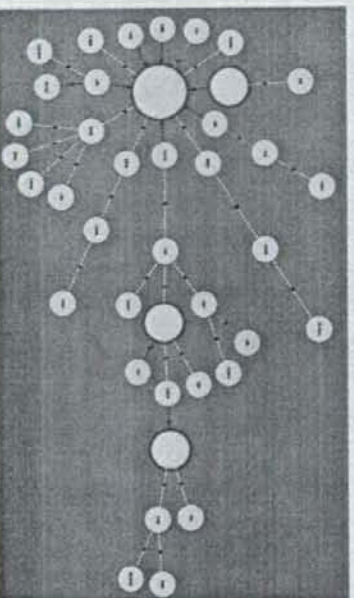


Hypothetical phylogenetic tree

MST (Minimum Spanning Tree)



MST for 46 Clinical Strains



By BIONUMERICS ver 4.6

19 loci VNTR compare with 16 loci VNTR

VNTR loci	HGI	HGI	
QUB-26	0.86780		
MIRU26	0.81695		
mtub21	0.80667		
Q1-B-18	0.80565		
QUB-11b	0.80455		
Q1-B-11a	0.70537		
MIRU10	0.61485		
etA	0.58653		
mtub04	0.58182		
VNTR2372	0.57710		
MIRU40	0.55819		
QUB-4156	0.54520		
mtub30	0.50452		
MIRU04	0.41977		
MIRU31	0.37627		
QUB-15	0.34520		
VNTR2074	0.22768		
MIRU16	0.27401		
etC	0.12938		

VNTR loci	HGI	
	Beijing strains (n=189)	All Isolates (n=224)
1 VNTR330	0.8674	0.87
2 QUB-11b	0.6888	0.741
3 QUB-26	0.6295	0.6689
4 MIRU26	0.6139	0.7005
5 Q1-B-18	0.6017	0.6975
6 Mtub21	0.5444	0.6543
7 Q1-B-11a	0.5183	0.6188
8 QUB4156c	0.4691	0.4587
9 Q1-B1895	0.365	0.3556
10 MIRU31	0.338	0.4803
11 ETR-J	0.2897	0.3757
12 Mtub04	0.2688	0.4207
13 MIRU10	0.2388	0.3965
14 Mtub24	0.2232	0.2369
16 MIRU16	0.1308	0.2185

Suggestion

- Can we selected 6-10 "core" VNTR loci which have high discriminatory power in China, Japan and Korea to genotype and compare clinical strains in these countries?

Acknowledgement

▪ Fudan University

▪ Shanghai CDC

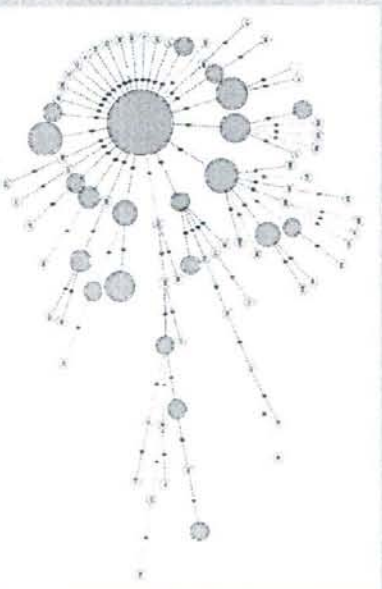
ca Peng Xu

ca Qian Gao

ca Jian Mei

Thank you!

MST of 173 MDR strains



Questions

- Some loci can not be recognized by the analyzer
 - ca E.g., VNTR 3820 ▶
- The measured value of the analyzer is smaller than actual, how to optimize? ▶
- The data analysis in different laboratories? ▶

Filter Set	ES	A	3	8	F	GS	?	5
Cell	SHR	LSHR	LSHR	JSHR	LSHR	LSHR	LSHR	LSHR
Gene Opt	SHC	JKC	HSC	WSC	JOC	YC	ITC	SHC
Yeast Opt	CHANDZ	DANDZ	VEP	HED	HEJ	HED	HEK	
Ind Opt	delC	RO	NOX	HEZ	NOX	FTT	RAMA	
Gene Opt	LT					LT		LT



Compare with Japanese Strains

VNTR loci	K1H207TKS				K1H501SSC				
	Expected	Analyzer	Agrose gel	Expected	Analyzer	Agrose gel	Expected	Analyzer	Agrose gel
QUP-18	992.2	10.4	1119.0	12.3	688.1	8.5	783.0	7.4	
Micb1	298.2	2.7	322.0	3.0	207.1	2.8	208.0	2.9	
QUB-11a	798.1	6.0	797.0	6.8	611.5	7.0	-	-	
QUB-26	878.2	6.7	1077.0	8.5	986.2	7.7	1111.0	9.8	
QUB-11b	358.5	3.9	359.0	4.2	202.0	2.0	211.0	2.1	
Mind6	633.2	6.1	636.0	6.2	288.1	0.9	303.0	1.3	
Mira4	312.2	2.6	312.0	2.9	229.2	1.6	248.9	1.9	
Mira16	487.0	2.7	515.0	3.6	494.1	0.9	620.0	1.2	
Mira40	487.9	2.5	626.0	3.1	440.4	1.6	480.0	1.9	
etfA	420.0	3.1	618.0	4.9	417.0	3.3	453.0	3.4	
etfC	201.8	3.1	286.0	3.7	227.9	2.1	382.0	3.6	
atd04	340.4	4.0	383.0	4.8	198.1	2.2	227.9	2.7	
VNTR207f	182.8	1.4	188.0	2.6	97.2	0.1	102.0	0.6	
VNTR207g	-	-	-	-	101.8	2.5	208.0	2.8	
VNTR207h	-	-	-	-	162.2	1.7	192.0	1.9	
QUB-15	713.0	6.9	-	-	578.0	4.4	588.0	5.2	
Mira10	361.2	2.6	398.0	3.5	308.0	2.5	358.0	4.0	
Mira-31	547.7	4.1	382.0	3.1	493.1	3.7	498.0	4.1	
Mind30	608.6	3.7	483.0	4.0	463.1	3.2	498.0	4.1	
QUB-115e	216.3	1.1	217.0	1.9	284.8	3.6	303.0	3.9	

Tuberculosis (TB) in China

- China has the world's second largest TB epidemic, with more than 1.3 million new cases of TB every year.
- More than 400 million people were infected with *M. tuberculosis*.
- Of the 37 notifiable communicable diseases in China, TB ranks first in terms of reported cases and deaths.



MDR TB in China

- China had the largest number of multidrug-resistant (MDR-TB) cases (139,894 in 2004), equaling a third of the entire world's MDR-TB cases.
- The prevalence of drug resistant TB in China was high. In some provinces:
Prevalence of MDR-TB in new cases > 10%
Prevalence of MDR-TB in retreatment cases > 30%

MDR and XDR TB in Shanghai, China

Shen Xin, Mei Jian

Shanghai Municipal Center for
Disease Control and Prevention
(Shanghai CDC)

January, 2009

Outline

- Tuberculosis (TB) and drug resistant TB in China
- MDR and XDR TB in Shanghai
- Summary

Objectives

- To determine the number and percentage of TB patients in Shanghai with MDR and XDR TB,
- To determine whether there is transmission of MDR and XDR strains of *M. tuberculosis* in Shanghai.

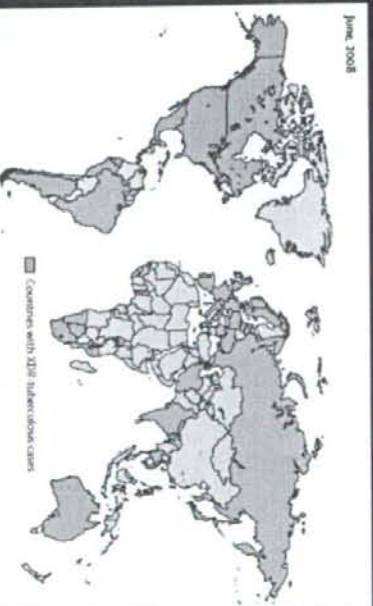
Methods

TB Cases in Shanghai, 2008

Population (million)	Pulmonary TB Cases
■ Total	7,140 Incidence rate: 40/100,000
■ Residents	3,863 Incidence rate: 28.4/100,000
■ Urban Migrants*	3,277 Incidence rate: >70/100,000

*Urban Migrants: People from poor rural areas in other provinces of China

XDR TB in the world



Lancet infectious diseases, 2008

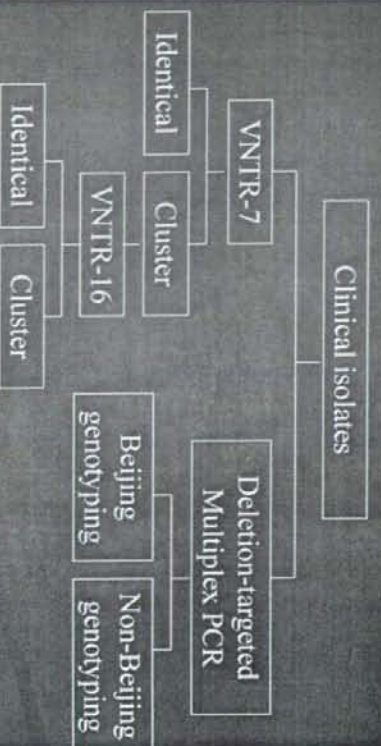
XDR TB in China?

- High prevalence of TB, and large number of MDR TB
- Long-term use of second-line anti-TB drugs
 - widely used for other bacterial infections
 - used for TB more frequently than before
 - easier to get at pharmacies than first-line drugs

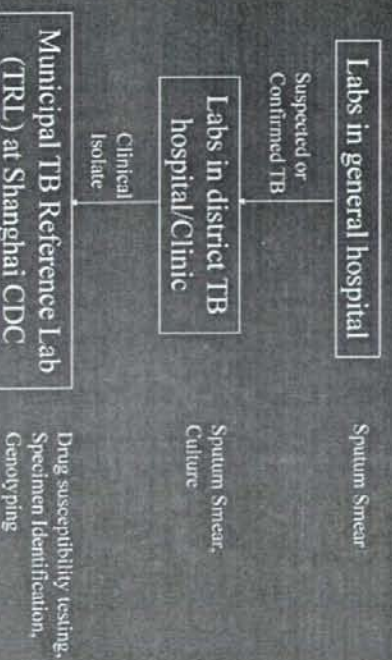
XDR-TB



Genotyping methods



Surveillance System and Laboratory Testing



Definitions

- **New case:** Patient without a previous history of TB treatment (Primary drug resistance)
- **Previously treated case:** Patient who had previously received at least 30 days of anti-tuberculosis treatment any time during their life (Acquired drug resistance)
- **MDR:** Resistance to at least isoniazid and rifampin
- **XDR:** resistance to at least isoniazid, rifampin, a fluoroquinolone and one of three injectable second-line drugs (capreomycin, kanamycin, and amikacin)
- **Pre-XDR:** resistance to isoniazid and rifampin and either a fluoroquinolone or a second-line injectable drug, but not both
- **Simple MDR:** resistance to just isoniazid and rifampin but not pre-XDR TB and XDR TB

Drug susceptibility testing

- | | |
|---------------------------|---------------------------------------|
| ■ First-line drugs | ■ Second-line drugs |
| ■ Isoniazid (0.2ug/ml) | ■ Ofloxacin (2.0ug/ml) |
| ■ Rifampin (40.0ug/ml) | ■ Kanamycin (30.0ug/ml) |
| ■ Streptomycin (4.0ug/ml) | ■ Capreomycin (40.0ug/ml) |
| ■ Ethambutol (2.0ug/ml) | ■ Amikacin (40.0ug/ml) |
| | ■ Para-aminosalicylic acid (1.0ug/ml) |

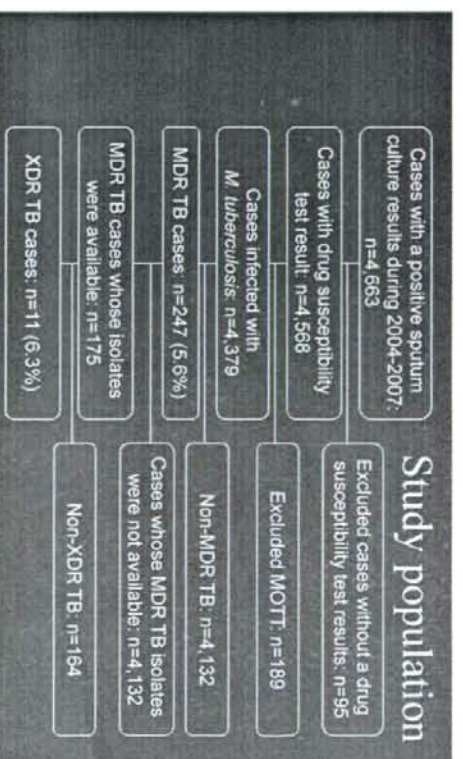
Treatment outcomes of MDR TB patients

	Simple MDR		Pre-XDR		XDR		Total
	n = 109 (%)	n = 55 (%)	n = 11 (%)	n = 175 (%)			
Cured	62(56.9)	29(52.7)	1(9.1)	92(52.6)			
Completed treatment	3(2.8)	2(3.6)	0	6(3.4)			
Died during treatment	6(5.5)	5(9.1)	1(9.1)	12(6.9)			
Still on treatment	30(27.5)	14(25.5)	8(72.7)	51(29.1)			
Lost to follow up	1(0.9)	1(1.8)	1(9.1)	3(1.7)			
Moved/transferred	7(6.4)	4(7.3)	0	11(6.3)			

Treatment outcomes of MDR TB patients, stratified by new cases versus previously treated cases

XDR	Treatment success		No treatment success		Odds ratio	95% CI	P
	n = 96	n = 79	n = 96	n = 79			
New	0 (0.0)	6 (100)	-	-	-	-	0.25
Retreatment	1 (20.0)	4 (80.0)	-	-	-	-	
Pre-XDR							
New	23 (71.9)	9 (28.1)	1	1	1	(1.3, 17.8)	<0.01
Retreatment	8 (34.8)	15 (65.2)	4.8	4.8	4.8	(1.3, 17.8)	<0.01
Simple MDR							
New	47 (71.2)	19 (28.8)	1	1	1	(1.6, 9.2)	<0.01
Retreatment	17 (39.5)	26 (60.5)	3.8	3.8	3.8	(1.6, 9.2)	<0.01
Total							
New	70 (67.3)	34 (32.7)	1	1	1	(1.8, 7.1)	<0.01
Retreatment	26 (36.7)	45 (63.3)	3.6	3.6	3.6	(1.8, 7.1)	<0.01

Results



Characteristics of MDR, pre-XDR and XDR TB patients

Characteristic	All MDR n = 175 (%)	Simple MDR n = 109 (%)	Pre-XDR n = 55 (%)	XDR n = 11 (%)	P
Age (years)					
15-29	35(20)	24(22)	11(20)	0	
30-44	53(30.3)	37(33.9)	13(23.6)	3(27.3)	
45-59	57(32.6)	26(23.9)	23(41.8)	8(72.7)	0.04
60-74	20(11.4)	14(12.8)	6(10.9)	0	
≥75	10(5.7)	8(7.4)	2(3.6)	0	
Sex					
Male	132(75.4)	84(77.1)	38(69.1)	10(90.9)	0.25
Female	43(24.6)	25(22.9)	17(30.9)	1(9.1)	
Treatment history					
New	105(60)	69(63.3)	30(54.5)	6(54.5)	0.52
Retreatment	70(40)	40(36.7)	25(45.5)	5(45.5)	
Status					
Resident	112(64)	70(64.2)	34(61.8)	8(72.7)	0.79
Migrant	63(36)	39(35.8)	21(38.2)	3(27.3)	

Acknowledgement

- Shanghai CDC
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Genotypes of *M. tuberculosis*

- 87.3% (165/189) of the MDR isolates and 90.9% (10/11) of the XDR isolates were Beijing genotype strains.
- Among 175 MDR, 4 clusters were identified, each with 2 different patients. But no epidemiological links were detected. None of the isolates with XDR were in a genotype cluster.

Thank you!

Summary

- 5.6% of the TB cases were MDR in Shanghai.
 - 6.3% of the MDR TB were actually XDR.
 - >50% MDR and XDR TB patients were new cases.
 - New cases have better treatment outcome than previously treated cases.
- Ongoing transmission of MDR/XDR occurred in Shanghai.

325 isolates were collected from whole Japan

- (1) IS6110 RFLP analysis
 - (2) Spoligotyping
 - (3) VNTR analysis
- 16 loci : MIRU-VNTR (12 loci) and ETR (4 loci)
19 loci : QUB (5 loci) and the other loci

Comparing of PIC in each locus

No.	Locus	Allele	PIC*		The study			
			Allele number (n/N)	Information content (-log ₁₀ P)	12 VNTR	24 VNTR	13 VNTR	12MIRU +4ETR
1	21520	QUB 1/9	9/9	0.00	X	X	X	X
2	4622	QUB 2/8	8/8	0.00	X	X	X	X
3	2158	QUB 3/10	10/10	0.00	X	X	X	X
4	1935	MIRU 2/1	1/1	0.00	X	X	X	X
5	4156	4VNTR	8/8	0.00	X	X	X	X
6	2122	4VNTR	8/8	0.00	X	X	X	X
7	4624	MIRU 2/1	1/1	0.00	X	X	X	X
8	2024	MIRU 2/1	1/1	0.00	X	X	X	X
9	2026	MIRU 2/1	1/1	0.00	X	X	X	X
10	2028	MIRU 2/1	1/1	0.00	X	X	X	X
11	2030	MIRU 2/1	1/1	0.00	X	X	X	X
12	2125	ETRU 2/1	1/1	0.00	X	X	X	X
13	2126	ETRU 2/1	1/1	0.00	X	X	X	X
14	2491	MIRU 2/1	1/1	0.00	X	X	X	X
15	4622	MIRU 2/1	1/1	0.00	X	X	X	X
16	4623	MIRU 2/1	1/1	0.00	X	X	X	X
17	2029	MIRU 2/1	1/1	0.00	X	X	X	X
18	2028	4VNTR	8/8	0.00	X	X	X	X
19	1935	3VNTR	3/3	0.00	X	X	X	X
20	1944	MIRU 2/1	1/1	0.00	X	X	X	X
21	2028	MIRU 2/1	1/1	0.00	X	X	X	X
22	2029	MIRU 2/1	1/1	0.00	X	X	X	X
23	2027	4VNTR	8/8	0.00	X	X	X	X
24	2028	MIRU 2/1	1/1	0.00	X	X	X	X
25	2029	MIRU 2/1	1/1	0.00	X	X	X	X
26	2027	MIRU 2/1	1/1	0.00	X	X	X	X
27	2028	MIRU 2/1	1/1	0.00	X	X	X	X
28	2029	MIRU 2/1	1/1	0.00	X	X	X	X
29	2027	MIRU 2/1	1/1	0.00	X	X	X	X
30	2028	MIRU 2/1	1/1	0.00	X	X	X	X
31	2029	MIRU 2/1	1/1	0.00	X	X	X	X
32	2027	MIRU 2/1	1/1	0.00	X	X	X	X
33	2028	4VNTR	8/8	0.00	X	X	X	X
34	2029	4VNTR	8/8	0.00	X	X	X	X
35	2027	4VNTR	8/8	0.00	X	X	X	X
36	2028	4VNTR	8/8	0.00	X	X	X	X
37	2029	4VNTR	8/8	0.00	X	X	X	X
38	2027	4VNTR	8/8	0.00	X	X	X	X
39	2028	4VNTR	8/8	0.00	X	X	X	X
40	2029	4VNTR	8/8	0.00	X	X	X	X
41	2027	4VNTR	8/8	0.00	X	X	X	X
42	2028	4VNTR	8/8	0.00	X	X	X	X
43	2029	4VNTR	8/8	0.00	X	X	X	X
44	2027	4VNTR	8/8	0.00	X	X	X	X
45	2028	4VNTR	8/8	0.00	X	X	X	X
46	2029	4VNTR	8/8	0.00	X	X	X	X
47	2027	4VNTR	8/8	0.00	X	X	X	X
48	2028	4VNTR	8/8	0.00	X	X	X	X
49	2029	4VNTR	8/8	0.00	X	X	X	X
50	2027	4VNTR	8/8	0.00	X	X	X	X
51	2028	4VNTR	8/8	0.00	X	X	X	X
52	2029	4VNTR	8/8	0.00	X	X	X	X
53	2027	4VNTR	8/8	0.00	X	X	X	X
54	2028	4VNTR	8/8	0.00	X	X	X	X
55	2029	4VNTR	8/8	0.00	X	X	X	X
56	2027	4VNTR	8/8	0.00	X	X	X	X
57	2028	4VNTR	8/8	0.00	X	X	X	X
58	2029	4VNTR	8/8	0.00	X	X	X	X
59	2027	4VNTR	8/8	0.00	X	X	X	X
60	2028	4VNTR	8/8	0.00	X	X	X	X
61	2029	4VNTR	8/8	0.00	X	X	X	X
62	2027	4VNTR	8/8	0.00	X	X	X	X
63	2028	4VNTR	8/8	0.00	X	X	X	X
64	2029	4VNTR	8/8	0.00	X	X	X	X
65	2027	4VNTR	8/8	0.00	X	X	X	X
66	2028	4VNTR	8/8	0.00	X	X	X	X
67	2029	4VNTR	8/8	0.00	X	X	X	X
68	2027	4VNTR	8/8	0.00	X	X	X	X
69	2028	4VNTR	8/8	0.00	X	X	X	X
70	2029	4VNTR	8/8	0.00	X	X	X	X
71	2027	4VNTR	8/8	0.00	X	X	X	X
72	2028	4VNTR	8/8	0.00	X	X	X	X
73	2029	4VNTR	8/8	0.00	X	X	X	X
74	2027	4VNTR	8/8	0.00	X	X	X	X
75	2028	4VNTR	8/8	0.00	X	X	X	X
76	2029	4VNTR	8/8	0.00	X	X	X	X
77	2027	4VNTR	8/8	0.00	X	X	X	X
78	2028	4VNTR	8/8	0.00	X	X	X	X
79	2029	4VNTR	8/8	0.00	X	X	X	X
80	2027	4VNTR	8/8	0.00	X	X	X	X
81	2028	4VNTR	8/8	0.00	X	X	X	X
82	2029	4VNTR	8/8	0.00	X	X	X	X
83	2027	4VNTR	8/8	0.00	X	X	X	X
84	2028	4VNTR	8/8	0.00	X	X	X	X
85	2029	4VNTR	8/8	0.00	X	X	X	X
86	2027	4VNTR	8/8	0.00	X	X	X	X
87	2028	4VNTR	8/8	0.00	X	X	X	X
88	2029	4VNTR	8/8	0.00	X	X	X	X
89	2027	4VNTR	8/8	0.00	X	X	X	X
90	2028	4VNTR	8/8	0.00	X	X	X	X
91	2029	4VNTR	8/8	0.00	X	X	X	X
92	2027	4VNTR	8/8	0.00	X	X	X	X
93	2028	4VNTR	8/8	0.00	X	X	X	X
94	2029	4VNTR	8/8	0.00	X	X	X	X
95	2027	4VNTR	8/8	0.00	X	X	X	X
96	2028	4VNTR	8/8	0.00	X	X	X	X
97	2029	4VNTR	8/8	0.00	X	X	X	X
98	2027	4VNTR	8/8	0.00	X	X	X	X
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103	2029	4VNTR	8/8	0.00	X	X	X	X
104	2027	4VNTR	8/8	0.00	X	X	X	X
105	2028	4VNTR	8/8	0.00	X	X	X	X
106	2029	4VNTR	8/8	0.00	X	X	X	X
107	2027	4VNTR	8/8	0.00	X	X	X	X
108	2028	4VNTR	8/8	0.00	X	X	X	X
109	2029	4VNTR	8/8	0.00	X	X	X	X
110	2027	4VNTR	8/8	0.00	X	X	X	X
111	2028	4VNTR	8/8	0.00	X	X	X	X
112	2029	4VNTR	8/8	0.00	X	X	X	X
113	2027	4VNTR	8/8	0.00	X	X	X	X
114	2028	4VNTR	8/8	0.00	X	X	X	X
115	2029	4VNTR	8/8	0.00	X	X	X	X
116	2027	4VNTR	8/8	0.00	X	X	X	X
117	2028	4VNTR	8/8	0.00	X	X	X	X
118	2029	4VNTR	8/8	0.00	X	X	X	X
119	2027	4VNTR	8/8	0.00	X	X	X	X
120	2028	4VNTR	8/8	0.00	X	X	X	X
121	2029	4VNTR	8/8	0.00	X	X	X	X
122	2027	4VNTR	8/8	0.00	X	X	X	X
123	2028	4VNTR	8/8	0.00	X	X	X	X
124	2029	4VNTR	8/8	0.00	X	X	X	X
125	2027	4VNTR	8/8	0.00	X	X	X	X
126	2028	4VNTR	8/8	0.00	X	X	X	X
127	2029	4VNTR	8/8	0.00	X	X	X	X
128	2027	4VNTR	8/8	0.00	X	X	X	X
129	2028	4VNTR	8/8	0.00	X	X	X	X
130	2029	4VNTR	8/8	0.00	X	X	X	X
131	2027	4VNTR	8/8	0.00	X	X	X	X
132	2028	4VNTR	8/8	0.00	X	X	X	X
133	2029	4VNTR	8/8	0.00	X	X	X	X
134	2027	4VNTR	8/8	0.00	X	X	X	X
135	2028	4VNTR	8/8	0.00	X	X	X	X
136	2029	4VNTR	8/8	0.00	X	X	X	X
137	2027	4VNTR	8/8	0.00	X	X	X	X
138	2028	4VNTR	8/8	0.00	X	X	X	X
139	2029	4VNTR	8/8	0.00	X	X	X	X
140	2027	4VNTR	8/8	0.00	X	X	X	X
141	2028	4VNTR	8/8	0.00	X	X	X	X
142	2029	4VNTR	8/8	0.00	X	X	X	X
143	2027	4VNTR	8/8	0.00	X	X	X	X
144	2028	4VNTR	8/8	0.00	X	X	X	X
145	2029	4VNTR	8/8	0.00	X	X	X	X
146	2027	4VNTR	8/8	0.00	X	X	X	X
147	2028	4VNTR	8/8	0.00	X	X	X	X
148	2029	4VNTR	8/8	0.00	X	X	X	X
149	2027	4VNTR	8/8	0.00	X	X	X	X
150	2028	4VNTR	8/8	0.00	X	X	X	X
151	2029	4VNTR	8/8	0.00	X	X	X	X
152	2027	4VNTR	8/8	0.00	X	X	X	X
153	2028	4VNTR	8/8	0.00	X	X	X	X
154	2029	4VNTR	8/8	0.00	X	X	X	X
155	2027	4VNTR	8/8	0.00	X	X	X	X
156	2028	4VNTR	8/8	0.00	X	X	X	X
157	2029	4VNTR	8/8	0.00	X	X	X	X
158	2027	4VNTR	8/8	0.00	X	X	X	X
159	2028	4VNTR	8/8	0.00	X	X	X	X
160	2029	4VNTR	8/8	0.00	X	X	X	X
161	2027	4VNTR	8/8	0.00	X	X	X	X
162	2028	4VNTR	8/8	0.00	X	X	X	X
163	2029	4VNTR	8/8	0.00	X	X	X	X
164	2027	4VNTR	8/8	0.00	X	X	X	X
165	2028	4VNTR	8/8	0.00	X	X	X	X
166	2029	4VNTR	8/8	0.00	X	X	X	X
167	2027	4VNTR	8/8	0.00	X	X	X	X
168	2028	4VNTR	8/8	0.00	X	X	X	X
169	2029	4VNTR	8/8	0.00	X	X	X	X
170	2027	4VNTR	8/8	0.00	X	X	X	X
171	2028	4VNTR	8/8	0.00	X	X	X	X
172	2029	4VNTR	8/8	0.00	X	X	X	X
173	2027	4VNTR	8/8	0.00	X	X	X	X
174	2028	4VNTR	8/8	0.00	X	X	X	X
175	2029	4VNTR	8/8	0.00	X	X	X	X
176	2027	4VNTR	8/8	0.00	X	X	X	X
177	2028	4VNTR	8/8	0.00	X	X	X	X
178	2029	4VNTR	8/8	0.00	X	X	X	X
179	2027	4VNTR						

VNTR profiles in case of coincidental occurrence
(8 cases, 20 isolates)

IS6110	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

7

JATA(12)-VNTR analysis of MDR-TB

3122 TB strains were isolated from all over Japan
in 2002. 55 TB strains in 3122 were made a
diagnosis as MDR-TB

- (1) IS6110 RFLP
- (2) VNTR
 - JATA (12)
 - Supply (15)

8

TB genotyping in Japan

- (1) By analyzing the TB collected from whole Japan, 70 % of TB isolates were Beijing genotype.
- (2) The percentage of cluster rate in IS6110 RFLP was 18.5%.
- (3) We established new promising loci of VNTR analyses for Beijing strains.

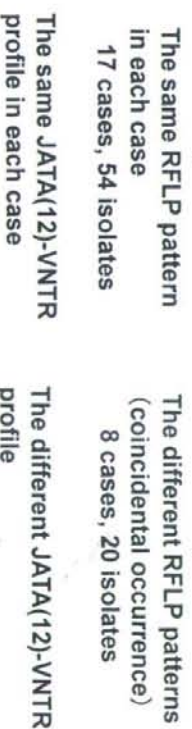
The discrimination power of JATA(12)-VNTR analysis is higher than that of IS6110 RFLP and Supply (15).

5

Application of the JATA(12)-VNTR analysis to clinical TB isolates

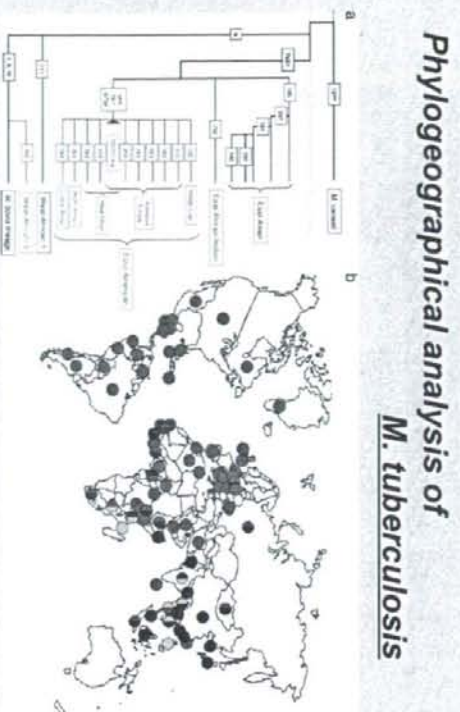
The infection by the identical strain was doubted
by contact investigation.

25 cases, 74 isolates



6

1. Phylogeographic analysis of BJ family
2. Phylogenetic information of BJ family from VNTR data
3. VNTR analysis for phylogeographic understanding of BJ family in Asia



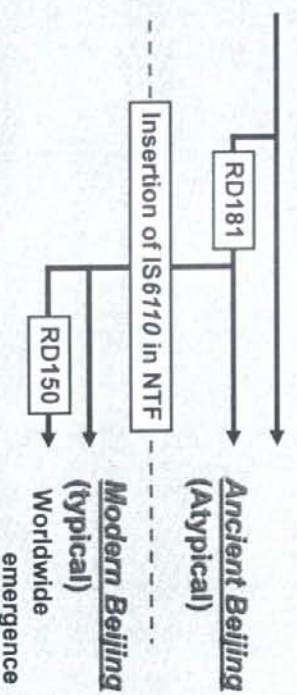
Phylogenetic analysis and VNTR genotyping for Beijing family in Asia

Takayuki Wada

*Department of Microbiology,
Osaka City Institute of Public
Health
and Environmental Sciences*

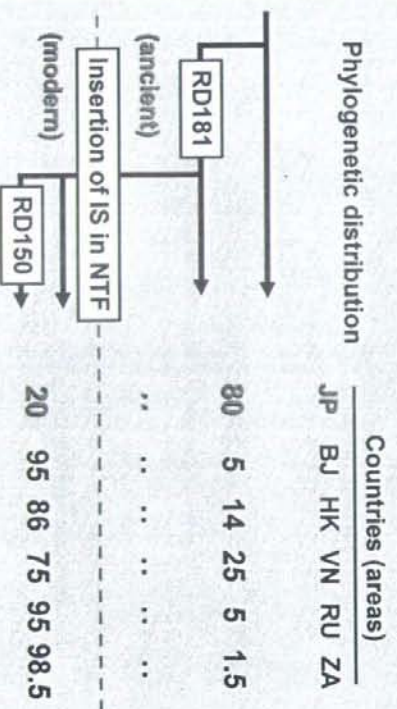
1. Phylogeographic analysis of BJ family
2. Phylogenetic information of BJ family from VNTR data
3. VNTR analysis for phylogeographic understanding of BJ family in Asia

Various genetic characteristics provide phylogenetic information

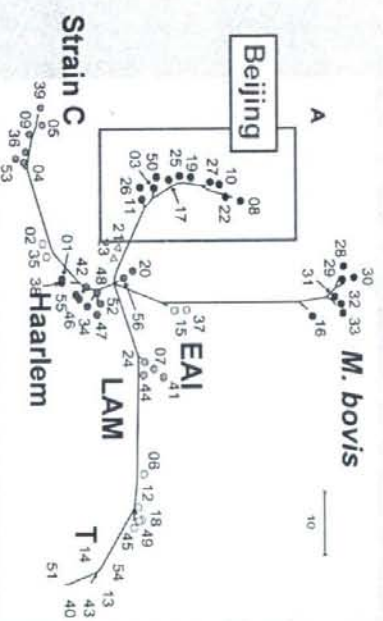


What kind of Beijing family strains has been predominant in Japan?

Phylogeographic distribution of the BJ family *M. tuberculosis*

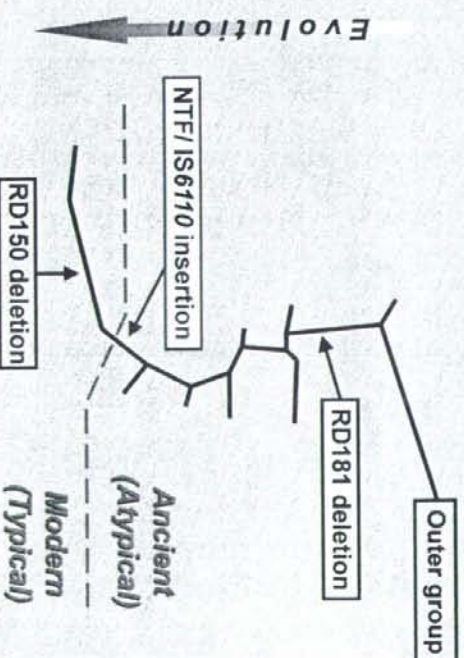


Phylogenetic analysis by SNP (Single Nucleotide Polymorphism)



Filliol, et al. (2006)
J Bacteriol 188(2), 759-772

Phylogenetic tree of the BJ family *M. tuberculosis*

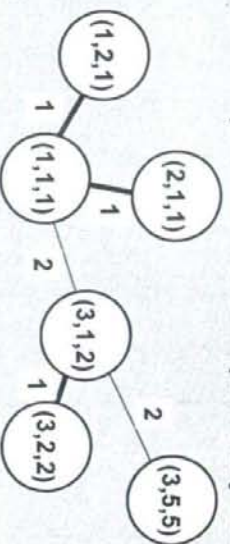


Phylogenetic tree based on VNTR by minimum spanning tree (MST)* is reliable (2)

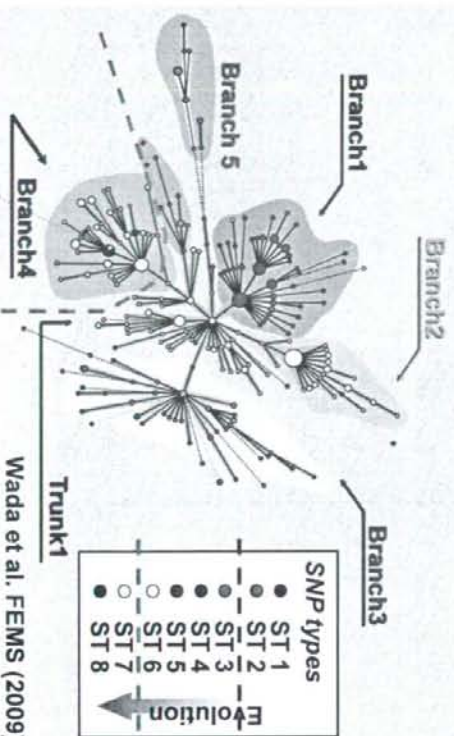
*What's MST?

- A graphic algorithm to cluster similar types.
- MST reconstructs a tree that connects all genetic profiles in such a way that the summed genetic distance of all branches is minimized.

(similar to maximum pulmonary method)



Phylogenetic analysis based on VNTR by minimum spanning tree (MST) is reliable (3)



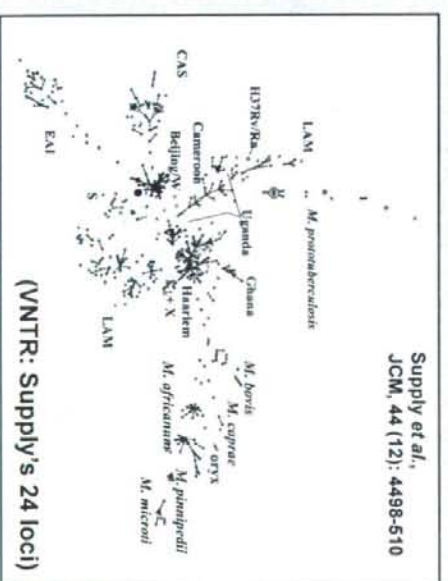
Wada et al. FEMS (2009)

1. Phylogeographic analysis of BJ family

2. Phylogenetic information of BJ family from VNTR data

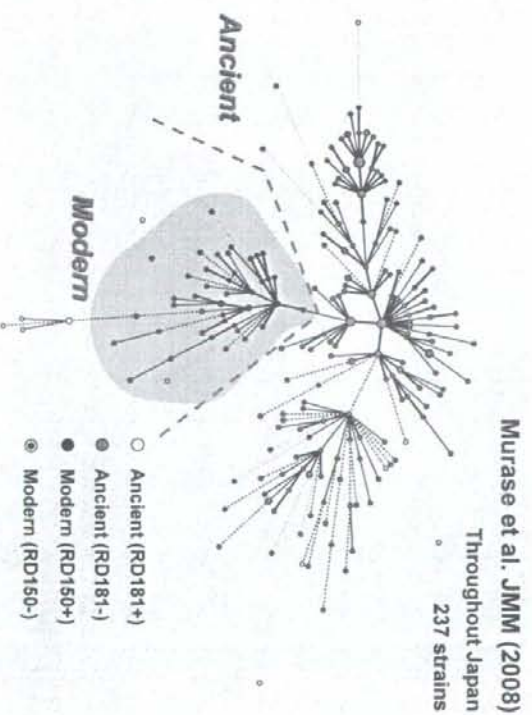
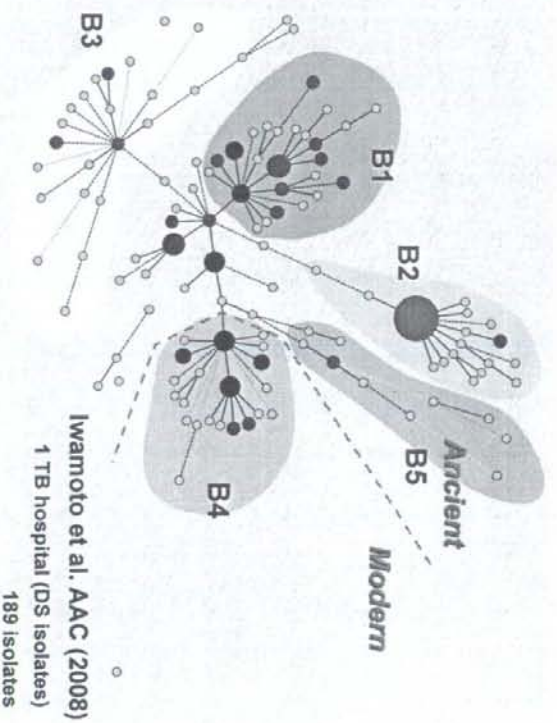
3. VNTR analysis for phylogeographic understanding of BJ family in Asia

Phylogenetic tree based on VNTR by minimum spanning tree (MST) is reliable (1)



Supply et al.,
JCM, 44 (12): 4498-510

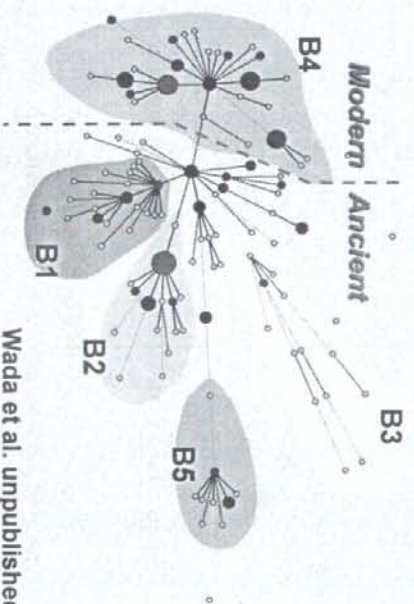
(VNTR: Supply's 24 loci)

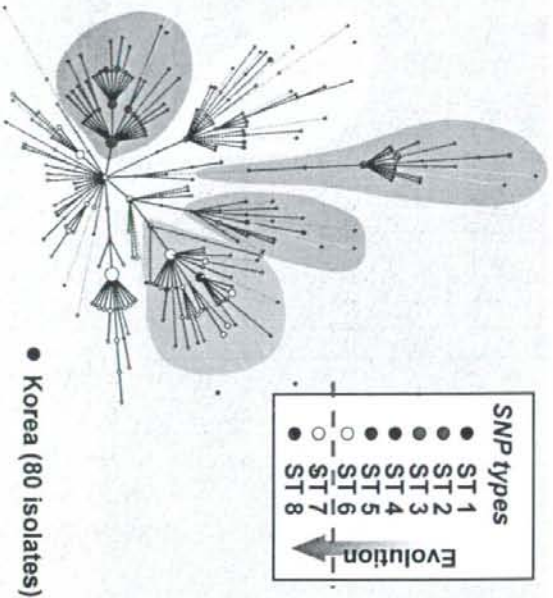
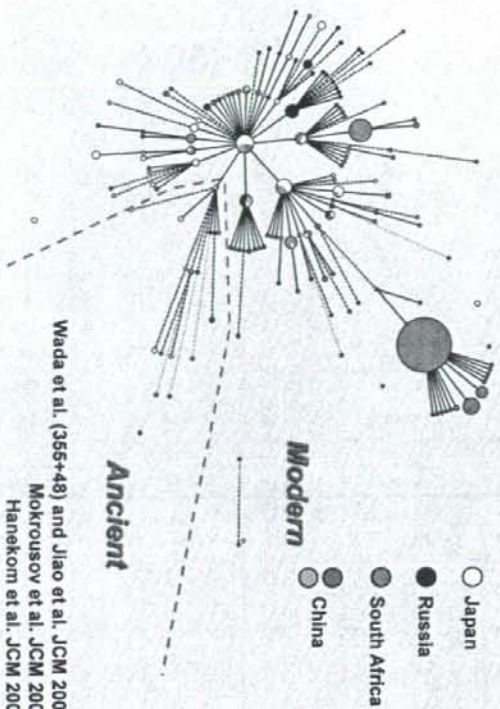


1. Phylogeographic analysis of BJ family

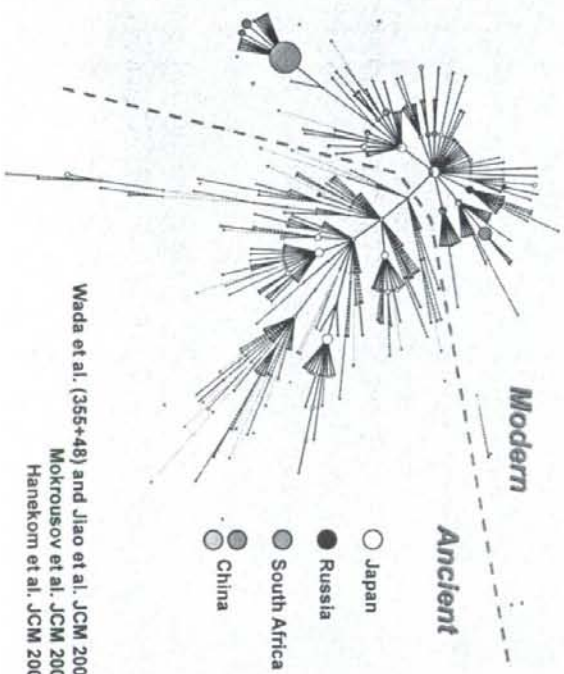
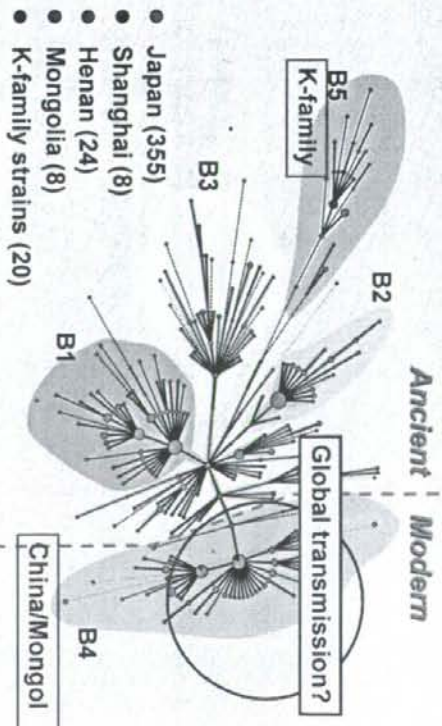
2. Phylogenetic aspects of VNTR about BJ family isolates in Japan

3. VNTR analysis for phylogeographic understanding of BJ family in East Asia





MST analysis can be applicable to international comparison of BJ strains



Next interests...

- 1) Which loci can be more discriminable in each population?
(e.g. V3820 in Japan)
- 2) The reasons of phylogeographic difference (genetic diversity) of BJ family (genomic comparison, etc.)
- 3) Continuous observation of population structure by VNTR in each area

Next interests...

- 4) Expansion of area
- 5) Other analysis such as MDS, coalescent theory

Thank you for your attention !!

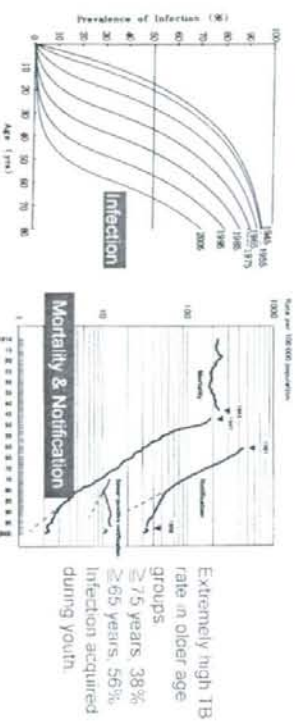
Population used in this study

A total of 904 *M. tuberculosis* isolates obtained from two medical centers between 2002-2007. They were from newly registered pulmonary TB patients.

Of these isolates, 711 strains were Beijing family strains. The details of 904 isolates by spoligotyping were: Beijing (78.7%), T1 (5.2%), T3-Osaka (2.5%), T2 (2.3%), EAI2 (1.4%), LAM9 (1.1%), Haarlem3 (0.9%), T3 (0.7%), others (7.3%).

Phylogenetic analysis: 15-VNTR & MST, RD181, NTF
Genotypic clonarity: 15-locus & 4-hypervariable loci
VNTR (11a, 3232, 3820, 4120)

Epidemiologic Characteristics in Japan



Gap between very high infection prevalence in the elderly and very low prevalence in the younger population. (at the age of 20, 50% in 1950 vs 1.3% in 2000)

The annual risk for infection was estimated to be 4% in the 1950s and 0.05% in the 1990s (reflecting an 11% annual rate of decrease from the 50s to the 70s).

The modern TB control measures (mass BCG vaccination, contact investigation, chemotherapy, etc.) has been started in 1951.

Spatial and temporal changing in population structure of *Mycobacterium tuberculosis* Beijing family strains

Tomotada Iwamoto
Kobe Institute of Health

MTB population dynamics

