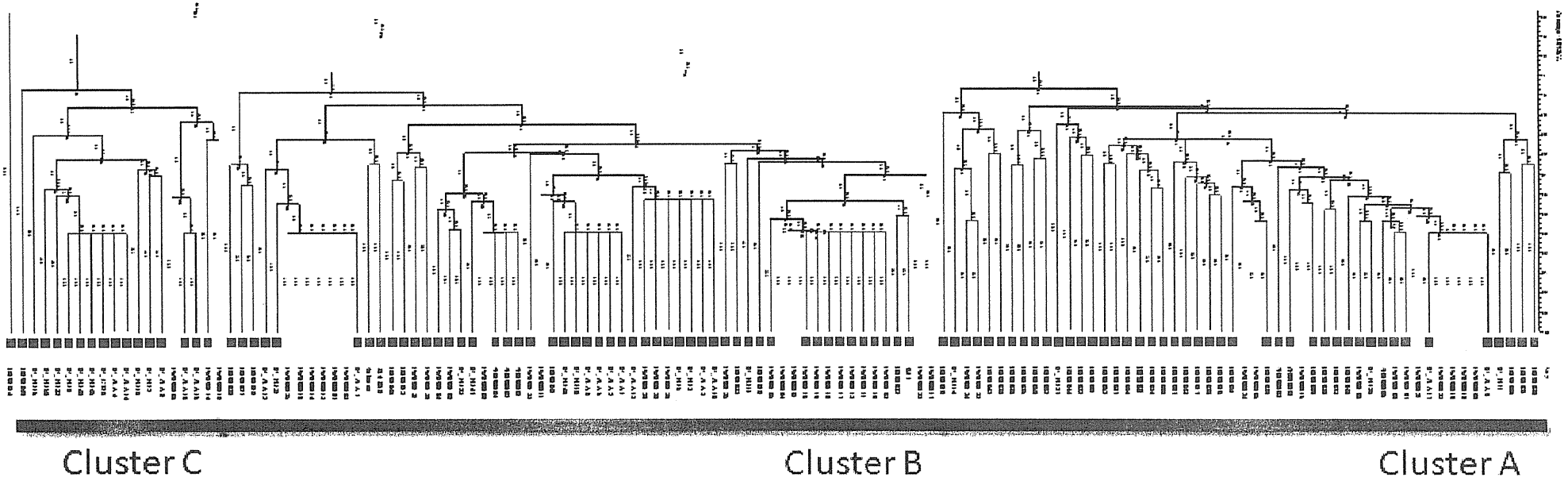


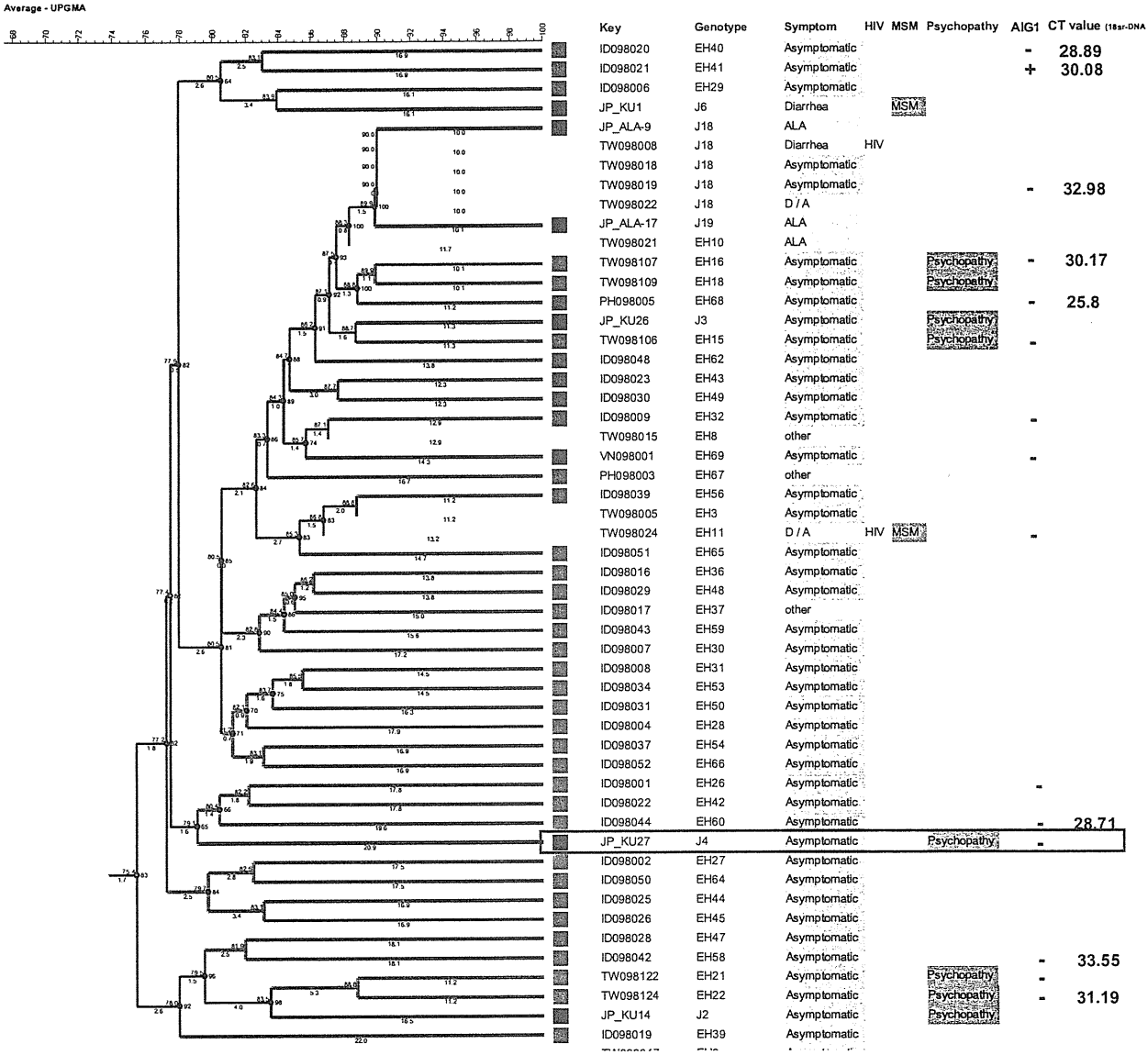

**Figure 3. The phylogenetic tree of Taiwanese, alien worker, and Japanese *E. histolytica* strains based on the six tRNA-linked STR loci by UPGMA method.**

Table 5、Evaluation of the reliability of grouping of Cluster A, B and C by jackknife method though random sampling *E. histolytica* strains from each cluster.

| | A | B | C | X |
|---|---|---|---|---|
| A | 86.5 | 0 | 0 | 0 |
| B | 0 | 98.4 | 5.6 | 0 |
| C | 0 | 1.6 | 94.4 | 0 |
| X | 13.5 | 0 | 0 | 100 |

Score more close to 100% indicates more similar.

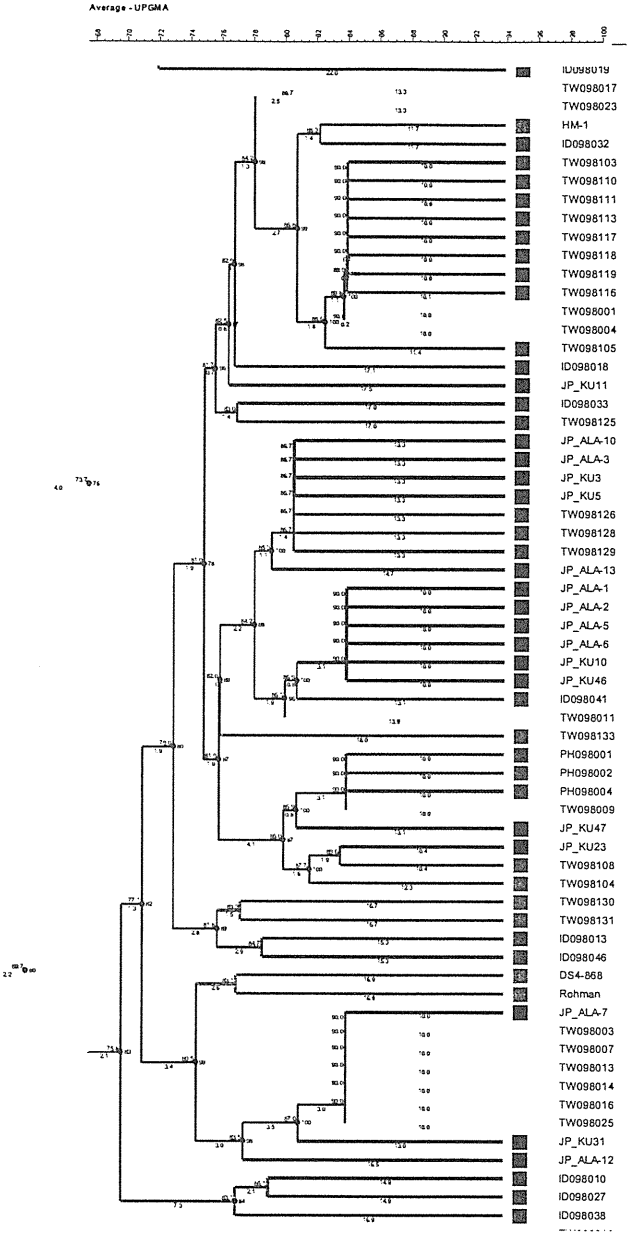
# Cluster A




| Key | Genotype | Symptom | HIV | MSM | Psychopathy | AIG1 | CT value (18sr-DNA real-time PCR) |
|---|---|---|---|---|---|---|---|
| ID098020 | EH40 | Asymptomatic | | | | - | 28.89 |
| ID098021 | EH41 | Asymptomatic | | | | + | 30.08 |
| ID098006 | EH29 | Asymptomatic | | | | | |
| JP_KU1 | J6 | Diarrhea | | MSM | | | |
| JP_ALA-9 | J18 | ALA | | | | | |
| TW098008 | J18 | Diarrhea | HIV | | | | |
| TW098018 | J18 | Asymptomatic | | | | | |
| TW098019 | J18 | Asymptomatic | | | | - | 32.98 |
| TW098022 | J18 | D / A | | | | | |
| JP_ALA-17 | J19 | ALA | | | | | |
| TW098021 | EH10 | ALA | | | | | |
| TW098107 | EH16 | Asymptomatic | | | Psychopathy | - | 30.17 |
| TW098109 | EH18 | Asymptomatic | | | Psychopathy | | |
| PH098005 | EH68 | Asymptomatic | | | | - | 25.8 |
| JP_KU26 | J3 | Asymptomatic | | | Psychopathy | | |
| TW098106 | EH15 | Asymptomatic | | | Psychopathy | - | |
| ID098048 | EH62 | Asymptomatic | | | | | |
| ID098023 | EH43 | Asymptomatic | | | | | |
| ID098030 | EH49 | Asymptomatic | | | | | |
| ID098009 | EH32 | Asymptomatic | | | | - | |
| TW098015 | EH8 | other | | | | | |
| VN098001 | EH69 | Asymptomatic | | | | - | |
| PH098003 | EH67 | other | | | | | |
| ID098039 | EH56 | Asymptomatic | | | | | |
| TW098005 | EH3 | Asymptomatic | | | | | |
| TW098024 | EH11 | D / A | HIV | MSM | | - | |
| ID098051 | EH65 | Asymptomatic | | | | | |
| ID098016 | EH36 | Asymptomatic | | | | | |
| ID098029 | EH48 | Asymptomatic | | | | | |
| ID098017 | EH37 | other | | | | | |
| ID098043 | EH59 | Asymptomatic | | | | | |
| ID098007 | EH30 | Asymptomatic | | | | | |
| ID098008 | EH31 | Asymptomatic | | | | | |
| ID098034 | EH53 | Asymptomatic | | | | | |
| ID098031 | EH50 | Asymptomatic | | | | | |
| ID098004 | EH28 | Asymptomatic | | | | | |
| ID098037 | EH54 | Asymptomatic | | | | | |
| ID098052 | EH66 | Asymptomatic | | | | | |
| ID098001 | EH26 | Asymptomatic | | | | - | |
| ID098022 | EH42 | Asymptomatic | | | | | |
| ID098044 | EH60 | Asymptomatic | | | | - | 28.71 |
| JP_KU27 | J4 | Asymptomatic | | | Psychopathy | - | |
| ID098002 | EH27 | Asymptomatic | | | | | |
| ID098050 | EH64 | Asymptomatic | | | | | |
| ID098025 | EH44 | Asymptomatic | | | | | |
| ID098026 | EH45 | Asymptomatic | | | | | |
| ID098028 | EH47 | Asymptomatic | | | | | |
| ID098042 | EH58 | Asymptomatic | | | | - | 33.55 |
| TW098122 | EH21 | Asymptomatic | | | Psychopathy | - | |
| TW098124 | EH22 | Asymptomatic | | | Psychopathy | - | 31.19 |
| JP_KU14 | J2 | Asymptomatic | | | Psychopathy | | |
| ID098019 | EH39 | Asymptomatic | | | | | |

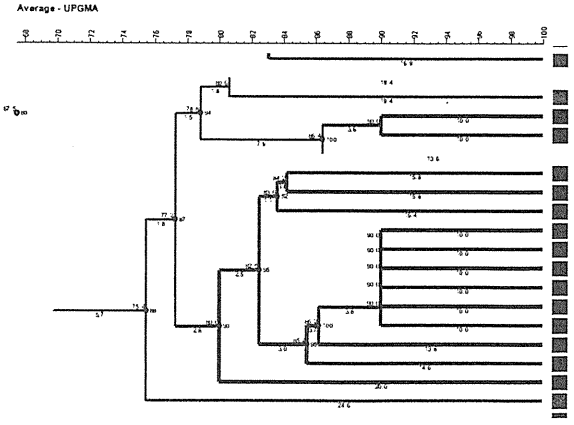
- Indonesian
- Japanese
- Filipino
- Taiwanese-Native
- Taiwanese-Institution
- Vietnamese
- Reference strain

# Cluster B




| Key | Genotype | | | AIG1 | CT value (18sr-DNA real-time PCR) |
|---|---|---|---|---|---|
| ID098019 | EH39 | Asymptomatic | | | |
| TW098017 | EH9 | Asymptomatic | | - | |
| TW098023 | EH9 | Diarrhea | | | |
| HM-1 | EH71 | Diarrhea | | + | |
| ID098032 | EH51 | Asymptomatic | | | |
| TW098103 | EH12 | Asymptomatic | Psychopathy | - | 27.13 |
| TW098110 | EH12 | Asymptomatic | Psychopathy | - | 25.25 |
| TW098111 | EH12 | Asymptomatic | Psychopathy | | |
| TW098113 | EH12 | Asymptomatic | Psychopathy | | |
| TW098117 | EH12 | Asymptomatic | Psychopathy | - | |
| TW098118 | EH12 | Asymptomatic | Psychopathy | | |
| TW098119 | EH12 | Asymptomatic | Psychopathy | | |
| TW098116 | EH20 | Asymptomatic | Psychopathy | | |
| TW098001 | EH1 | Asymptomatic HIV | | | |
| TW098004 | EH2 | Diarrhea | | - | 31.75 |
| TW098105 | EH14 | Asymptomatic | Psychopathy | - | 30.77 |
| ID098018 | EH38 | Asymptomatic | Psychopathy | | |
| JP_KU11 | J21 | ALA | MSM | | |
| ID098033 | EH52 | other | | | |
| TW098125 | EH23 | other | Psychopathy | | |
| JP_ALA-10 | J1 | ALA | | | |
| JP_ALA-3 | J1 | ALA | | | |
| JP_KU3 | J1 | Diarrhea | MSM | | |
| JP_KU5 | J1 | Asymptomatic | MSM | | |
| TW098126 | J1 | Diarrhea | Psychopathy | - | |
| TW098128 | J1 | Asymptomatic | Psychopathy | | |
| TW098129 | J1 | Asymptomatic | Psychopathy | | |
| JP_ALA-13 | J16 | ALA | | - | 28.45 |
| JP_ALA-1 | J8 | ALA | | | |
| JP_ALA-2 | J8 | ALA | | | |
| JP_ALA-5 | J8 | ALA | | | |
| JP_ALA-6 | J8 | ALA | | | |
| JP_KU10 | J8 | Diarrhea | MSM | | |
| JP_KU46 | J8 | Diarrhea | | | |
| ID098041 | EH57 | Asymptomatic | | | |
| TW098011 | EH7 | Asymptomatic HIV | | | |
| TW098133 | EH25 | Asymptomatic | Psychopathy | | |
| PH098001 | EH5 | Asymptomatic | | - | 26.02 |
| PH098002 | EH5 | Asymptomatic | | | |
| PH098004 | EH5 | Asymptomatic | | - | 29.89 |
| TW098009 | EH5 | Diarrhea | | - | 33.16 |
| JP_KU47 | J14 | Diarrhea HIV | | | |
| JP_KU23 | J11 | Diarrhea | MSM | | |
| TW098108 | EH17 | Asymptomatic | Psychopathy | - | |
| TW098104 | EH13 | Asymptomatic | Psychopathy | - | |
| TW098130 | EH24 | Asymptomatic | Psychopathy | - | |
| TW098131 | EH24 | Asymptomatic | Psychopathy | | |
| ID098013 | EH34 | Asymptomatic | | | |
| ID098046 | EH61 | Asymptomatic | | | |
| DS4-868 | EH70 | Diarrhea | | | |
| Rohman | EH72 | Asymptomatic | | | |
| JP_ALA-7 | J20 | ALA | | | |
| TW098003 | J20 | Asymptomatic HIV | | + | 29.57 |
| TW098007 | J20 | Diarrhea | | + | 25.32 |
| TW098013 | J20 | Asymptomatic HIV | | | |
| TW098014 | J20 | Diarrhea HIV | MSM | | |
| TW098016 | J20 | Asymptomatic | | + | 24.15 |
| TW098025 | J20 | ALA HIV | | - | 33.57 |
| JP_KU31 | J5 | Asymptomatic | MSM | | |
| JP_ALA-12 | J22 | ALA | | | |
| ID098010 | EH33 | Asymptomatic | | + | 30.04 |
| ID098027 | EH46 | Asymptomatic | | + | 27.64 |
| ID098038 | EH55 | Asymptomatic | | | |

# Cluster C




| Key | Genotype | | | AIG1 | CT value (18sr-DNA real-time PCR) |
|---|---|---|---|---|---|
| ID098038 | EH55 | Asymptomatic | | | |
| TW098010 | EH6 | Diarrhea | | - | 33.54 |
| TW098114 | EH19 | Asymptomatic | Psychopathy | - | |
| JP_ALA-15 | J23 | ALA | | | |
| JP_ALA-16 | J23 | ALA | | | |
| TW098006 | EH4 | other HIV | | + | 26.62 |
| JP_ALA-8 | J17 | ALA | | | |
| JP_KU2 | J7 | Diarrhea | MSM | | |
| JP_KU16 | J10 | Diarrhea | MSM | | |
| JP_ALA-14 | J13 | ALA | | | |
| JP_ALA-4 | J13 | ALA | | | |
| JP_C726 | J13 | ALA | | | |
| JP_KU45 | J13 | Diarrhea | | | |
| JP_KU48 | J13 | ALA | | | |
| JP_KU8 | J13 | D / A | MSM | | |
| JP_KU32 | J12 | Diarrhea | MSM | | |
| JP_KU50 | J15 | Diarrhea | | | |
| JP_KU15 | J9 | Diarrhea | MSM | | |
| ID098049 | EH63 | other | | + | 22.5 |

**Figure 4. The analysis of AIG1 genes of symptomatic and asymptomatic *E. histolytica* strains from both Taiwanese and alien workers.**

# Drug-resistance mechanism, pathogenesis and genomics of tuberculosis: Pyrazinamide resistance and *pncA* gene mutations in *Mycobacterium tuberculosis*

Mei-Hua Wu, Szu-Peng Wang, Yun-Ling Chang, Ting-Fang Wang, Ruwen Jou

Reference laboratory of Mycobacteriology, Research and Diagnostic Center, Centers for Disease Control

E-mail:

Mei-Hua Wu: meihua@cdc.gov.tw

Szu-Peng Wang, spwang@cdc.gov.tw

Yun-Ling Chang, yunling@cdc.gov.tw

Ting-Fang Wang, tingfang@cdc.gov.tw

Ruwen Jou: rwj@cdc.gov.tw

Corresponding author: Ruwen Jou

Mailing address: 161 Kun-Yang Street, Nan-Kang, Taipei, 115, Taiwan.

E-mail address: rwj@cdc.gov.tw

Key words: Drug resistance, Pyrazinamide, *Mycobacterium tuberculosis*

## Abstract

Pyrazinamide (PZA) is a potent first-line tuberculosis (TB) drug that can shorten the duration of TB treatment. Drug-resistant mechanism of PZA is still not fully understood. The purposes of this collaborative study were to investigate prevalence of PZA resistance and genetic characteristics of the *pncA* gene conferring PZA resistance, and to explore other drug-resistant mechanism. In this study, we analyzed *pncA* gene sequencing results of 442 *Mycobacterium tuberculosis* isolates including 281 (63.6%) multidrug-resistant (MDR) isolates. Concordance between phenotypic and genotypic PZA susceptibility results was 98.5%. Of the 422 *M. tuberculosis* isolates, 30.5% (135/442) isolates had *pncA* gene mutations conferring PZA-resistance. Of the 135 PZA-resistant strains, 29.6% were isolated from MDR-TB cases. The hot-spot regions of mutations were at codon -11 and in codons 200-260. PZA resistance in non-MDR-TB cases was 0.9% (4/161); while in MDR-TB cases were approximately 30%. Besides, PZA-resistant isolate without any mutation in the *pncA* gene identified in this study will be further analyzed focusing on Rv1330. Sequences of other genes of pyrazinoic acid efflux proteins or undefined *pncA* regulatory proteins could be further analyzed.

## Introduction

Pyrazinamide (PZA) is a sterilizing tuberculosis (TB) drug and when added to regimens containing rifampicin, it can kill persisting *Mycobacterium tuberculosis* bacilli during the initial intensive phase of chemotherapy. PZA also helps to shorten the duration of TB treatment. Pervious studies revealed that PZA is a prodrug and requires conversion to its active form, pyrazionic acid (POA), by the bacterial enzyme pyrazimamidase (PZase). Conventional PZA susceptibility testing required to be performed at low pH conditions which inhibit the *in vitro* growth of *M. tuberculosis*. Therefore, PZA susceptibility testing on solid medium is of limited value, and none of clinical laboratories provide such service in Taiwan. Since the liquid culture-based method, such as BACTAC MGIT 960 (Becton Dickinson Biosciences, Sparks, MD) was considered to be a reference method, the National Reference Laboratory at Taiwan Centers for Diseases Control adopted the method to facilitate the management of multidrug-resistant (MDR) TB cases, *M. bovis* infected cases, and on special requests.

PZAase is encoded by the 561-bp gene *pncA*. Mutations in the *pncA* gene results in reduce or loss of the PZAase activities, and thus considered to be the primary drug-resistant mechanism in *M. tuberculosis*. *pncA* gene mutations have been observed in PZA-resistant *M. tuberculosis* and naturally PZA-resistant *M. bovis* isolates. Molecular detection of PZA-resistance related mutations could be used for the early detection of resistant isolates, and ensures the prescription of appropriate regimens in the intensive phase of treatment. Nevertheless, PZA-resistant isolates without *pncA* gene mutations still exist.

In this study, we analyzed the proportion of PZA-resistant among TB and MDR-TB cases and evaluated the role of *pncA* gene mutations as markers for detection of PZA resistance in *M. tuberculosis*. In addition, we identified PZA-resistant isolates without *pncA* gene mutations for further investigation on other resistant mechanisms.

## Materials and Methods

**Study population.** We received *M. tuberculosis* isolates from clinical mycobacteriology laboratories in Taiwan. One isolates was selected from individual TB case. In this study, we analyzed 442 *M. tuberculosis* isolates (Table 1).

**Drug susceptibility testing.** The agar proportion method on either Middlebrook 7H10 or 7H9 (Creative Microbiologicals or Sancordon, Taiwan), and BACTEC$^{TM}$ MGIT$^{TM}$ 960 SIRE Kits (Becton Dickinson Diagnostic Systems, Sparks, MD) with a liquid culture system were used. The critical first-line drug concentrations for the agar proportion method on 7H10 were 0.2 μg/ml and 1.0 μg/ml for INH, 1.0 μg/ml for RMP, 5.0 μg/ml and 10μg/ml for ethambutol (EMB), 2.0 μg/ml, 10μg/ml for streptomycin (SM). Growth on the control medium was compared to growth on the drug-containing medium to determine susceptibility. PZA susceptibility testing was done using MGIT liquid culture containing 100 μg/ml PZA and Wayne test (pyrazinamidase test) on 7H9 broth base containing 100 mg/L PZA, 2 μg/ml sodium pyruvate and 15g/L of agar. The DST results were categorized as resistant or susceptible. The tests were validated by comparison to the susceptibility of *M. tuberculosis* H37Rv included in the same DST. MDR was defined as *M. tuberculosis* isolates resistant to at least INH and RMP.

**DNA sequencing of the *pncA* gene.** The primer set was used to analyze the variation at the *pncA* gene. A 651-bp fragment targeting the *pncA* mutation was amplified and sequenced with the oligonucleotide primers *pncA* -F (5′-GCT GGT CAT GTT CGC GAT CG-3′) and *pncA* -R (5′-CAG GAG CTG CAA ACC AAC TCG-3′) . The PCR reactions were performed as follows: 35 cycles at 94°C for 1 min; annealing at 60°C for 30 sec; and elongation at 72°C for 1 min.. Thereafter, the PCR products were analyzed with an ABI Veriti automated sequencer (Applied Biosystems, USA), and

the sequence data were assembled and edited using the Sequencher 4.7 Demo software.

## Results

### PZA susceptibility testing.

In this study, we analyzed PZA susceptibility and *pncA* gene sequencing results of 442 *M. tuberculosis* isolates including 63.6% (281/442) MDR-TB and 36.4% (161/442) non- MDR-TB cases (Table 1). To verify the use of sequencing as an assay for rapid detection of PZA resistance, we performed 3 methods, MGIT, Wayne and gene sequencing, in parallel using a panel of 96 MDR *M. tuberculosis* isolates. Concordance between phenotypic and genotypic PZA susceptibility testing results was 98.5%.

### *pncA* gene sequencing.

Of the 422 *M. tuberculosis* isolates, 30.5% (135/442) isolates had *pncA* gene mutations conferring PZA-resistance. Of the 135 PZA-resistant strains, 29.6% were isolated from MDR-TB cases and 0.9% (4/161) from non-MDR-TB cases ($P < 0.01$).

Mutation patterns among PZA-resistant isolates were highly diversified. Of the 135 PZA-resistant isolates, we identified 83 patterns with various frequencies (Figure 1). The Predominant mutations were 7.8% (12/153) at codon 226 (A to C), 6.7% (9/153) at codon 214 (T to C) and 4.6% (7/153) at codon -11 (A to G). Overall, hot-spot regions of mutations were at codon -11 and in codons 200-260 (Figure 1).

Besides, PZA-resistant isolates without any mutation in the *pncA* gene identified in this study will be sent to the Department of bacteriology laboratory II at the NIID, Japan for further genetic analysis focused on Rv1330.

## Discussion

In the first year of this collaborative project, we used *pncA* gene sequencing to evaluate the feasibility of mutations as markers for detecting PZA resistance, and identify PZA-resistant isolates without *pncA* mutations for further studies at NIID.

We found high concordance of 98.5% between conventional drug susceptibility testing and genotypic method compared to that of other studies (72-97%). We confirmed that sequencing of the *pncA* gene can provide consistent results as compared to other conventional PZA susceptibility tests.

In this study, PZA resistance in new TB cases was 0.9%; while in MDR-TB cases was approximately 30%. MDR–TB cases had acquired resistant resulting from either pervious treatment or direct infection of PZA-resistant isolates. For better management and treatment of MDR-TB cases, the TB program needs to have more subtle control strategies to prevent acquired PZA-resistance. Nevertheless, efflux pump mechanism might also contribute to high proportion of PZA resistance among MDR-TB cases. Further studies are suggested.

In this study, highly diversified mutation patterns were observed in the *pncA* gene conferring PZA resistance. We could take advantage of this characteristic as a measure for preliminary screening of a probable TB cluster. Since no significant or high-frequency hot-spot regions of the *pncA* mutations was identified, whole *pncA* gene analysis was recommended for designing a rapid molecular PZA susceptibility testing. In addition, based on this finding, a diagnostic such as microarray assay could be developed for rapid detection of PZA resistance.

Even through PZA-resistant isolates without any mutation in the *pncA* gene identified in this study were sent to NIID for further genetic analysis focused on Rv1330, no mutation was found. Sequences of other genes of POA efflux proteins or undefined *pncA* regulatory proteins could be further analyzed.

## Acknowledgements

This work was supported by a grant (DOH100-DC-2019) from Centers for Diseases Control, Taiwan and a grant-in-aid of Ministry of Health, Labor and Welfare, the Government of Japan (H23-Shinkou- shitei-020).

## References

1. Ando H., Mitarai S., Kondo Y.et al., Pyrazinamide resistance in multidrug-resistant *Mycobacterium tuberculosis* isolates in Japan, Euro Soc of Clin Microbio and Infect Dis, 2009, 16, 1155–1171

2. Nizova A. V., Stepanshina V. N., Maiskaya N. V. et al., Determination of drug resistance of the clinical strains of *Mycobacterium tuberculosis* to pyrazinamide. Mol Genet, Microbio and Virol., 23(4), 184-188.

3. Zhang Y, Mitchison D, The curious characteristics of pyrazinamide: a review. Int. J. Tuberc. Lung Dis., 2003, 7 (1): 6–21.

4. Scorpio A, Zhang Y, Mutations in *pncA*, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nature Medicine, 1996, 2 (6): 662–7.

5. Niuris C. Mirabal, Sergio L. Yzquierdo, Dihadenys Lemus, et al., Evaluation of colorimetric methods using nicotinamide for rapid detection of pyrazinamide resistance in *Mycobacterium tuberculosis*, J of Clin Microbio, 2010, 2729–2733.

6. Muthaiah M, Jagadeesan S, Ayalusamy N, et al., Molecular Epidemiological Study of Pyrazinamide-Resistance in Clinical Isolates of *Mycobacterium tuberculosis* from South India. Int J Mol Sci. 2010, 11(7):2670-80.

7. Dormandy J, Somoskovi A, Kreiswirth B N, et al. Discrepant results between pyrazinamide susceptibility testing by the reference BACTEC 460TB method and pncA DNA sequencing in patients infected with multidrug-resistant W-Beijing *Mycobacterium tuberculosis* strains. Chest. 2007, 131(2):497-501.

8. Jonmalung J, Prammananan T, Leechawengwongs M, Chaiprasert A., Surveillance of pyrazinamide susceptibility among multidrug-resistant *Mycobacterium tuberculosis* isolates from Siriraj Hospital, Thailand. BMC Microbiol. 2010, 20;10:223.

9. Sreevatsan S, Pan X, Zhang Y, et al., Mutations associated with pyrazinamide resistance in *pncA* of *Mycobacterium tuberculosis* complex organisms. Antimicrob Agents Chemother. 1997, 41(3):636-40.

10. Pfyffer GE, Palicova F, Rüsch-Gerdes S., Testing of susceptibility of *Mycobacterium tuberculosis* to pyrazinamide with the nonradiometric BACTEC MGIT 960 system. J Clin Microbiol. 2002, 40(5):1670-4.

11. Martin A, Takiff H, Vandamme P, et al. A new rapid and simple colorimetric method to detect pyrazinamide resistance in *Mycobacterium tuberculosis* using nicotinamide. J Antimicrob Chemother. 2006 58(2):327-31.

12. Shenai S, Rodrigues C, Sadani M, et al., Comparison of phenotypic and genotypic methods for pyrazinamide susceptibility testing. Indian J Tuberc. 2009, 56(2):82-90.

13. Chedore P, Bertucci L, Wolfe J, et al., Potential for false resistance to pyrazinamide when using the BACTEC MGIT 960 System for susceptibility testing of *Mycobacterium tuberculosis.* J. Clin. Microbiol. doi:10.1128/JCM.01775-09.

## Publication list for this work

NIL

**Tables and Figures**

Figure 1 Major mutations in the *pncA* gene and their frequencies

(a)

| Mutation Codon | Number of Isolates |
|---|---|
| 226 | 12 |
| -11 | 9 |
| 214 | 9 |
| 452 | 5 |
| 23 | 4 |
| 260 | 4 |
| 394 | 4 |
| 419 | 4 |
| 309 | 3 |
| 39 | 2 |
| 40 | 2 |
| 137 | 2 |
| 160 | 2 |
| 202 | 2 |
| 204 | 2 |
| 211 | 2 |
| 225 | 2 |
| 233 | 2 |
| 261 | 2 |
| 290 | 2 |
| 304 | 2 |
| 308 | 2 |
| 310 | 2 |
| 323 | 2 |
| 410 | 2 |
| 416 | 2 |
| 421 | 2 |
| 451 | 2 |
| 469 | 2 |

(b)

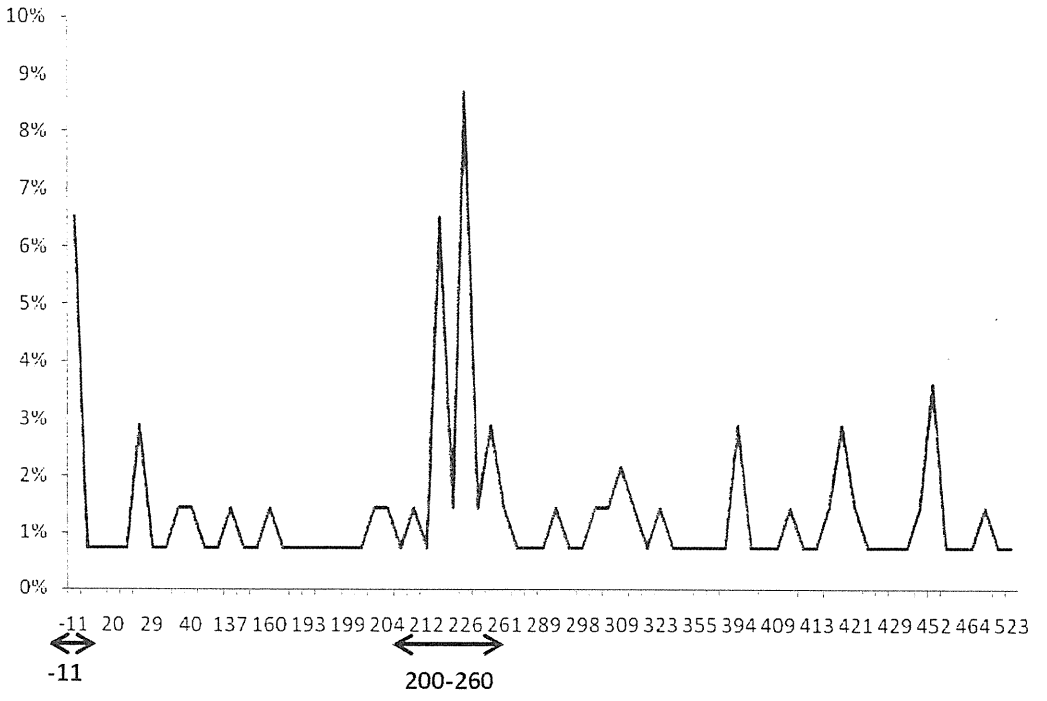

10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
-11 20 29 40 137 160 193 199 204 212 226 261 289 298 309 323 355 394 409 413 421 429 452 464 523
-11
200-260

Table 1 Distribution of PZA resistance in 442 tuberculosis cases

| | *pncA* | | Number of cases |
|---|---|---|---|
| | Mutation | Wild-type | |
| MDR TB | 131 (29.6%) | 150 (33.9%) | 281 |
| Non-MDR TB | 4 (0.90%) | 158 (35.7%) | 161 |
| Total | 135 (30.5%) | 308 (69.5%) | 442 |

# Molecular studies on virulence and drug resistance of leprosy:

# Laboratory diagnosis of leprosy

Wei-Lin Huang, Rai-Che Chien, Ruwen Jou

Reference laboratory of Mycobacteriology, Research and Diagnostic Center, Centers for Disease Control

E-mail:

Wei-Lun Huang: hwl@cdc.gov.tw

Rai-Che Chien : josh.022@yahoo.com.tw

Ruwen Jou: rwj@cdc.gov.tw

Corresponding author: Ruwen Jou

Mailing address: 161 Kun-Yang Street, Nan-Kang, Taipei, 115, Taiwan.

E-mail address: rwj@cdc.gov.tw

Key words: Identification, Drug resistance, Leprosy