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Research paper

pncA gene mutations in reporting pyrazinamide resistance among the MDR-TB suspects

Xiaoyuan Wu[a], Wei Lu[b], Yan Shao[b], Honghuan Song[b], Guoli Li[b], Yan Li[b], Limei Zhu[b], Cheng Chen[b,c,*]

[a] Central Laboratory, Nanjing Chest Hospital, Medicine School of Southeast University, Nanjing, Jiangsu, China
[b] Department of Chronic Communicable Disease, Center for Disease Control and Prevention of Jiangsu Province, Nanjing, China
[c] Key Laboratory of Public Health Safety (Fudan University), Ministry of Education, Shanghai, China

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ABSTRACT

Mutations in *pncA* gene contributing to PZA resistance was not clearly elucidated in China. To reveal the correlated mutations of *pncA* gene on pyrazinamide (PZA) resistance. 148 *Mycobacterium tuberculosis* clinical isolates were included from multi-drug resistant tuberculosis suspects. The MGIT 960 test and microscopic observation drug susceptibility (MODS) assay were adopted for PZA phenotype drug susceptibility test. 120 isolates with consistent MGIT 960 and MODS results were selected for *pncA* gene sequencing. 68 samples (56.7%) were resistant to PZA while leaving 52 PZA susceptible samples. Out of the 68 PZA resistant isolates, 49 (72.1%) harbored mutations of *pncA*, and 4 (7.7%) of the 52 PZA susceptible samples harbored mutations of *pncA* as well. Compared to the phenotype drug resistant pattern of PZA, the mutations of *pncA* gene reached a sensitivity of 0.72 to report PZA resistance and a specificity of 0.92 to predict PZA susceptibility. Those mutations, Gln10Pro, Asp12Ala, Tyr41Stop, Gly97Asp, Val128Gly and FSC131(ins) exceeding 5% of the total PZA resistant isolates of each, might be helpful but not adequate in PZA molecular susceptibility test design and development.

1. Introduction

It was reported that the rate of PZA resistance ranges from 3 to 42.1%, and PZA resistance rate was as high as 81.3% among rifampicin resistant strains (Zignol et al., 2016). In China, PZA resistant rate was as high as 43.5% among multi-drug resistant (MDR) tuberculosis (TB) isolates (Xu et al., 2016), which is a great challenge for the successful MDR-TB treatment, as PZA was commonly considered as an effective component for the MDR-TB treatment regimen (WHO, 2016).

Currently, the phenotypic assay was the only recommended method for PZA resistance detection on clinical isolates, such as the MGIT 960 test (Aono et al., 2002). Meanwhile, other phenotypic method, such as MODS was also useful in PZA resistance detection (Wu et al., 2015). As we know, PZA will be effective in killing *M. tuberculosis* under the acid condition. However, over inoculation will induce the increasing level of PH, and finally results in a false positive result of PZA susceptibility test (Zhang et al., 2002). Thus, developing a reliable and proficiency-undepend method is necessary for PZA susceptibility test.

pncA gene of *M. tuberculosis* was considered as the main factor controlling the bacillus susceptibility to pyrazinamide (Unissa et al., 2010), and mutations of *pncA* gene were highly contributed in the mechanism of PZA resistance (Chang et al., 2011). Until now, dozens of mutations for *pncA* gene were reported in Europe (Miotto et al., 2014), but the mutations of *pncA* gene contributing to PZA resistance was not clearly defined in China. More importantly, not all the mutations of *pncA* gene conferring a resistance to PZA. PZA resistance was reported in association with MDR-TB (Pierre-Audigier et al., 2012), so revealing the mutations of *pncA* gene associated with PZA resistance among the MDR-TB is firstly urgent in China.

2. Methods

2.1. Sample collection

Sputum samples of MDR-TB suspects with chemotherapy treatment history were collected from the inpatient of Nanjing Chest Hospital from Dec 2013 to April 2014, and the clinical isolates belonging to Mycobacterium TB complex (MTBC) were identified by the p-nitrobenzoic acid method (Giampaglia et al., 2007). Finally, a total of 148 MTBC clinical isolates from MDR-TB suspects were included. This study

* Corresponding author at: Department of Chronic Communicable Disease, Center for Disease Control and Prevention of Jiangsu Province, 172 Jiangsu Rd., Nanjing, China.
E-mail address: chencheng128@gmail.com (C. Chen).

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was approved by the ethics committee of Jiangsu provincial center for disease control and prevention, and informed consent was obtained from all subjects. All methods were carried out in accordance with relevant guidelines and regulations, and all experimental protocols were approved by the ethics committee of Jiangsu provincial center for disease control and prevention.

2.2. Microscopic Observation Drug Susceptibility (MODS) assay and MGIT 960 test

The procedure of clinical strain isolation and manipulation of MODS assay can be referred to the previous study (Wu et al., 2015). Six wells containing pyrazinamide concentrations of 400, 200, 100, 50, 25 and 12.5 mg/L were prepared, and the critical concentration of 100 mg/L was used for PZA resistance detection. The MGIT 960 method for pyrazinamide susceptibility test was performed as previously described (Cui et al., 2013), and the final concentration of pyrazinamide was 100 mg/L for testing.

2.3. Strain DNA extraction and pncA gene sequencing

The new growth strain on Löwenstein-Jensen (LJ) media was scraped in a tube with TE buffer for DNA extraction. Each tube containing cultures was boiled in the 95 °C metal bath for 30 min and then incubated on ice for 5 min. After centrifugation at 12000 rpm for 5 min, the supernatant was kept for PCR amplification. The *pncA* gene of *M. tuberculosis* was amplified and sequenced for potential mutations compared with the sequence of *M. tuberculosis* H37Rv *pncA* gene (Rv2043c, Accession Number: NC_000962.3). The primers used for *pncA* gene amplification were as the follows: forward (5′-CTG GTC ATG TTC GCG ATC G-3′), and reverse (5′-CCA ACA GTT CAT CCC GGT TC-3′). The PCR product was 694 bp length, including a 103 bp upstream sequence and 30 bp downstream sequence of the *pncA* gene. The Applied Biosystems 3730xl DNA Analyzer was used for the sequencing.

2.4. Statistical analysis

pncA gene sequencing was compared against a composite reference standard based on both phenotypic methods MODS and MGIT 960. The sensitivity and specificity were calculated to show the accuracy of *pncA* gene mutations in predicting PZA resistance. Meanwhile, mutations of *pncA* genes were classified into two categories: (1) Category A for those mutations occurred in no less than 5% of the mutant PZA resistant samples; (2) Category B for those mutations occurred in less than 5% of the mutant PZA resistant samples. Analyses were performed by SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA). All the data are available for this manuscript.

3. Results

All the 148 clinical isolates were selected from the MDR-TB suspects. Finally, one-hundred and twenty-seven samples with validated results by both MODS and MGIT were included. The MGIT 960 and MODS results showed that 3 isolates were MGIT 960 resistant but MODS sensitive, and 4 isolates were MGIT 960 sensitive but MODS resistant. Those seven isolates with inconsistent phenotypic results were excluded. Thus, one-hundred and twenty isolates with consistent MGIT 960 and MODS results were chosen for *pncA* gene sequencing, and the sequence of H37Rv strain was adopted as the reference. As shown in Table 1, according to the phenotypic methods, sixty-eight samples (56.7%) were resistant to PZA while leaving 52 samples susceptible to PZA. Out of the 68 PZA resistance samples, 49 (72.1%) harbored mutations of *pncA*. Compared to the phenotype drug resistant pattern of PZA, the mutations of *pncA* gene reached a sensitivity of 0.72 to report PZA resistance and a specificity of 0.92 to predict PZA susceptibility.

All the mutations of the 53 strains were listed in Table 2. According to the mutation frequency among all the 49 PZA resistant strains, harboring mutations, we classified those mutation frequencies no less than 5% as category A mutation and those less than 5% as category B mutation. As shown in Table 2, a total of forty-six mutations were discovered in the 53 stains. Meanwhile, six mutations were belonging to the category A mutation: Gln10Pro, Asp12Ala, Tyr41Stop, Gly97Asp, Val128Gly, FSC131(ins), and leaving the rest 37 mutations as category B mutation.

According to the results of Miotto et al. (Miotto et al., 2014), twenty-one mutations were simultaneously found in our study, which were belonging to the very high confidence (19 mutations), high confidence (one mutation) and unclear in conferring PZA resistance (one mutation) according to Miotto's classification. Meanwhile, the rest twenty-five mutations, including one category A mutation Tyr41Stop, were not reported in Miotto's study. However, seven of the 25 mutations were found inducing distinct amino acid change compared to Miotto's study: Ile6Ser, Ile6Ile, Val7Val, Phe13Phe, Phe58Phe, Phe81Val, Val139Met, and one different nucleotide change (code 136 by insertion of T base) induced sequence frameshift.

Among the 52 PZA susceptible stains, four stains harbored *pncA* gene mutations. The corresponding mutations were as follows: three category B mutations (Leu4Trp, Ile6Ser, FSC174(ins)), and one category A mutation (FSC131 (ins)). The numbers of mutation for *pncA* gene were illustrated in Fig. 1. The four PZA susceptible strains harboring PZA mutations were conducted phenotypic drug susceptibility for confirmation. For mutation *pncA* Leu4Trp, which was found in a PZA susceptible strain in our study, was discovered in PZA resistant strains by Miotto's study. However, the same codon mutation Leu4Ser was found as a resistant mutation in both studies. These results indicated that mutation Leu4Trp might play an ambiguous role in PZA resistance.

4. Discussion

In this study, we found 92.5% *pncA* gene mutant clinical isolates were found resistant to PZA, and all the mutations reached a sensitivity of 0.72 and a specificity of 0.92 in reporting PZA resistance and susceptibility. Meanwhile, nearly 30% of the PZA resistance isolates contained a wildtype *pncA* gene, indicating other genes or mechanisms might be responsible for PZA resistance. However, the positive predictive value of those mutations reached above 90%, which seemed helpful in determining PZA resistance. Meanwhile, Five of the six mutations exceeding 5% of the overall PZA resistant isolates harboring mutations respectively, which were previously revealed by Miotto et al. as “very high confidence”. (Gln10Pro, Asp12Ala, Gly97Asp, Val128Gly, FSC131(ins)) (Miotto et al., 2014), were indispensable in molecular drug susceptible test design. Even though mutation Tyr41Stop was not reported by Miotto's study, it may have potential in PZA molecular test design reported by a previous study in China as well (Hou et al., 2000).

Sequencing was in advantage of detecting PZA resistance based on *pncA* gene mutations, and the next generation sequencing (NGS) method was reported having increased sensitivity in detecting *pncA* gene mutations regardless of low-level *pncA* heteroresistance when compared to the Sanger sequencing (Maningi et al., 2015; Operario et al., 2017). However, the prior limitation for sequencing method in PZA resistance detection was unfeasibility in the routine settings. Thus, a PCR-based method seems quite practical in the peripheral care providers.

Currently, several studies had reported a PCR-based method for detecting PZA resistance. Such as the high-resolution melt analysis and Taqman-Integrated microfluidic card (IMC) (Pholwat et al., 2015; Ramirez et al., 2010). However, there is no desirable accuracy in detecting PZA resistance for those methods, and the main reason could be the larger amount of scattered mutations located in *pncA* gene (Sandgren et al., 2009). Just as Miotto et al. revealed (Miotto et al., 2014), and not all the mutations convey a resistance of PZA, which further increased the difficulty in designing and optimizing the PCR-

Table 1
2*2 table to show the distribution of *pncA* gene mutations among the PZA^S and PZA^R clinical isolates.

pncA gene	No. of isolates		Sensitivity	Specificity	PPV	NPV
	PZA^R (n = 68)	PZA^S (n = 52)				
Mutant	49	4	0.72 (0.60–0.82)	0.92 (0.81–0.98)	0.92(0.81–0.98)	0.72(0.59–0.82)
Wild type	19	48				

PPV: positive predictive value; NPV: negative predictive value; PZA^S: PZA susceptible; PZA^R: PZA resistance.

based molecular susceptible test. Thus, identifying the hot-spot area of *pncA* gene is quite necessary for the molecular method design and development.

In our study, six mutations ranked as category A mutation may have the potential in predicting PZA resistance. However, the prediction ability of PZA resistance relied not only on the high frequency of mutations, but also on the mutant effect on *pncA* gene expression. Several previous studies had engaged in clarifying the functions on *pncA* mutations. Yoon et al. had reported that *pncA* residues (Gly97, Thr135) might be important for *pncA* activity and/or proper protein folding (Yoon et al., 2014). Another study conducted by Vats et al. demonstrated the mechanism of PZA resistance developed due to K96R mutation occurring in the *pncA* catalytic region (Vats et al., 2015). Nevertheless, compared to the large number of mutations of *pncA* gene, the functional studies establishing PZA resistance for those *pncA* gene mutations were limited.

Our study found four mutations induced synonymous amino acid change, which were not found in Miotto's study. Further inspection of

Table 2
Mutations of *pncA* gene for the 53 mutant strains (including 49 PZA-R strains and 4 PZA-S).

Nucleotide change	Nucleotide position in *pncA* gene	Results of mutation	Amino acid change	Mutations in PZA^R (%)n = 49	Mutations in PZA^S (%)n = 4	Mutation category	Miotto et al., (2014)
T > C	2	ATG1ACG	Met1Thr	2.04%		B	Very high confidence
T > C	11(1)	TTG4TCG	Leu4Ser	2.04%		B	Very high confidence
T > G	11(2)	TTG4TGG	Leu4Trp	/	25.00%	/	Very high confidence
T > G	17	ATC6AGC	Ile6Ser	/	25.00%	/	Not reported
C > T	18	ATC6ATT	Ile6Ile	2.04%		B	Not reported
C > T	21	GTC7GTT	Val7Val	2.04%		B	Not reported
A > C	29	CAG10CCG	Gln10Pro	6.12%		A	Very high confidence
A > C	35	GAC12GCC	Asp12Ala	8.16%		A	Very high confidence
Insertion37 GA	37	FSC12 (ins)		2.04%		B	Not reported
C > T	39	TTC13TTT	Phe13Phe	2.04%		B	Not reported
Deletion C	51	FSC17 (del)		2.04%		B	Not reported
Insertion C	52	FSC17 (ins)		4.08%		B	Not reported
C > G	123	TAC41TAG	Tyr41Stop	6.12%		A	Not reported
Deletion ACCCGG	158–163	FSC53–55		2.04%		B	Not reported
C > G	161	CCG54CGG	Pro54Arg	4.08%		B	Very high confidence
C > T	174	TTC58TTT	Phe58Phe	4.08%		B	Not reported
T > C	175	TCC59CCC	Ser59Pro	2.04%		B	Not reported
A > C	188	GAC63GCC	Asp63Ala	2.04%		B	Unclear in conferring PZA^R
T > C	199	TCG67CCG	Ser67Pro	2.04%		B	Very high confidence
G > T	203	TGG68TTG	Trp68Leu	2.04%		B	Very high confidence (Multi)
Insertion G	221	FSC74(ins)		2.04%		B	Not reported
T > G	241	TTC81GTC	Phe81Val	2.04%		B	Not reported
G > A	290	GGT97GAT	Gly97Asp	6.12%		A	Very high confidence
A > C	298	ACC100CCC	Thr100Pro	2.04%		B	Very high confidence
A > G	329	GAC110GGC	Asp110Gly	2.04%		B	Not reported
T > G	383	GTC128GGC	Val128Gly	8.16%		A	Very high confidence
G > T	391	GTC131TTC	Val131Phe	2.04%		B	Not reported
Insertion GG	392	FSC131(ins)		6.12%	25.00%	A	Very high confidence
G > C	395	GGT132GCT	Gly132Ala	2.04%		B	Very high confidence
T > C	398	ATT133ACT	Ile133Thr	2.04%		B	Very high confidence
A > C	403	ACC135CCC	Thr135Pro	2.04%		B	Very high confidence
Insertion T	408	FSC136(ins)		4.08%		B	Not reported
G > A	415	GTG139ATG	Val139Met	2.04%		B	Not reported
A > C	422	CAG141CCG	Gln141Pro	2.04%		B	Very high confidence
Deletion (C)	437	FSC146(del)		2.04%		B	Not reported
Deletion (C)	456	FSC152(del)		2.04%		B	Not reported
T > G	476	CTG159CGG	Leu159Arg	2.04%		B	Very high confidence
Deletion (G)	489	FSC163(del)		4.08%		B	Not reported
Insertion C	522	FSC174(ins)		/	25.00%	/	Not reported
G > A	525	ATG175ATA	Met175Ile	2.04%		B	high confidence
A > C	535(1)	AGC179CGC	Ser179Arg	2.04%		B	Not reported
A > G	535(2)	AGC179GGC	Ser179Gly	2.04%		B	Not reported
T > C	539(1)	GTC180GCC	Val180Ala	2.04%		B	Very high confidence
T > G	539(2)	GTC180GGC	Val180Gly	2.04%		B	Very high confidence
G > A	541	GAG181AAG	Glu181Lys	2.04%		B	Not reported
G > T	554	AGC185ATC	Ser185Ile	2.04%		B	Not reported

PZA^S: PZA susceptible; PZA^R: PZA resistance.

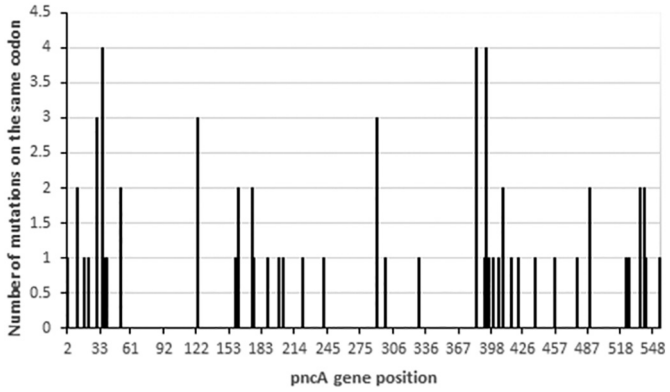

Fig. 1. The frequency of the *pncA* gene mutations for the 53 strains.

the 4 mutations revealed that they were located on the same isolate (strain ID: PZA41). Meanwhile, mutation Glu181Lys of the same strain induced nonsynonymous amino acid change, which might be responsible for the resistance to PZA.

In this study, nearly 30% of the PZA resistance isolates were not found in association with *pncA* gene mutation, which implied that other genes or mechanisms might be responsible for PZA resistance. Previous studies had indicated that panD (Werngren et al., 2017; Zhang et al., 2013) and rspA (Simons et al., 2013; Tan et al., 2014) genes were involved in the mechanism of PZA resistance.

Several limitations of this study should be mentioned. First, the inclusion of a relatively small sample size of MDR-TB suspects might not be adequate to reveal those low-frequency mutations, and those mutations found in our study should be tested in the general TB population. Second, the relationship between those enrolled samples was not evaluated by clustering rate, which might not exclude the possibility of recent transmission, but the consecutive inclusion of the MDR-TB suspects would be valuable to demonstrate the current situation of PZA resistance patterns. Third, the Sanger sequencing method used in this study might underestimate the low-level *pncA* resistance.

In conclusion, this study adopted both MGIT 960 and MODS to confirm PZA susceptibility for each stain, which ensured a high accuracy of the phenotypic assay for PZA resistance detection. And five of the six category A mutations, which were previously revealed by Miotto et al. as well (Miotto et al., 2014), might be useful in molecular test design. However, the “hot spot” mentioned is limited and is likely to cover less than 20% of resistant cases in the population considered. Nevertheless, our study indicated that 54.3% (25/46) of the mutations were not reported by Miotto et al., which indicated a different *pncA* gene mutation patterns existed across regions.

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Conflict of interest

All the authors declared no conflict of interest.

Ethical approval

This study was approved by the ethics committee of Jiangsu Provincial Center for Disease Control and Prevention.

Informed consent

The informed consent was obtained from all the enrolled participants.

References

Aono, A., Hirano, K., Hamasaki, S., Abe, C., 2002. Evaluation of BACTEC MGIT 960 PZA medium for susceptibility testing of Mycobacterium tuberculosis to pyrazinamide (PZA): compared with the results of pyrazinamidase assay and Kyokuto PZA test. Diagn. Microbiol. Infect. Dis. 44, 347–352.
Chang, K.C., Yew, W.W., Zhang, Y., 2011. Pyrazinamide susceptibility testing in mycobacterium tuberculosis: a systematic review with meta-analyses. Antimicrob. Agents Chemother. 55, 4499–4505.
Cui, Z.L., Wang, J., Lu, J.M., Huang, X.C., Zheng, R.J., Hu, Z.Y., 2013. Evaluation of methods for testing the susceptibility of clinical mycobacterium tuberculosis isolates to pyrazinamide. J. Clin. Microbiol. 51, 1374–1380.
Giampaglia, C.M., Martins, M.C., Chimara, E., Oliveira, R.S., de Oliveira Vieira, G.B., Marsico, A.G., Mello, F.C., de Souza Fonseca, L., Kritski, A., da Silva Telles, M.A., 2007. Differentiation of Mycobacterium tuberculosis from other mycobacteria with rho-nitrobenzoic acid using MGIT960. Int. J. Tuberc. Lung Dis. 11, 803–807.
Hou, L., Osei-Hyiaman, D., Zhang, Z., Wang, B., Yang, A., Kano, K., 2000. Molecular characterization of pncA gene mutations in Mycobacterium tuberculosis clinical isolates from China. Epidemiol. Infect. 124, 227–232.
Maningi, N.E., Daum, L.T., Rodriguez, J.D., Mphahlele, M., Peters, R.P., Fischer, G.W., Chambers, J.P., Fourie, P.B., 2015. Improved detection by next-generation sequencing of pyrazinamide resistance in mycobacterium tuberculosis isolates. J. Clin. Microbiol. 53, 3779–3783.
Miotto, P., Cabibbe, A.M., Feuerriegel, S., Casali, N., Drobniewski, F., Rodionova, Y., Bakonyte, D., Stakenas, P., Pimkina, E., Augustynowicz-Kopec, E., Degano, M., Ambrosi, A., Hoffner, S., Mansjo, M., Werngren, J., Rusch-Gerdes, S., Niemann, S., Cirillo, D.M., 2014. Mycobacterium tuberculosis pyrazinamide resistance determinants: a multicenter study. MBio 5.
Operario, D.J., Koeppel, A.F., Turner, S.D., Bao, Y., Pholwat, S., Banu, S., Foongladda, S., Mpagama, S., Gratz, J., Ogarkov, O., Zhadova, S., Heysell, S.K., Houpt, E.R., 2017. Prevalence and extent of heteroresistance by next generation sequencing of multidrug-resistant tuberculosis. PLoS One 12, e0176522.
Pholwat, S., Liu, J., Stroup, S., Gratz, J., Banu, S., Rahman, S.M., Ferdous, S.S., Foongladda, S., Boonlert, D., Ogarkov, O., Zhdanova, S., Kibiki, G., Heysell, S., Houpt, E., 2015. Integrated microfluidic card with TaqMan probes and high-resolution melt analysis to detect tuberculosis drug resistance mutations across 10 genes. MBio 6, e02273.
Pierre-Audigier, C., Surcouf, C., Cadet-Daniel, V., Namouchi, A., Heng, S., Murray, A., Guillard, B., Gicquel, B., 2012. Fluoroquinolone and pyrazinamide resistance in multidrug-resistant tuberculosis. Int. J. Tuberc. Lung Dis. 16, 221–223.
Ramirez, M.V., Cowart, K.C., Campbell, P.J., Morlock, G.P., Sikes, D., Winchell, J.M., Posey, J.E., 2010. Rapid detection of multidrug-resistant Mycobacterium tuberculosis by use of real-time PCR and high-resolution melt analysis. J. Clin. Microbiol. 48, 4003–4009.
Sandgren, A., Strong, M., Muthukrishnan, P., Weiner, B.K., Church, G.M., Murray, M.B., 2009. Tuberculosis drug resistance mutation database. PLoS Med. 6, e2.
Simons, S.O., Mulder, A., van Ingen, J., Boeree, M.J., van Soolingen, D., 2013. Role of rpsA gene sequencing in diagnosis of pyrazinamide resistance. J. Clin. Microbiol. 51, 382.
Tan, Y., Hu, Z., Zhang, T., Cai, X., Kuang, H., Liu, Y., Chen, J., Yang, F., Zhang, K., Tan, S., Zhao, Y., 2014. Role of pncA and rpsA gene sequencing in detection of pyrazinamide resistance in mycobacterium tuberculosis isolates from Southern China. J. Clin. Microbiol. 52, 291–297.
Unissa, A.N., Selvakumar, N., Hassan, S., 2010. Insight to pyrazinamide resistance in Mycobacterium tuberculosis by molecular docking. Bioinformation 4, 24–29.
Vats, C., Dhanjal, J., Goyal, S., Gupta, A., Bharadvaja, N., Grover, A., 2015. Mechanistic analysis elucidating the relationship between Lys96 mutation in Mycobacterium tuberculosis pyrazinamidase enzyme and pyrazinamide susceptibility. BMC Genomics 16 (Suppl. 2), S14.
Werngren, J., Alm, E., Mansjo, M., 2017. Non-pncA Gene-mutated but pyrazinamide-resistant mycobacterium tuberculosis: why is that? J. Clin. Microbiol. 55, 1920–1927.
WHO, 2016. WHO Treatment Guidelines for Drug-Resistant Tuberculosis.
Wu, X., Lu, W., Shao, Y., Liu, Q., Shi, X., Wang, X., Li, C., Zhu, L., Chen, C., 2015. The indirect microscopic observation drug susceptibility assay demonstrated high concordance with the indirect MGIT method for pyrazinamide susceptibility testing. J. Antimicrob. Chemother. 70, 2295–2299.
Xu, P., Wu, J., Yang, C., Luo, T., Shen, X., Zhang, Y., Nsofor, C.A., Zhu, G., Gicquel, B., Gao, Q., 2016. Prevalence and transmission of pyrazinamide resistant Mycobacterium tuberculosis in China. Tuberculosis (Edinb) 98, 56–61.
Yoon, J.-H., Nam, J.-S., Kim, K.-J., Ro, Y.-T., 2014. Characterization of pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis isolates from Korea and analysis of the correlation between the mutations and pyrazinamidase activity. World J. Microbiol. Biotechnol. 30, 2821–2828.
Zhang, Y., Permar, S., Sun, Z., 2002. Conditions that may affect the results of susceptibility testing of Mycobacterium tuberculosis to pyrazinamide. J. Med. Microbiol. 51, 42–49.
Zhang, S., Chen, J., Shi, W., Liu, W., Zhang, W., Zhang, Y., 2013. Mutations in panD encoding aspartate decarboxylase are associated with pyrazinamide resistance in Mycobacterium tuberculosis. Emerg. Microbes Infect. 2.
Zignol, M., Dean, A.S., Alikhanova, N., Andres, S., Cabibbe, A.M., Cirillo, D.M., Dadu, A., Dreyer, A., Driesen, M., Gilpin, C., Hasan, R., Hasan, Z., Hoffner, S., Husain, A., Hussain, A., Ismail, N., Kamal, M., Mansjo, M., Mvusi, L., Niemann, S., Omar, S.V., Qadeer, E., Rigouts, L., Ruesch-Gerdes, S., Schito, M., Seyfaddinova, M., Skrahina, A., Tahseen, S., Wells, W.A., Mukadi, Y.D., Kimerling, M., Floyd, K., Weyer, K., Raviglione, M.C., 2016. Population-based resistance of Mycobacterium tuberculosis isolates to pyrazinamide and fluoroquinolones: results from a multicountry surveillance project. Lancet Infect. Dis. 16, 1185–1192.