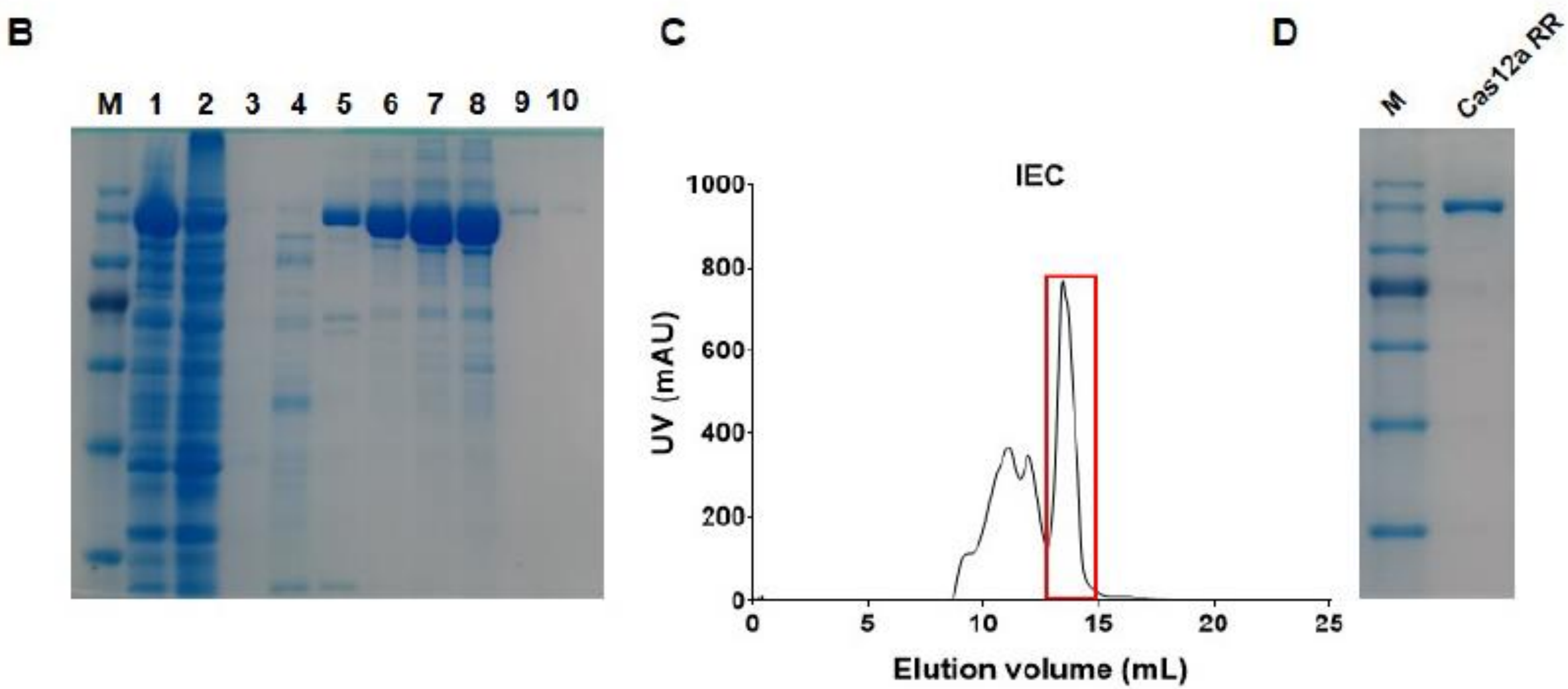
Xiao G, et al. Eur J Clin Microbiol Infect Dis. 2020

Cas12a mutant-based module for detection of Mtb streptomycin resistance



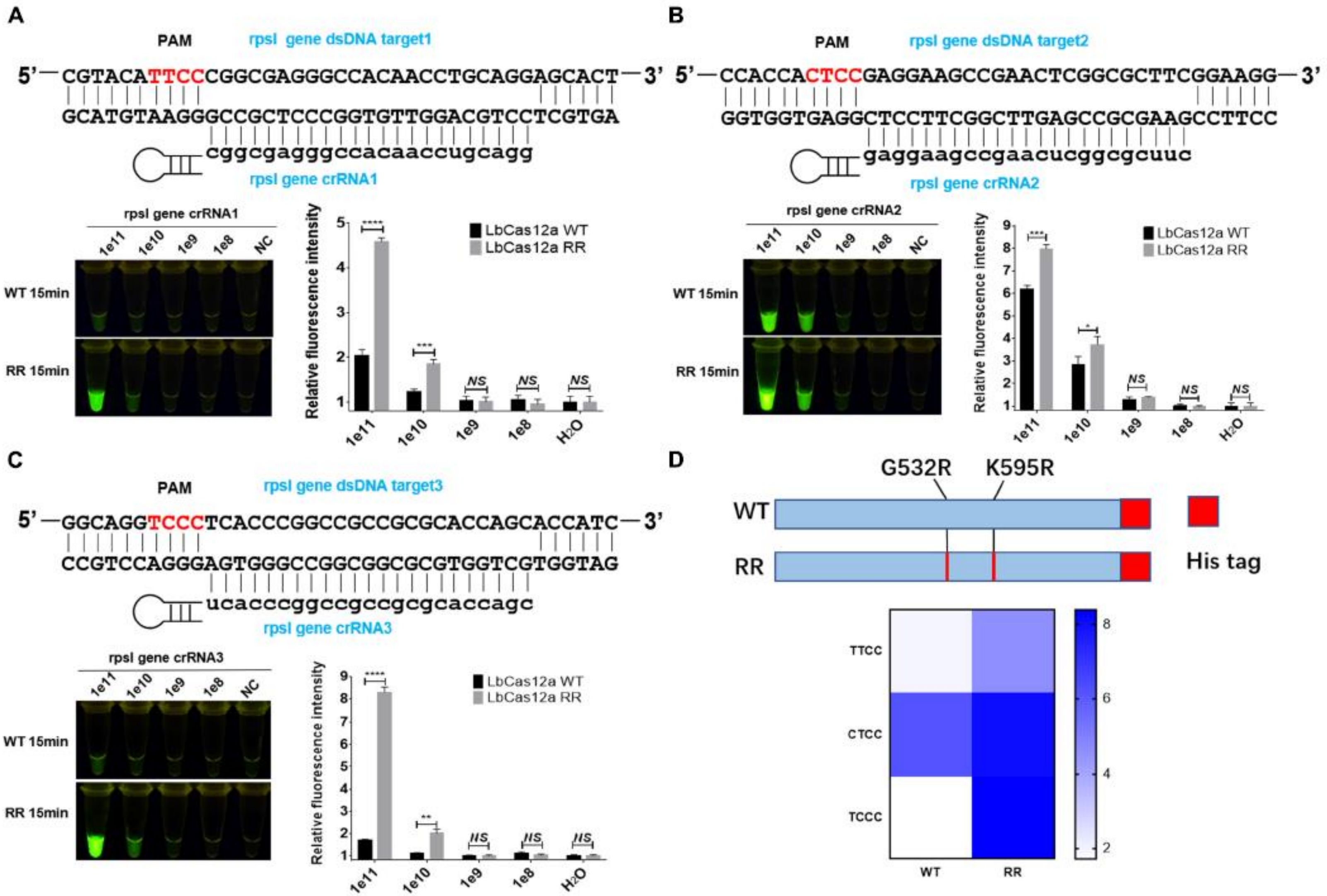
Liu P, et al. Front Microbiol. 2022

Cas12a mutant protein purification



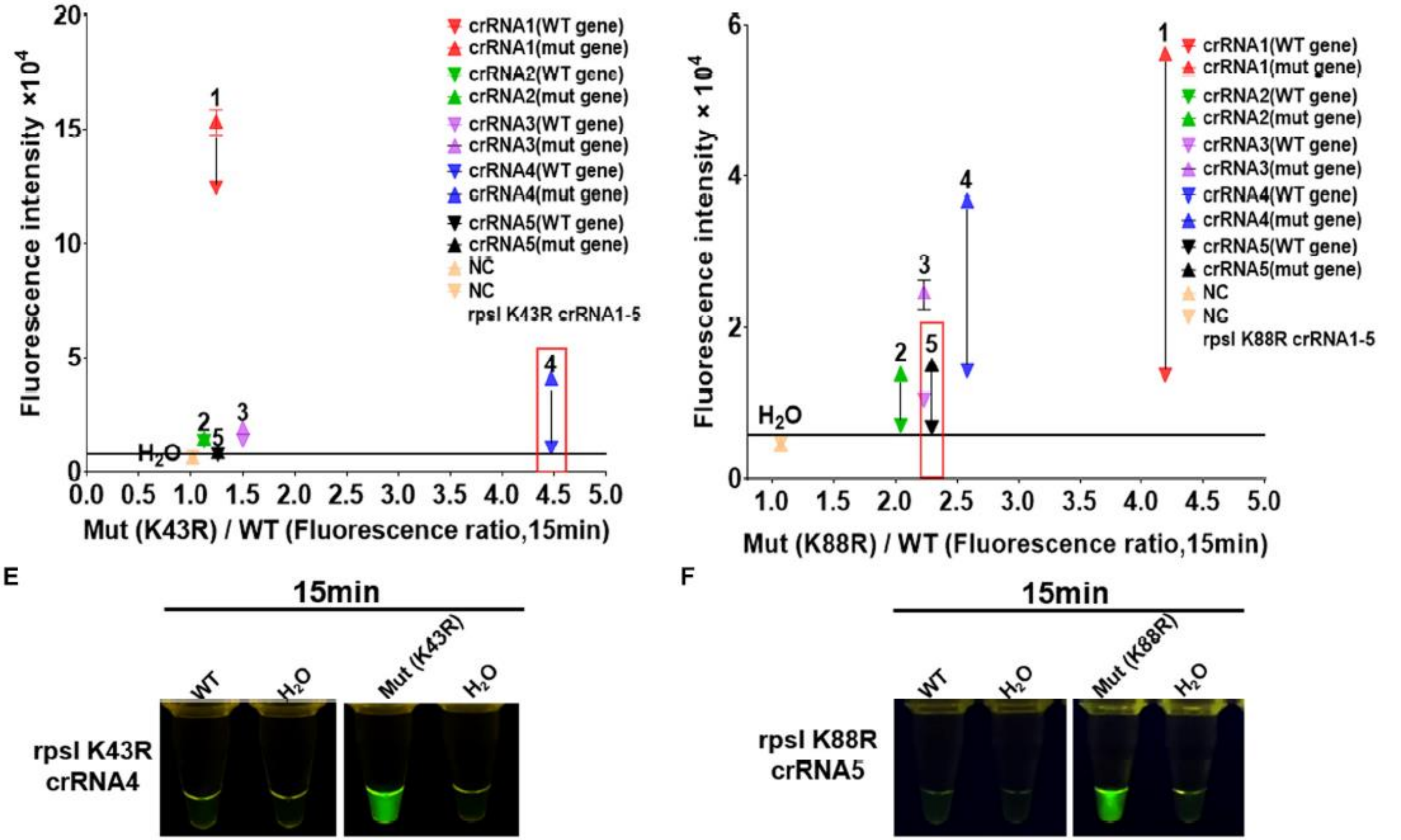
Liu P, et al. Front Microbiol. 2022

Cas12a mutant protein recognizes non-traditional PAM



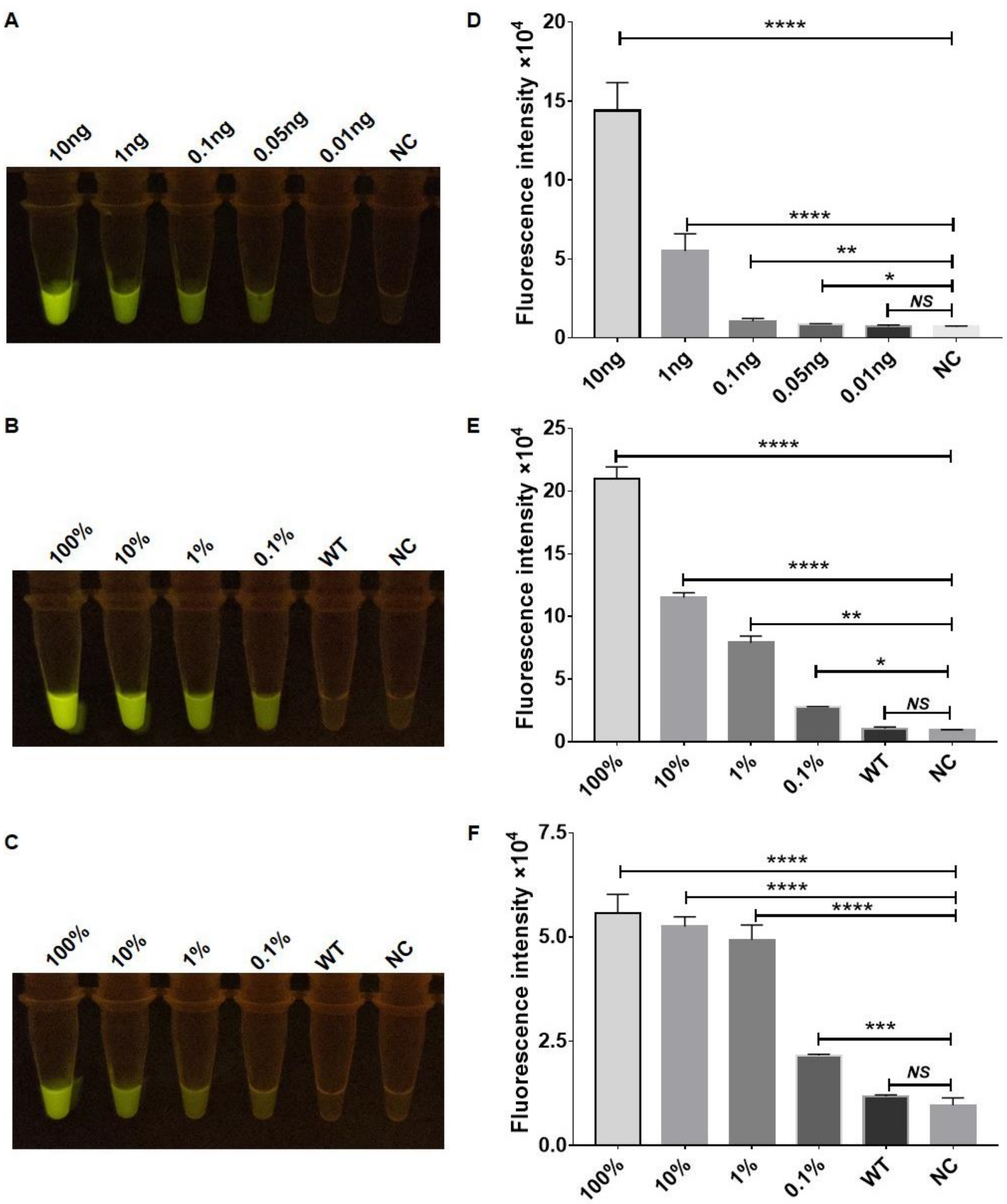
Liu P, et al. Front Microbiol. 2022

Screening of the crRNA at rpsl K43R and rpsl K88R mutation

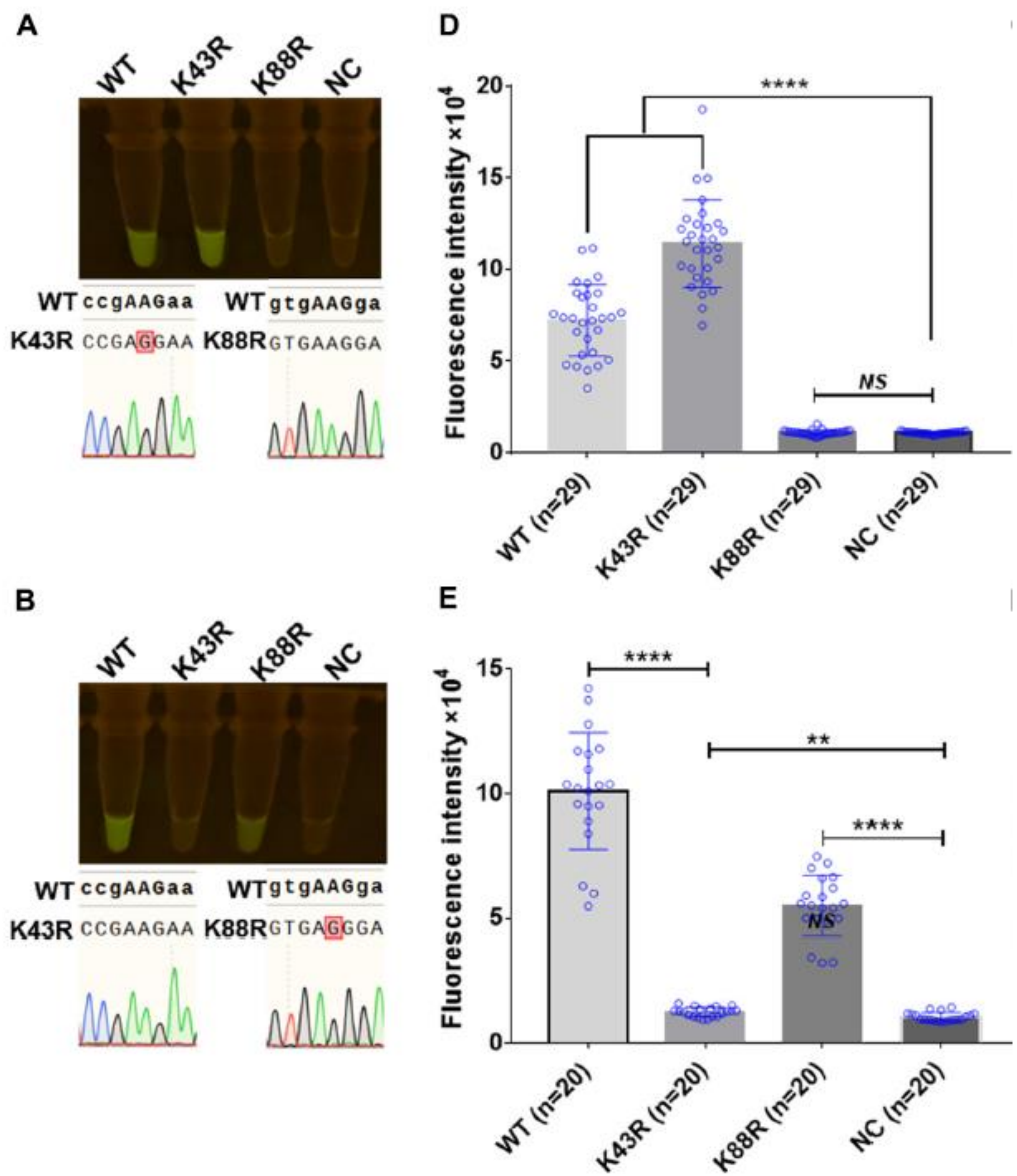


Liu P, et al. Front Microbiol. 2022

High sensitivity and specificity of Cas12a mutant-based module



High sensitivity and specificity of Cas12a mutant-based module

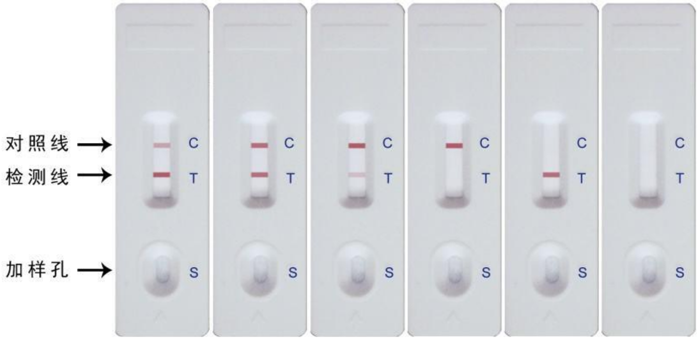


Liu P, et al. Front Microbiol. 2022

Future direction

●**WGS**: **Aim to characterize mutation profile within 1 week**

●**CRISPR: Aim to enable accurate testing at the Point of Care**



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End TB, We Together!