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Original Article

Genetic surveillance and outcomes of pyrazinamide and fluoroquinolones-resistant tuberculosis in Taiwan

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Abstract *Background:* Pyrazinamide (PZA) and fluoroquinolone (FQ), particularly moxifloxacin (MXF), are essential drugs in the World Health Organization (WHO) recommended short-course regimen to treat drug-susceptible tuberculosis (TB).
Methods: To understand the extent of PZA and MXF susceptibility in general TB cases in Taiwan, we conducted retrospective analyses of 385 conservative *Mycobacterium tuberculosis* complex (MTBC) isolates identified from 4 TB laboratories in different regions of Taiwan. The case information was obtained from the TB registry. Genotypic drug susceptibility testing (DST) was performed by sequencing drug-resistance associated genes, PZA (*pncA*) and FQ (*gyrA*, and *gyrB*). Phenotypic DST was determined using the Bactec MGIT 960 system or the agar proportion method. Genotyping was carried out using spacer oligonucleotide typing.
Results: In this study, 4.7% (18/385) cases' isolates harbored *pncA* mutations and 7.0% (27/385) cases' isolates harbored *gyrA* or *gyrB* mutation. Notably, *pncA* mutation was associated with Beijing family genotypes ($P = 0.028$), East African-Indian (EAI) genotypes ($P = 0.047$) and MDR-TB ($P < 0.001$). Whereas, *gyrA* or *gyrB* mutation was associated with EAI genotypes ($P = 0.020$) and MDR-TB ($P = 0.006$). In addition, a statistically significant difference was found between the favorable outcomes using active and inactive PZA ($P = 0.009$) in 38 case isolates with any *pncA*, *gyrA*, or *gyrB* mutation.
Conclusion: We concluded that routine PZA and FQ susceptibility tests are recommended for guiding the treatment of TB.

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Introduction

Tuberculosis (TB) is an aerosol-transmitted disease caused by *Mycobacterium tuberculosis* complex, mainly *M. tuberculosis* and *Mycobacterium bovis*. The World Health Organization (WHO) estimated 6.4 million new TB cases occurred in 2021. Moreover, there were estimated 0.45 million multidrug-resistant (MDR)-TB, defined as resistance at least to isoniazid (INH) and rifampin (RIF), or RIF-resistant (RR-TB) cases.[1] In Taiwan, 7062 new TB cases were notified, including 82 (1.2%) MDR-TB and 41 (0.6%) RR-TB cases confirmed in 2021.[2]

In 2022, WHO recommended a short-course treatment of drug-susceptible (DS)-TB cases aged 12 years or older as a treatment option. The 4-month regimen comprising 2 months of rifapentine (RFP), INH, pyrazinamide (PZA), and moxifloxacin (MXF), followed by 2 months of INH, RFP, and MXF (2HPMZ/2HPM).[3]

PZA is a potent TB drug in shortening TB therapy.[4] It is a prodrug requiring activation by the enzyme pyrazinamidase (PZase), encoded by the *pncA* gene in *M. tuberculosis*.[4,5] PZA resistance is mainly caused by *pncA* mutations and the detection of resistance-conferring mutations in the *pncA* gene using DNA sequencing is the most reliable method for the detection of PZA resistance.[4—7] Besides, phenotypic drug susceptibility testing (pDST) of PZA is the culture-based MGIT 960 system recommended by WHO.[7]

Fluoroquinolones (FQs), including MXF, are the key component of the treatment of MDR-TB by targeting DNA gyrase to disrupt DNA replication. FQs resistance is primarily associated with mutations within the quinolone resistance-determining region (QRDR) of the *gyrA* (codons 74 to 113) and *gyrB* (codons 500 to 538) genes, respectively.[8] Two WHO-endorsed commercial nucleic acid amplification tests, the GenoType MTBDRsl (Hain Lifescience, Nehren, Baden-Württemberg, Germany) and the Xpert MTB/XDR assay (Cepheid, Sunnyvale, CA, United States), which detect FQs resistance by identifying the QRDR region of the *gyrA* and *gyrB* genes.[9] In addition, pDST for MXF resistance using the agar proportion method (APM) or MGIT 960 system was recommended by WHO.

The susceptibility testing to PZA and FQs is not routinely performed for *M. tuberculosis* isolates of general TB cases in clinical laboratories in Taiwan. Nevertheless, surveillance data of PZA and FQs resistance were provided for MDR and RR-TB cases by the reference laboratory at Taiwan Centers for Disease Control (TCDC).[10] Since population-representative surveillance data of resistance to PZA and FQs is scarce, we conducted genetic surveillance and outcome analysis of PZA and MXF-resistant TB for facilitating the uptake of the short regimen for DS-TB treatment.

Methods

Study design and clinical isolates

This is a retrospective population-based cohort study of 385 TB cases confirmed by conservative isolates of 4 TCDC-authorized clinical TB labs in 2019. One initial *M. tuberculosis* isolate was analyzed for each case. The characteristics and treatment outcomes of cases were obtained from the TB Registry. We assessed treatment outcomes to classify the cases as cured, treatment completed, treatment failure, died, or lost to follow-up according to the internationally recommended outcome definitions. Cured and treatment completed were categorized as favorable outcomes.[11]

Phenotypic drug susceptibility testing

M. tuberculosis isolates were subjected to DST using the APM with 7H10 medium (Becton, Dickinson and Company, Sparks, MD, USA). Drug resistance was defined as the growth of 1% of colonies in a drug-containing medium. According to WHO recommendations, the critical concentrations of MXF in the 7H10 medium is 0.5 μg/ml.[7] PZA (100 μg/ml) resistance was tested using the Bactec MGIT 960 system as described previously.[7] Growth on a control medium was compared to growth on the corresponding drug-containing medium to determine susceptibility. The DST results were used to categorize the isolates as resistant or susceptible. The tests were validated by determining the susceptibility of *M. tuberculosis* H37Rv. MDR-TB is defined as an *M. tuberculosis* isolate that is resistant to at least INH and RIF.

Genotypic drug susceptibility testing

The *pncA* gene and its promoter were amplified using a pair of specific primers (*pncA*-F, 5′-GCTGGTCATGTTCGCGATCG-3′, *pncA*-R, 5′-CGCTTGCGGCGAGCGCTCCA-3′) to detect mutations associated with PZA resistance. The *gyrA* and *gyrB* genes were amplified using pairs of specific primers (*gyrA*-F, 5′-GATGACAGACACGACGTTGC-3′, *gyrA*-R, 5′-AGCATCTC-CATCGCCAACG-3’; *gyrB*-F, 5′-AAGACCAAGTTGGGCAACAC-3′, *gyrB*-R, 5′-CTGCCACTTGAGTTTGTACA-3′). PCR conditions were as follows: 95 °C denaturation for 10 min, 35 cycles of 95 °C denaturation for 1min, annealing for 1min at 65 °C for *pncA* and *gyrA*, 62 °C for *gyrB*, and extension at 72 °C for 1min, and a final extension cycle at 72 °C for 6min. PCR products were verified using a high-performance DNA analyzer (QIAxcel Advanced System, Qiagen, Hilden, North Rhine-Westphalia, Germany) and sent for sequencing

(Genomics, New Taipei City, Taiwan). Sequences were analyzed using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, Mich, USA) by comparison with the *M. tuberculosis* H37Rv sequence (NC_000962.3).

Genotyping

Spacer oligonucleotide typing (spoligotyping) analysis was used for genotyping. A commercially available kit (Ocimum Biosolutions, Hyderabad, Telangana, India) was used as described previously.[12] Briefly, the amplified DNA was hybridized onto a membrane that was covalently precoated with a set of 43 spacer oligonucleotides derived from the spacer sequences of *M. tuberculosis* H37Rv and *M. bovis* P3. The Immobilon® ECL Detection system (Millipore Corporation, Billerica, MA, United States) was used for the final image detection. The spoligotypes were compared with the SITVIT global database (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/).

Statistical analysis

Data entry and analysis were done using Microsoft Excel. The chi-squared test or Fisher's exact test (when expected cell size <5) was used for the univariate analysis of categorical variables. P-values of <0.05 were considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated to estimate the correlation between the variables (http://vassarstats.net/).

Results

Characteristics of the study population

Characteristics of the study population was summarized in Table 1 and Fig. 1. In this cohort study, we enrolled 385 TB cases including 94, 49, 165, and 77 cases identified from northern, central, southern, and eastern Taiwan, respectively (Fig. 1). Two hundred sixty-six (69.1%) cases were male, and 198 (51.4%) cases were aged more than 65 years old. Three hundred sixty-six (95.1%) and 19 (4.9%) were new and previously treated cases, respectively. Three major genotypes were 129 (33.9%), 116 (30.4%), and 49 (12.9%) of the Beijing family, East-Africa-India family, and Haarlem genotypes, respectively. Genotypes not belonging to 3 major genotypes were all classified as other genotypes in Table 1. Besides, we found 322 (83.6%), 12 (3.1%), and 51 (13.3%) isolates were pan-susceptible, MDR, and other resistant, respectively. Notably, 18 (4.7%) isolates harbored *pncA* mutation, and 27 (7.0%) cases had *gyrA* or *gyrB* mutation. Furthermore, 2 cases' isolates had concurrent *pncA*/*gyrA*, and 5 cases' isolates had concurrent *pncA*/*gyrB* mutations.

Associations with mutations, drug resistance and treatment outcomes

The *pncA*, *gyrA*, *gyrB* mutation types, treatment outcomes, drug resistance profiles, and regimens were shown in Table 2. Of the 38 cases with any *pncA*, *gyrA* or *gyrB* mutation, 29 (76.3%) and 9 (23.7%) were non-MDR-TB and MDR-TB cases, respectively.

PZA resistance

In this survey, *M. bovis* was identified in all 5 isolates harboring *pncA* H57D mutation. A discordant DST result was detected in one phenotypically PZA-susceptible isolate harboring *pncA* L35R. Of the 5 phenotypically PZA-resistant isolates, 4 isolates harbored a novel mutation, *pncA* P70 deletion, and 1 isolate harbored nt 120c deletion. Of the 18 (4.7%) isolates harboring *pncA* mutations, 4 (22.2%) were Beijing family genotypes. Nevertheless, no association between Beijing family genotypes and *pncA* mutations was found. Of the 12 (3.1%) MDR-TB cases, 6 cases had isolates harboring *pncA* mutations. Notably, *pncA* mutation was associated with MDR-TB cases ($P < 0.001$, OR = 34.78 [95% CI, 9.371 to 129.067]) (Table 1).

FQs resistance

For *gyrA* mutations, we found 2 isolates harboring novel mutations, M33L and V55M (Table 2). Notably, 5 (1.4%) isolates harbored codon 90 or 94 mutations [A90V (3), D94N (1), and D94G (1)] were not routinely identified for FQs resistance except in our MDR/RR-TB laboratory service program provided by TCDC. In addition, we found 5 isolates harbored *gyrB* silent mutations, G476G (1), R534R (1), and G551R (3); and 3 isolates harbored *gyrB* novel mutations, G509S, A516G, and V535G. Of the 27 (7%) isolates that harbored *gyrA* or *gyrB* mutation, 14 (51.9%) isolates were Beijing family genotypes and 4 (14.8%) isolates were MDR. We observed that *gyrA* or *gyrB* mutation was associated with EAI genotypes ($P = 0.020$, OR = 0.230 [95% CI, 0.060 to 0.877]) and MDR-TB ($P = 0.006$, OR = 7.974 [95% CI, 2.202 to 28.870]) (Table 1).

Treatment outcome

Of the 385 patients, 347 cases' isolates had no *pncA*, *gyrA*, or *gyrB* mutation including 336 cases received medications. The average treatment duration of this group was 214 days (ranged from 1 to 1018 days) and the rate of cured/treatment completed was 75.89% (255/336). Whereas, the average treatment duration of the 38 cases with any *pncA*, *gyrA*, or *gyrB* mutation was 256.6 days (ranged from 6 to 744 days) and the rate of cured/treatment completed was 65.79% (25/38). No significant difference between 2 treatment groups was found ($p = 0.079$).

The overall treatment outcomes of 385 TB cases were 280 (72.7%) favorable, 88 (22.9%) death, 15 (3.9%) transferred out, and 2 (0.5%) loss to follow-up (Table 3). Of the 38 cases' isolates with any *pncA*, *gyrA*, or *gyrB* mutation, 25 (65.8%) and 13 (34.2%) cases had favorable treatment outcomes and died, respectively. The treatment success rate of 38 TB cases (65.8%) was slightly lower than that of 385 general TB cases (72.7%), but no significant difference was observed ($P = 0.362$). On the other hand, the mortality rate (34.2%) of the 38 cases was higher than that of 385 general TB cases (22.9%), but no significant difference was found ($P = 0.117$). Of the 38 cases, the ratio of previously

Table 1 Characteristics of study population.

Characteristics	Total no (%)	No. (%) isolates		Odds ratio (95% CI)	*p*-value	No. (%) isolates		Odds ratio (95% CI)	*p*-value
	385 (100.0)	*pncA* mutation 18 (4.7)	*pncA* wild-type 367 (95.3)			*gyrA or gyrB* mutation 27 (7.0)	*gyrA or gyrB* wild-type 358 (93.0)		
Gender									
Male	266 (69.1)	14 (77.8)	252 (68.7)	reference		18 (66.7)	248 (69.3)	reference	
Female	119 (30.9)	4 (22.2)	115 (31.3)	0.626 (0.202–1.944)	0.413	9 (33.3)	110 (30.7)	1.127 (0.491–2.588)	0.777
Age									
≦25	23 (6.0)	1 (5.6)	22 (6.0)	0.591 (0.070–4.968)	>0.999	4 (14.8)	19 (5.3)	2.737 (0.749–10.003)	0.219
26-44	52 (13.5)	0 (0.0)	52 (14.2)	0.000	0.056	4 (14.8)	48 (13.4)	1.083 (0.311–3.774)	>0.999
45-64	112 (29.1)	8 (44.4)	104 (28.3)	reference		8 (29.6)	104 (29.1)	reference	
≧65	198 (51.4)	9 (50.0)	189 (51.5)	0.619 (0.232–1.653)	0.335	11 (40.7)	187 (52.2)	0.765 (0.298–1.961)	0.578
Nationality									
Taiwanese	355 (92.2)	18 (100.0)	337 (91.8)	reference		26 (96.3)	329 (91.9)	reference	
Foreigner	30 (7.8)	0 (0.0)	30 (8.2)	0.000	0.381	1 (3.7)	29 (8.1)	0.436 (0.057–3.333)	0.505
Case category									
New	366 (95.1)	15 (83.3)	351 (95.6)	reference		25 (92.6)	341 (95.3)	reference	
Previously treated	19 (4.9)	3 (16.7)	16 (4.4)	4.388 (1.152–16.707)	0.052	2 (7.4)	17 (4.7)	1.605 (0.351–7.3405)	0.634
Acid-fast bacillus smear									
Positive	199 (51.7)	13 (72.2)	186 (50.7)	2.502 (0.874–7.162)	0.077	13 (48.1)	186 (52.0)	0.848 (0.387–1.857)	0.680
Negative	184 (47.8)	5 (27.8)	179 (48.8)	reference		14 (51.9)	170 (47.5)	reference	
Genotype									
Beijing family	129 (33.9)	4 (22.2)	125 (34.4)	0.277 (0.083–0.931)	0.028*	14 (51.9)	115 (32.5)	1.055 (0.435–2.557)	0.920
East-Africa-India family	116 (30.4)	4 (22.2)	112 (30.9)	0.310 (0.092–1.041)	0.047*	3 (11.1)	113 (31.9)	0.230 (0.060–0.877)	0.020*
Haarlem	49 (12.9)	1 (5.6)	48 (13.2)	0.181 (0.022–1.470)	0.094	1 (3.7)	48 (13.6)	0.181 (0.022–1.470)	0.094
Other genotypes	87 (22.8)	9 (50.0)	78 (21.5)	reference		9 (33.3)	78 (22.0)	reference	
Drug resistance pattern									
Pan-susceptible	322 (83.6)	9 (50.0)	313 (85.3)	reference		19 (70.4)	303 (84.6)	reference	
Isoniazid-resistance	26 (6.8)	2 (11.1)	24 (6.5)	2.898 (0.593–14.176)	0.195	2 (7.4)	24 (6.7)	1.329 (0.292–6.047)	0.000
Rifampin-resistance	5 (1.3)	1 (5.6)	4 (1.1)	8.694 (0.881–85.801)	0.145	1 (3.7)	4 (1.1)	3.987 (0.425–37.444)	0.272
Multidrug-resistance	12 (3.1)	6 (33.3)	6 (1.6)	34.778 (9.371–129.067)	<0.001*	4 (14.8)	8 (2.2)	7.974 (2.202–28.870)	0.006*
Other drug-resistance	19 (4.9)	0 (0.0)	19 (5.2)	0.000	>0.999	1 (3.7)	18 (5.0)	0.886 (0.112–6.996)	>0.999

95% CI, 95% Confidence interval.

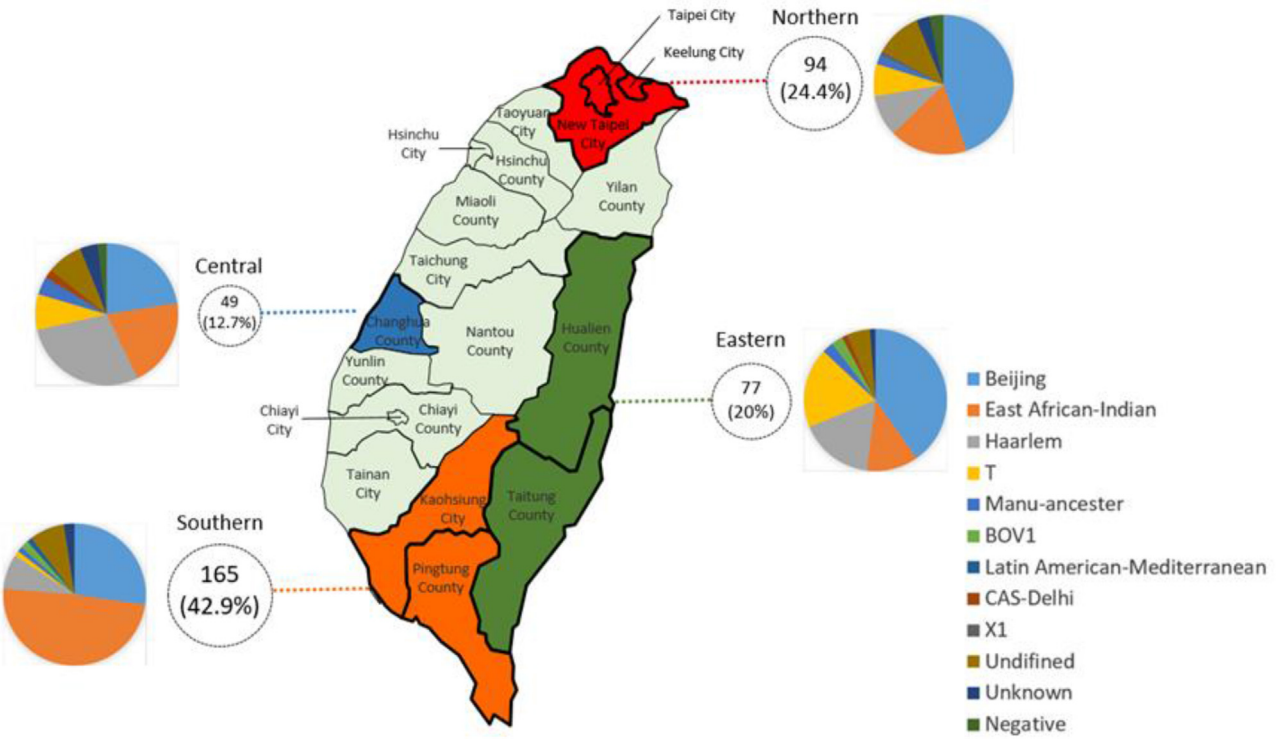

Figure 1. Distribution of the study populations. Numbers and percentages of the study cases distributed in four regions (outlined with thick lines) were presented in circles. The distribution of spoligotypes was shown in pie charts.

Table 2 The *pncA*, *gyrA*, *gyrB* mutations types, treatment outcomes, drug resistance profiles and regimens of 38 cases.

Case	*pncA* mutation type	PZA gDST[b]	*gyrA* mutation type	*gyrB* mutation type	FLQ gDST[b]	Treatment outcome	Drug resistance profile[c]	Regimen (active drugs)[c]
1	L35R	R[b]	wt[a]	wt	S[b]	Treatment completed	-	INH RIF EMB PZA
2	K48E	R	wt	wt	S	Died	INH RIF EMB PZA STR RFB ETO	MXF LFX KAN DCS CLO LZD BDQ
3	H57D	R	wt	N428N, A442S	S	Cured	PZA	INH RIF EMB
4	H57D	R	wt	N428N, A443S	S	Cured	PZA	INH RIF EMB RFB MXF
5	H57D	R	wt	N428N, A444S	S	Treatment completed	PZA	INH RIF EMB
6	H57D	R	wt	N428N, A445S	S	Died	PZA	INH RIF EMB
7	H57D	R	wt	N428N, A446S	S	Died	INH PZA	RIF EMB
8	S65S	S	wt	wt	S	Died	-	INH RIF EMB PZA STR
9	W68R	R	wt	wt	S	Cured	INH RIF PZA RFB	EMB MXF KAN PTO DCS CLO LZD
10	P70 del[d]	R	wt	wt	S	Cured	PZA	INH RIF EMB
11	P70 del[d]	R	wt	wt	S	Cured	PZA	INH RIF EMB
12	P70 del[d]	R	wt	wt	S	Died	PZA	INH RIF EMB
13	P70 del[d]	R	wt	wt	S	Died	INH PZA	RIF EMB
14	T76P	R	wt	wt	S	Cured	INH RIF EMB PZA	MXF KAN CAP DCS CLO PAS

Table 2 (*continued*)

Case	*pncA* mutation type	PZA gDST[b]	*gyrA* mutation type	*gyrB* mutation type	FLQ gDST[b]	Treatment outcome	Drug resistance profile[c]	Regimen (active drugs)[c]
15	T76P	R	D94G	wt	R	Died	INH RIF EMB PZA STR RFB MXF LVX ETO	AMK DCS CLO LZD BDQ
16	G78V	R	G88C	wt	R	Cured	INH RIF EMB PZA STR RFB MXF LVX ETO	KAN AMK DCS CLO LZD PAS BDQ DLM
17	nt 120c del[d]	R	wt	wt	S	Died	INH RIF EMB PZA RFB	MXF KAN PTO DCS
18	V163G	R	wt	wt	S	Died	INH RIF EMB PZA STR RFB	MXF LFX KAN PTO CLO LZD
19	wt	S	M33L[d]	wt	U[b]	Cured	-	INH RIF EMB PZA
20	wt	S	V55M[d]	wt	U[e]	Treatment completed	INH	RIF EMB PZA RFB MXF
21	wt	S	A90V	wt	R	Cured	-	INH RIF EMB PZA
22	wt	S	A90V	wt	R	Cured	-	INH RIF EMB PZA
23	wt	S	A90V	wt	R	Cured	-	INH RIF PZA
24	wt	S	D94G	wt	R	Cured	EMB	INH RIF PZA
25	wt	S	D94G	wt	R	Cured	INH RIF EMB RFB MXF LVX ETO	PZA KAN DCS CLO PAS BDQ
26	wt	S	D94N	wt	R	Died	-	INH RIF EMB
27	wt	S	D94G, wt	wt	R	Died	RIF RFB MXF LVX	INH EMB PZA KAN PTO DCS CLO
28	wt	S	D111D	wt	S	Cured	-	INH RIF EMB PZA
29	wt	S	D111D	wt	S	Cured	-	INH RIF EMB PZA
30	wt	S	wt	G476G	S	Cured	-	INH RIF EMB PZA RFB
31	wt	S	wt	G509S[d]	U[e]	Treatment completed	-	INH RIF EMB PZA
32	wt	S	wt	A516G[d]	U[e]	Died	-	INH RIF EMB
33	wt	S	wt	T529N	U[e]	Cured	INH RIF EMB STR RFB MXF ETO	PZA LFX KAN DCS CLO BDQ
34	wt	S	wt	R534R	S	Cured	-	INH RIF EMB PZA
35	wt	S	wt	V535G[d]	U[e]	Cured	-	INH RIF EMB PZA
36	wt	S	wt	G551R	S	Cured	-	INH RIF EMB PZA STR
37	wt	S	wt	G551R	S	Cured	-	INH RIF EMB PZA
38	wt	S	wt	G551R	S	Died	-	INH RIF EMB PZA

[a] wt, wild-type.
[b] gDST, genotypic drug susceptibility testing; R, resistance; S, susceptible; U, undetermined.
[c] INH, isoniazid; RIF, rifampicin; EMB, ethambutol; PZA, pyrazinamide; STR, streptomycin; RFB, rifabutin; MXF, moxifloxacin; LVX, levofloxacin; KAN, kanamycin; AMK, amikacin; CAP, capreomycin; PTO, prothionamide; DCS, D-cycloserine; CLO, clofazimine; LZD, linezolid; PAS, para-aminosalicylic acid; ETO, ethionamide; BDQ, bedaquiline; DLM, Delamanid.
[d] Novel mutation.

treated cases among death cases (15.4%) was higher than that of 385 general TB cases (8.0%).

We analyzed the associations of core drugs with the outcomes of 38 cases with PZA or FQs resistant gene mutations (Table 4). The treatment outcomes were 25 (65.8%) favorable and 13 (34.2%) death. In Table 4, active drugs are defined as cases susceptible to the drugs used for treatment, and inactive drugs as either case resistant to the drugs or drugs not used for treatment. Of the 25 cases with favorable treatment outcomes, 17 cases were treated with a PZA-containing regimen. Most importantly, statistically significant differences were observed between active and inactive PZA ($P = 0.009$, OR = 7.083 [95% CI, 1.519 to 33.033]). Moreover, we found no statistically significant differences between active and inactive INH ($P = 0.270$), RIF ($P = 0.263$), FQs ($P > 0.999$), and second-line injectable drugs including amikacin, capreomycin and kanamycin ($P = 0.263$).

Discussion

Taiwan has a low DR-TB burden with >80% of new pan-susceptible TB notified each year. In line with WHO-recommended short-course treatment for DS-TB, we conduct a cohort baseline survey for facilitating programmatic action. Since PZA and FQs DST was not routinely

Table 3 Treatment outcomes of 385 tuberculosis cases.

Treatment outcome	Total no. (%)	No. (%) of isolates with indicated gene mutation type							
		pncA		Risk Ratio	*P* value	*gyrA* or *gyrB*		Risk Ratio	*P* value
	[385 (100.0)]	mutation [18 (4.7)]	wild-type [367 (95.3)]			mutation [27 (7.0)]	wild-type [358 (93.0)]		
Cured	209 (54.3)	7 (38.9)	202 (55.0)	0.929 (0.862–1.001)	0.024	17 (63.0)	192 (53.6)	1.002 (0.931–1.078)	>0.999
Treatment completed	71 (18.4)	2 (11.1)	69 (18.8)	0.924 (0.852–1.002)	0.113	3 (11.1)	68 (19.0)	0.961 (0.889–1.040)	0.514
Died	88 (22.9)	9 (50.0)	79 (21.5)	reference		7 (25.9)	81 (22.6)	reference	
Transferred out	15 (3.9)	0 (0.0)	15 (4.1)	0.898 (0.837–0.963)	0.350	0 (0.0)	15 (4.2)	0.921 (0.866–0.979)	0.382
Loss to follow-up	2 (0.5)	0 (0.0)	2 (0.5)	0.898 (0.837–0.963)	>0.999	0 (0.0)	2 (0.6)	0.921 (0.866–0.979)	>0.999

provided in clinical diagnosis of TB other than MDR/RR-TB cases in Taiwan, this is the first study on genetic surveillance of PZA and FQs-resistant *M. tuberculosis* in general TB cases. This study revealed genetic surveillance of PZA and FQs resistance among general TB cases in Taiwan was 4.4% (17/385) and 2.9% (11/385), respectively. We observed statistically significant differences between the favorable outcomes using active and inactive PZA (P = 0.009, OR = 7.083 [95% CI, 1.519 to 33.033]) in 38 cases' isolates with any *pncA*, *gyrA*, or *gyrB* mutation. Since we found 2.7% (10/368) and 1.4% (5/368) non-MDR/RR-TB isolates harbored non-synonymous *pncA* and *gyrA* mutations, routine PZA and FQs susceptibility are suggested to be implemented in the TB control program.

Our phenotypic drug-resistance surveillance of MDR-TB from 2008 to 2019 revealed that the resistant rates of PZA and FQs in MDR-TB cases were 28.9% and 12.6% in Taiwan, respectively.[10] Previous studies revealed that the *pncA* mutation rate among general TB cases varied widely among different countries and settings, with rates of 12.6% in Azerbaijan, 5.1% in Bangladesh, 42.1% in Belarus, 3.0% in Pakistan, and 3.1–3.9% in South Africa.[13] Other PZA-resistant surveys demonstrated that the PZA phenotypic resistance rate among general TB cases was 1.8% in California and 15% in China, respectively.[14,15] Whereas, among MDR-TB isolates, 31.5%, 53%, and 58.3% were PZA-resistant in Korea, Sub-Saharan Africa, and Zambia, respectively.[16–18]

One phenotypically PZA-susceptible isolate with *pncA* L35R mutation is classified as "not associated with resistance-interim" in the WHO Mutation Catalog, occurring mainly in PZA-S isolates (n = 23), but also seen in one phenotypic PZA-R isolate.[19] Furthermore, 4 isolates harboring *pncA* P70 deletion identified from southern Taiwan were EAI2-Manila genotype, which is predominantly distributed in Southeastern Asia, such as Thailand, Vietnam, and the Philippines that are major migrants originated in Taiwan.[20–22] However, the aforementioned 4 domestic cases were in 4 distinct clusters determined using the Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) genotyping method (data not shown). One domestic case' isolate with CAS 1-Delhi sublineage harboring silent mutation, *pncA* S65S, as a putative genetic marker for Central Asian (CAS)-lineage isolates was consistent with the finding of a previous study.[23]

M. bovis isolates are intrinsically resistant to PZA and can concurrently be resistant to INH and/or RIF. This could lead to inadequate treatment and pose challenges to TB control efforts. In this study, 5 (1.3%) isolates harboring *pncA* H57D were identified as *M. bovis*. The human bovine TB identification rate was similar to the rate of 1.0% (202/20,972) from 2008 to 2019 in Taiwan.[24] PZA DST is an unmet need to be deployed to clinical laboratories to improve the screening and identification of *M. bovis* and concurrently enhance the One-Health approach for zoonotic bovine TB surveillance.[24]

In this study, *gyrA* or *gyrB* mutation was associated with MDR-TB and EAI-Malina genotypes, but not Beijing family genotypes. Our MDR-TB survey conducted in 2019 revealed that *gyrA* and *gyrB* mutation rate was 11.4% (10/88) and 3.4% (3/88), respectively, and no association between *gyrA* or *gyrB* mutations and Beijing family genotypes was found

Table 4 Associations of active or inactive core drugs with outcomes of 38 tuberculosis cases with *pncA*, *gyrA* or *gyrB* mutation.

Drug[a]		No. (%) with Outcome			
	Total No. (%)	Favorable	Died	Odds ratio (95% CI)[b]	*P* value
Isoniazid	38 (100.0)	25 (65.8)	13 (34.2)		
Active	26 (68.4)	19 (76.0)	7 (53.8)	2.714 (0.653—11.289)	0.270
Inactive	12 (31.6)	6 (24.0)	6 (46.2)	reference	
Rifampicin	38 (100.0)	25 (65.8)	13 (34.2)		
Active	28 (73.7)	20 (80.0)	8 (61.5)	2.500 (0.566—11.051)	0.263
Inactive	10 (26.3)	5 (20.0)	5 (38.5)	reference	
Pyrazinamide	38 (100.0)	25 (65.8)	13 (34.2)		
Active	20 (52.6)	17 (68.0)	3 (23.1)	7.083 (1.519—33.033)	0.009
Inactive	18 (47.4)	8 (32.0)	10 (76.9)	reference	
Fluoroquinolone	38 (100.0)	25 (65.8)	13 (34.2)		
Active	8 (21.1)	5 (20.0)	3 (23.1)	0.833 (0.165—4.212)	>0.999
Inactive	30 (78.9)	20 (80.0)	10 (76.9)	reference	
Second-line injectable drugs	38 (100.0)	25 (65.8)	13 (34.2)		
Active	10 (26.3)	5 (20.0)	5 (38.5)	0.400 (0.091—1.768)	0.263
Inactive	28 (73.7)	20 (80.0)	8 (61.5)	reference	

[a] Active, susceptible to the drug used for treatment; inactive, either resistant to the drug or drug not used for treatment.
[b] Results of univariate analysis. 95% CI, 95% Confidence interval.

(data not shown). Previous studies revealed that the rate of resistance to MXF was 3.6%, 4.5%, 14.6%, 8.1%, and 0.9—1.0% in Azerbaijan, Bangladesh, Belarus, Pakistan, and South Africa, respectively.[13] In Australia, the FQs resistance rate was 0.6% in non-MDR TB cases.[25] A Korean study revealed that the resistance rate to any FQs was 0.8% and 26.2% in non-MDR-TB and MDR-TB cases, respectively.[26]

We revealed associations of PZA prescription with favorable treatment outcomes in general TB cases. However, previous studies showed that PZA resistance was not associated with treatment success in MDR-TB patients.[18,27] On the contrary, other studies demonstrated that genotypic PZA resistance was associated with earlier culture conversion or better treatment success rate in MDR-TB cases suggesting PZA susceptibility is crucial in clinical management of MDR-TB.[28—30] Moreover, 17 cases treated with regimens containing PZA had favorable treatment outcomes, excluding 2 MDR-TB cases, PZA was never used without RIF or INH (Table 2), it seems impossible to separate the effectiveness of PZA.

One limitation of this study was that the sample size was too small to accurately represent the entire population being studied even though we observed statistically significant differences between the favorable outcomes using active and inactive PZA in 38 cases isolates with any *pncA*, *gyrA*, or *gyrB* mutations. In addition, this study focused only on the *pncA* mutations for the detection of PZA resistance, while other PZA resistance-associated mutations such as *panD* and *rspA* genes were recently reported to correlate with PZA resistance.[31]

In conclusion, we found that *pncA*, *gyrA* or *gyrB* mutations are not rare. Depending on the resources available, routine universal DST diagnoses of PZA resistance are recommended for better treatment and management of general TB cases. Low FQs resistant rate suggested that the FQs-based short-course regimen for DS-TB may be feasible. The findings of this study provided optimized strategies for updating diagnostic algorithms and treatment regimens for the Tuberculosis Program.

Institutional Review Board and informed consent statement

This study was approved by the Institutional Review Board of the Taiwan Centers for Disease Control, Ministry of Health and Welfare (TwCDC IRB No. 109205 and TwCDC IRB No. 110108). The study analyzed only archived *M.* tuberculosis isolates, and written informed consent of the participants was waived. All methods were performed following the relevant guidelines and regulations.

Author contributions

R.J. designed the research. H.T.H., W.H.L., and T.H.C. performed the experiments. R.J., W.H.L., and H.T.H. analyzed the results and wrote the manuscript.

Declaration of competing interest

None.

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