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**Prevalence and molecular characteristics of drug-resistant
mycobacterium tuberculosis in Hainan, China: From 2014 to
2019**

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Abstract
Background: The emergence of antimicrobial resistance against *mycobacterium tuberculosis* has become the major concern in global tuberculosis control due to its limited therapy options and high mortality. However, the clinical and molecular

characteristics of drug-resistant strains vary in different geographical areas. Hainan Island located in southern China, is a high drug-resistant tuberculosis burden area. This study aimed to determine the dynamic changes of drug-resistance patterns and drug-related gene mutation types of *mycobacterium tuberculosis* in Hainan from 2014 to 2019.

Results: A total of 1484 culture-confirmed *mycobacterium tuberculosis* were included in this study. It was found that the proportions of drug resistance to isoniazid and rifampin were 31.3% and 31.1% respectively. Overall, the proportion of multidrug resistant *mycobacterium tuberculosis* was 24.9%. Multivariate logistic regression analysis showed that age and the treatment history were independent influencing factors of drug resistant tuberculosis. The proportions of drug-resistant tuberculosis in retreatment patients were considerably higher than those in new patients. The most common mutation types of isoniazid were Ser315 →Thr (66.3%), and the most common mutation types of rifampin were Ser531 →Leu (41.5%).

Conclusions: Our data suggests that the prevalence of drug resistant TB remains high in Hainan, and the risks for developing drug resistance with diversified and complicated mutation types increased significantly in retreatment patients. These results contribute to the knowledge of the prevalence of drug resistance in Hainan Province and also expand the molecular characteristics of drug resistance in China.

Keywords: *Mycobacterium tuberculosis*; CapitalBio™ DNA microarray; Drug sensitivity test; Molecular-characteristics; Drug-resistant rate

1 Introduction

Mycobacterium tuberculosis (MTB) is a main pathogen of tuberculosis (TB). It can affect almost all human organs, especially the lung [1-3]. The emergence of Drug-resistant *mycobacterium tuberculosis* (DR-TB), particularly multidrug-resistant *mycobacterium tuberculosis* (MDR-TB) and extensively drug-resistant *mycobacterium tuberculosis* (XDR-TB), has been identified as one of the major obstacles to effective TB control in many countries [4, 5]. It was estimated that 10.0 million (range, 9.0-11.1 million) people fell ill with TB in 2018, according to a newest report from the WHO. And there were approximately half a million (range, 417 000-556000) new cases of rifampin-resistant TB, of which 78% were MDR-TB.

DR-TB is usually associated with delayed diagnosis, prolonged or ineffective treatment or direct transmission of drug-resistant strains from one individual to another[6, 7].The magnitude and pattern of drug resistance varied greatly with the region because of the huge size of the country, the diverse population density, and the unbalanced economic development in China[8]. Hainan is the southernmost island in China. Separated from the mainland China by the Qiongzhou Strait, the population mobility of Hainan Province is lower than that of other Provinces. MTB of Hainan Province may demonstrate a unique genetic evolution due to the unique geographical location. Unfortunately, thus far, the true magnitude of DR-TB of Hainan Province was not well described to date and should be explored to facilitate control of the TB

epidemic in this region and throughout China.

To better understand the clinical and molecular characteristics of *mycobacterium tuberculosis* isolates, we analyzed all strains collected from TB inpatients admitted to the Second Affiliated Hospital of Hainan Medical University from 2014 to 2019. Clinical information, drug-resistant phenotypes and drug-resistance associated mutation types were compared. This study was to evaluate the clinical characteristics and changes in molecular epidemiology of DR-TB.

2 Results

2.1 Demographic and clinical characteristics

We analyzed the demographic and clinical information of culture-confirmed *mycobacterium tuberculosis* in Hainan from January 1, 2014 to December 31, 2019. A total of 1484 *mycobacterium tuberculosis* strains were included, and 923 of which were tested for rifampin and isoniazid drug-resistance gene mutation types by DNA microarray (Figure 1).

Figure 1. Inclusion and Exclusion of the study objects. MTB: *mycobacterium tuberculosis*; NTB: non-tuberculosis; DST: drug susceptibility test

Totally 223, 171, 107, 190, 331 and 462 isolates were tested for drug sensitivity from 2014 to 2019, respectively. The mean age of patients was 48 ± 17.5 (range: 1-95) years old and the gender ratio was 4.6. Gender and contact history showed no statistically significant change using the Chi-square test for trends ($P > 0.05$) while, age and treatment history changed significantly ($P < 0.05$). Compared with results in 2014, the proportion of patients aged between 25 to 64 was significantly higher in 2015 ($P = 0.028$), the proportion of patients aged over 44 was significantly higher in 2019 ($P = 0.032$), and the proportion of retreatment cases was significantly higher in 2016 ($P < 0.001$). What is worth mentioning is that, the proportion of new cases in 2019 (61.3 %) was higher than that in 2014 (55.6%) (Table 1).

Table 1 Characteristics of study population from 2014 to 2019

2.2 Drug susceptibility patterns

Changes of drug-resistance pattern of MTB from 2014 to 2019 were shown in Table 2. Analysis of the 1484 culture-confirmed TB cases showed that the isoniazid resistant TB rate was 31.3%, the rifampin resistant TB rate was 31.1%, the MDR TB rate was 24.9%, and the XDR-TB rate was 2.2%. In addition, any-drug-resistant TB accounted for 25.8% of new cases and 67.5% of retreatment cases. The isoniazid resistant TB accounted for 14.8% of new cases and 52.0% of retreatment cases. The rifampin resistant TB accounted for 11.4% of new cases and 55.7% of retreatment cases. MDR-TB accounted for 8.6% of new cases and 45.2% of retreatment cases. XDR-TB accounted for 0.4% of new cases and 4.6% of retreatment cases. For the

entire study cohort (1484 cases), the longitudinal changes in overall percentage of rifampin (RIF) resistance, kanamycin (KAR) resistance and protionamide (PTO) resistance overtime showed a statistically significant increase using the Chi-square test for trends (Table 2).

To have a better understanding of the epidemic trends in tuberculosis cases with different treatment histories, we explored the changes of drug resistance rate in newly treated patients and retreatment patients respectively. There was no statistical significance in changes of the drug resistance rate in the new TB cases ($P>0.05$), while in retreatment TB cases, RIF resistance increased at an annual rate of 0.05% (Chi-square test for trends: $\chi^2=14.257$, $P=0.014$), PTO resistance increased at an annual rate of 0.18% (Chi-square test for trends: $\chi^2=35.332$, $P<0.001$)(Figure 2).

Table 2 Evaluation and comparison of drug resistance rate of anti-tuberculosis drugs in 2014-2019

Abbreviation: INH, isoniazid; RIF, rifampin; EMB, ethambutol; STR, streptomycin; first-line drug resistance, including isoniazid, rifampin, ethambutol and streptomycin; MDR, multi-drug resistant; CPM, capreomycin; KAR, kanamycin; OFX, ofloxacin; PTO, protionamide; XDR, extensively drug-resistant

Figure 2 Trends of different drug-resistance patterns among 1484 culture-confirmed TB cases in Hainan, 2014 to 2019. In new cases, for INH resistance ($\chi^2=2.813$, $P=0.729$); for RIF resistance ($\chi^2=3.181$, $P=0.672$); for MDR-TB ($\chi^2=4.210$, $P=0.520$); for XDR-TB ($\chi^2=4.383$, $P=0.496$). In retreatment cases, for INH resistance ($\chi^2=9.512$, $P=0.090$); for RIF resistance ($\chi^2=14.257$, $P=0.014$); for MDR-TB ($\chi^2=10.328$, $P=0.066$); for XDR-TB ($\chi^2=7.670$, $P=0.175$). Note: *new cases, **retreatment cases. Abbreviation: INH-R, isoniazid resistance; RIF-R, rifampin resistance; MDR-TB, multidrug resistant mycobacterium tuberculosis; XDR-TB, extensively drug resistant mycobacterium tuberculosis

2.3 Factors associated with drug-resistance TB

Both univariate and multivariate analysis showed that age and treatment history were influencing factors for TB patients being resistant to any anti-TB drug, multidrug-resistant or extensive drug-resistant ($P<0.05$) (Table3). The risk of drug-resistance was associated with lower age. For those aged >64 years, the odd ratio to any drug-resistance, MDR and XDR were, respectively, 0.602 (95%CI: 0.397, 0.913), 0.277 (95%CI: 0.157, 0.486) and 0.287 (95%CI: 0.117, 0.702) times of those under 25 years old ($P=0.017$ for any tested-drug; $P<0.001$ for MDR; $P=0.006$ for XDR). Furthermore, the risk of drug-resistant in retreatment cases was significantly higher than that in new cases, and their risk of resistance to any tested-drug, multi-drug-resistant, extensive drug-resistant were respectively, 5.958(95%CI: 4.738, 7.492), 12.753(95%CI: 9.349, 17.397) and 16.498(95%CI: 9.816, 27.728) times

higher than that in new cases ($P < 0.001$ for any tested-drug, $P < 0.001$ for multi-drug-resistant, $P < 0.001$ for extensive drug-resistant). Compared to people with no exposure history, those who have exposure history may have higher drug resistance rates, and their risk of resistance to any detected drugs were 1.428 (95% CI: 1.000, 2.039) times higher than that of the people without contact history ($P = 0.050$).

Table 3 Factors associated with drug-resistance TB

Note: * indicates that the difference is statistically significant compared with 2014. Abbreviation: 95%CI means 95% confidence interval

2.4 Detection of drug resistance-associated mutations by DNA microarray

The gene mutation profile of *tuberculosis* resistant strains was further investigated. A total of 923 strains were tested for resistance gene mutations by DNA microarray and the mutation sites were shown in Table 4.

In the isoniazid resistance mutation sites, *KatG* and promoter *inhA* were 70.8% (204/288) and 8.0% (23/288), and combinatorial mutation of *KatG+inhA* was 1.0% (3/288), respectively. *KatG* was the most common mutation, and its mutation was all higher than 79.0% from 2017 to 2019. In 2019, the mutation rate of *inhA* was significantly increased (15.5%, 15/97, $P = 0.006$). In addition, the combinatorial mutation of *KatG+inhA* was only found in 2019.

Among rifampin resistance mutation sites, *rpoB531* (43.6%, 167/383), *rpoB526* (18.3, 70/383) and *rpoB511* (7.0, 27/383) were the most frequent. The mutation rates of *rpoB53* isolates from 2017 to 2019 were significantly higher than those from 2014 ($P < 0.05$).

The mutation rates of *rpoB + inhA* and *rpoB + rpoB + KatG* in MDR-TB strains were 3.3% (8/242). The *rpoB + KatG+ inhA* mutation was only found in three isolates in 2019. While the *rpoB + KatG* mutation rate was 71.1% (172 / 242), *rpoB531 + KatG315* mutation in MDR-TB isolates was significantly higher in 2018 (51.2%, 22/43, $P = 0.028$) and 2019 (52.7 %, 39/74, $P = 0.010$) than in 2014 (7.7%, 3 / 39).

This study also compared the mutation sites of drug-resistant strains from 2014 to 2019, and some new drug-resistant gene combinations were detected. For isoniazid, combinatorial mutation of *Ser315→Thr + inhAT_15* emerged. For rifampin, combinatorial mutation of *Gln513→Pro*, *Leu511→Pro + Asp516→Val* appeared. For MDR-TB strains, *Leu511→Pro*, *Asp516→Val + Ser315→Thr* were also found, and the drug-resistant mutation sites were constantly diversified.

Table 4 Evolution of drug resistance mutation sites of mycobacterium tuberculosis to first-line anti tuberculosis drugs isoniazid and rifampin in 2014-2019

Note: * indicates that the difference is statistically significant compared with 2014

3 Discussion

In 2017, the Southeast Asia and the Western Pacific region saw the largest number of new TB cases, accounting for 62% of all new cases worldwide. China is one of the three countries with the highest drug-resistant TB in the world. Previous studies have shown that the clinical and molecular characteristics of drug-resistant strains in China vary from region to region[9, 11-18]. Hainan is the only tropical island in China. Its unique tropical climate and relatively low population mobility may affect the infection and drug resistance of tuberculosis. Determining the change in the TB drug-resistance rate over time and its current status in Hainan are essential to adequately administer anti-TB regimens and achieve successful treatment. This study was a large population and long-term-based retrospective study conducted in Hainan Province, China. To our knowledge, this is the first study providing comprehensive assessment of the dynamic changes of drug resistance rate and the mutation sites of isoniazid and rifampin resistance in Hainan Province.

The overall percentage of retreatment cases was 44.4%, while, a review showed that the median percentage of TB patients experiencing an episode of recurrent TB after treatment completion was 3.4% (interquartile range [IQR] 1.6-6.0, range 0.4-16.7) [19], suggesting that the retreatment cases is still a big challenge in controlling the TB epidemic in Hainan.

The present study show that 44.3% (658/1484) of patients had drug-resistant disease, meanwhile the proportions of MDR- and XDR-TB among patients were 24.9% and 2.2%, nearly two times the proportions presented in the data from a China Clinical Tuberculosis Centre in 2017[20]. According to a WHO report, the global MDR/RR-TB (multidrug resistant tuberculosis / rifampin resistant tuberculosis) rate was 3.4% (95%CI: 2.5%-4.4%) for new cases and 18% (95%CI: 7.6%-31%) for retreatment cases in 2018, while in China, the MDR/RR-TB rate was 7.1% (95%CI: 5.6%-8.7%) for new cases and 21% (95%CI: 21%-21%) for retreatment cases in the same year. However, our data showed that, in 2019, the MDR/RR-TB rate was 11.4% for new cases and 55.7% for retreatment cases in Hainan, which was significantly higher than the average rate of both the global and China's. It showed a serious epidemic of drug-resistant tuberculosis in Hainan.

The changes of drug resistance rate of new cases were not statistically significant. However, the resistance rates of first-line anti-tuberculosis drugs and second-line anti-tuberculosis drugs increased significantly in retreatment patients with rifampin resistant TB increased from 45.9% in 2014 to 60.3% in 2019 and protionamide increased at an annual rate of 0.18%. A higher risk of drug resistance was found among retreatment patients, similar results could be found in other reports[21, 22]. This implies that acquired-drug resistance may play an increasing role in the DR-TB epidemic in Hainan. Hence, some appropriate strategies must be implemented to increase continuity of treatment and reduce the rate of treatment default.

We also found that people older than 64 years of age had a lower risk of any drug-resistant TB, MDR-TB and XDR-TB. This is consistent with the conclusion of a systematic review of European studies which concluded that MDR-TB cases are more

likely to occur in patients younger than 65 years of age [23, 24]. The higher risk of getting MDR-TB in people under 65 years may be attributed to the use of RIF for anti-TB treatment from around 1965. TB cases in older patients are usually considered as the infecting strains may be more ancient, and carry a lower risk of becoming resistant to drug, the frequency of DR-TB peaked in young adulthood and the age profile of DR-TB was in agreement with other reports[25].

Several recent studies have examined the contribution of *KatG* and *inhA* promoter mutations in drug-resistant TB isolates, and the results revealed significant geographic diversity across regions[26-28]. This study found that the most common mutation of MDR-TB was 34.7% (*Ser531* → *Leu*+ *Ser315* → *Thr*), however, a study in Brazil showed that was 41.7% [29]. In our study, the mutation rate in *KatG315* was 71.9%, which was higher than the mutation rates reported in Poland (66.0%) and Hebei Province, China (69.9%)[30, 31]. The most common mutation of rifampin was *rpoB531* (43.6%, 167 / 383), which was lower than the results of Isakova Jet al(64.8%)[32]. The regional differences in the frequencies of mutations associated with resistance may reflect the diversity in molecular characteristics of DR-TB isolates circulating in geographically distinct areas, and also provide insights for the development of molecular-based diagnostic tests.

Another interesting finding was that a combined mutation of *KatG+inhA*, which was rarely reported before, was identified in the study. Moreover, a simultaneous mutation in *rpoB+KatG+inhA* was also identified, indicating that *tuberculosis* strains were constantly mutating. These data might be helpful in the design and development of new anti-*mycobacterium tuberculosis* drugs. There were still some resistant isolates harboring no mutation within the sequenced regions. This implied that these isolates probably harbored mutations outside the sequenced area or that the resistance may be caused by other mechanisms, such as efflux pumps[33].

Due to the limitations of retrospective data collection, the education background, socioeconomic status, and living conditions of the patients involved in this study were not well described and recorded. The interplay of these factors and how could it affect the epidemic of drug-resistant TB are somewhat neglected. Well-designed studies with comprehensive and detailed research data in China should thus be conducted in the future.

4 Conclusions:

Despite these limitations, the trends of different drug resistance patterns overtime were examined and a better understanding of the epidemic characteristics of TB cases in Hainan was obtained. First, the drug-resistant TB rate remains high throughout the study. Second, the age and treatment history were independent risk factors of TB drug resistance. Third, different mutation rates and patterns are identified.

5 Materials and Methods

5.1 Study Population and Data Collection

This study was carried out from January 2014 to December 2019 at the Second Affiliated Hospital of Hainan Medical University, which serves as the sole specialized

TB hospital in Hainan. Information for all patients (age, gender, TB contact history, and TB treatment history, etc.) was collected and recorded. Only one isolate per patient was collected and tested.

5.2 Laboratory pretreatment

Pulmonary samples were collected by expectoration, gastric aspiration, and sputum induction. Extra pulmonary samples (pleural fluid, spinal fluid, and lymph nodes) were collected by pleural tap, lumbar puncture, lymph node biopsy, fine needle aspiration, and other techniques. The patients' samples were placed in a microcentrifuge tube, and processed for smear and culture. To identify the presence of acid-fast bacilli, we used *Ziehl-Neelsen* staining (Baso, Zhuhai, China) for smear microscopy. Each sample was inoculated into the acidic modified Lowenstein-Jensen (Cell Biotech Co., Ltd., Hainan, China) culture medium. Strain isolation and identification were performed in a tuberculosis reference laboratory of the Second Affiliated Hospital of Hainan Medical University. All operations strictly comply with standard biosecurity and institutional safety procedures.

5.3 Drug sensitivity test

Following cultivation, the MTB was assessed for drug sensitivity using a Lowenstein-Jensen culture medium and the following drug concentrations: isoniazid (0.2 µg/mL), rifampin (40 µg/mL), ethambutol (2.0 µg /mL), streptomycin (4.0 µg/mL), capreomycin (2 µg/mL), kanamycin (40 µg/mL), ofloxacin (30 µg/mL), and protionamide (40 µg/mL). Isolates with growth proportion for >1% on medium containing anti-TB drugs compared with the growth on drug free medium were considered to be resistant to those drugs[9].

5.4 Detection by CapitalBio™ DNA microarray

This study was based on MTB drug resistance gene detection kit (CapitalBio™ DNA microarray method, Beijing CapitalBio Technology, 301035), which can specifically detect the mutations on the promoter of *rpoB*, *KatG* and *inhA*. Laboratory operations were performed according to the manufacturer's instructions [10].

5.5 Statistical analysis

The Chi-square test or the Fisher's exact test were chosen to assess the difference between different groups. Univariate and multivariate analysis were used to evaluate the influencing factors related to drug resistance of *tuberculosis* by SPSS 21.0 software as well. A two-tailed $P < 0.05$ was considered statistically significant.

6 Abbreviations:

CPM: capreomycin

DR-TB: Drug-resistant mycobacterium tuberculosis

EMB: ethambutol

INH: isoniazid

KAR: kanamycin

MDR-TB: multidrug-resistant mycobacterium tuberculosis

MDR: multi-drug resistant

MTB: mycobacterium tuberculosis

PTO: protionamide

RIF: rifampin

STR: streptomycin

TB: tuberculosis

XDR-TB: extensively drug-resistant mycobacterium tuberculosis

XDR: extensively drug-resistant

7 Declarations

7.1 Ethical approval and consent to participate

The study was approved by the Ethic Committee of the Second Affiliated Hospital of Hainan Medical University, Hainan, China. Informed consent was obtained from research subjects, and patient records were anonymized and identified before analysis.

7.2 Consent for publication

Not applicable.

7.3 Availability of data and materials

The oligonucleotide probes and primers used in this study available from the corresponding author on reasonable request.

7.4 Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7.5 Funding:

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7.6 Authors' contributions

All authors contribute equally to this article. All authors read and approved the final manuscript.

7.7 Acknowledgements

Not applicable.

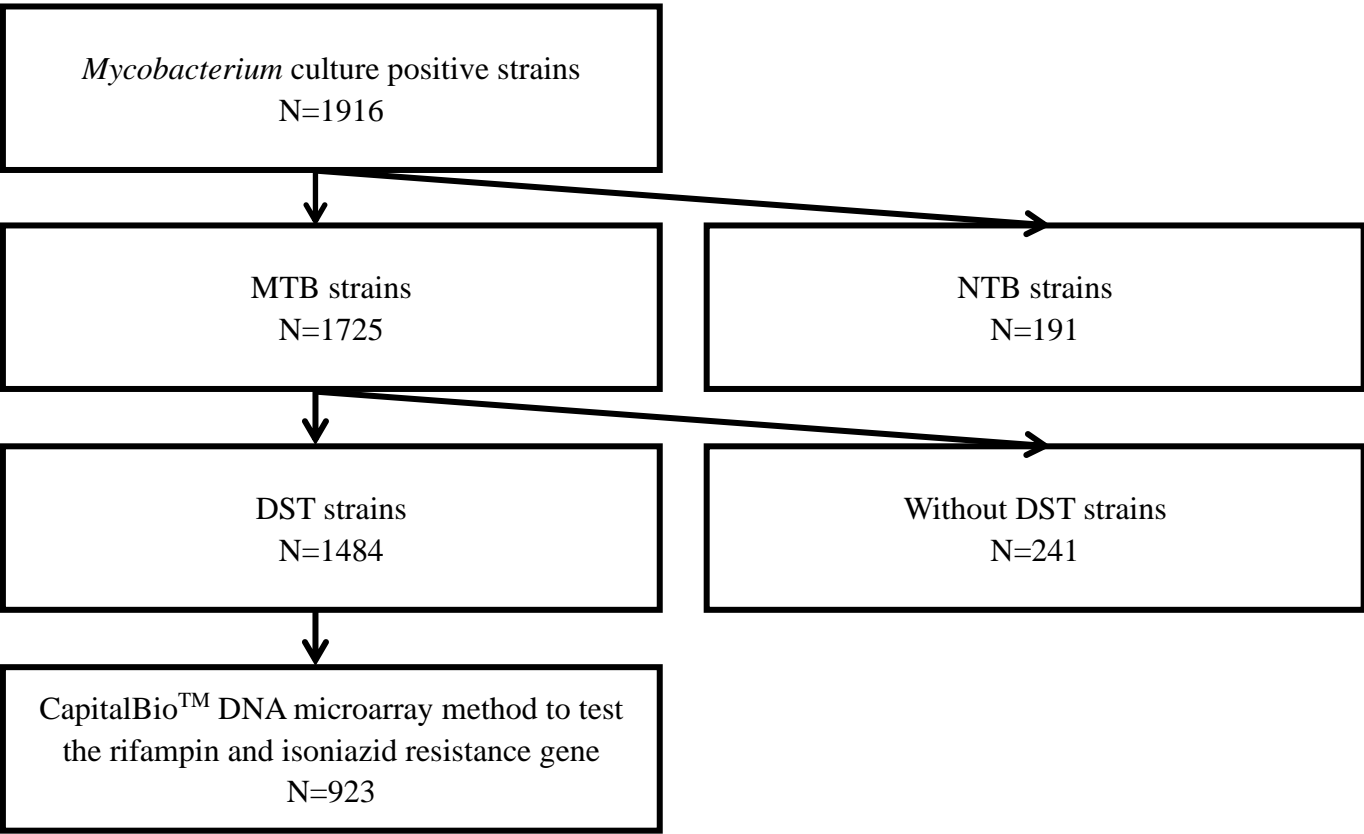


Figure 1. Inclusion and Exclusion of the study objects. MTB: *mycobacterium tuberculosis*; NTB: *non-tuberculosis*; DST: drug susceptibility test.

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Running Title: Characteristics of drug-resistant *Mycobacterium tuberculosis*

Table 1 Characteristics of study population from 2014 to 2019

| Characteristics | Total | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | χ^2 | <i>P</i> |
|-------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------|----------|
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | | |
| Gender | | | | | | | | | |
| Male | 1217(82.0) | 181(81.2) | 147(86.0) | 84(78.5) | 157(82.6) | 262(79.2) | 386(83.5) | 5.433 | 0.365 |
| Female | 267(18.0) | 42(18.8) | 24(14.0) | 23(21.5) | 33(17.4) | 69(20.8) | 76(16.5) | | |
| Age group(years) | | | | | | | | | |
| ~25 | 205(13.8) | 42(18.8) | 18(10.5) | 17(15.9) | 34(17.9) | 43(13.0) | 51(11.0) | 25.692 | 0.041 |
| ~44 | 375(25.3) | 55(24.7) | 55(32.2) | 26(24.3) | 43(22.6) | 88(26.6) | 108(23.4) | | |
| ~64 | 635(42.8) | 88(39.5) | 78(45.6) | 48(44.9) | 81(42.6) | 130(39.3) | 210(45.5) | | |
| >64 | 269(18.1) | 38(17.0) | 20(11.7) | 16(15.0) | 32(16.8) | 70(21.1) | 93(20.1) | | |
| Treatment history | | | | | | | | | |
| New cases | 825(55.6) | 125(56.1) | 88(51.5) | 39(36.4) | 94(49.5) | 196(59.2) | 283(61.3) | 27.727 | <0.001 |
| Retreatment cases | 659(44.4) | 98(43.9) | 83(48.5) | 68(63.6) | 96(50.5) | 135(40.8) | 179(38.7) | | |
| Contact history | | | | | | | | | |
| No | 1313(88.5) | 206(92.4) | 154(90.1) | 90(84.1) | 167(87.9) | 285(86.1) | 411(89.0) | 7.745 | 0.171 |
| Yes | 171(11.5) | 17(7.6) | 117(9.9) | 17(15.9) | 23(12.1) | 46(13.9) | 51(11.0) | | |
| Total | 1484 (100.0) | 223 (100.0) | 171 (100.0) | 107 (100.0) | 190 (100.0) | 331 (100.0) | 462 (100.0) | | |

Table 2 Evaluation and comparison of drug resistance rate of anti-*tuberculosis* drugs in 2014-2019

| Category | 2014 n (%) | 2015 n (%) | 2016 n (%) | 2017 n (%) | 2018 n (%) | 2019 n (%) | Total n (%) | χ^2 | <i>P</i> |
|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|----------|----------|
| All TB cases | | | | | | | | | |
| Any drug-resistance | 95(42.6) | 85(49.7) | 60(56.1) | 96(50.5) | 127(38.4) | 195(42.2) | 658(44.3) | 16.820 | 0.005 |
| INH | 71(31.8) | 67(39.2) | 42(39.3) | 68(35.8) | 85(25.7) | 132(28.6) | 465(31.3) | 16.349 | 0.006 |
| RIF | 60(26.9) | 63(36.8) | 48(44.9) | 68(35.8) | 86(26.0) | 136(29.4) | 461(31.1) | 20.521 | 0.001 |
| EMB | 23(10.3) | 12(7.0) | 20(18.7) | 26(13.7) | 26(7.9) | 41(8.9) | 148(10.0) | 15.941 | 0.007 |
| STR | 48(21.5) | 39(22.8) | 24(22.4) | 50(26.3) | 56(16.9) | 98(21.2) | 315(21.2) | 6.977 | 0.222 |
| MDR | 54(24.2) | 50(29.2) | 38(35.5) | 53(27.9) | 63(19.0) | 111(24.0) | 369(24.9) | 15.430 | 0.009 |
| Any second-line drug resistance | 42(18.8) | 43(25.1) | 34(31.8) | 46(24.2) | 47(14.2) | 96(20.8) | 308(20.8) | 20.435 | 0.001 |
| CPM | 2(0.9) | 3(1.8) | 3(2.8) | 7(3.7) | 6(1.8) | 21(4.5) | 42(2.8) | 10.443 | 0.064 |
| KAR | 6(2.7) | 5(2.9) | 7(6.5) | 13(6.8) | 6(1.8) | 14(3.0) | 51(3.4) | 13.119 | 0.022 |
| OFX | 38(17.0) | 41(24.0) | 33(30.8) | 42(22.1) | 43(13.0) | 76(16.5) | 273(18.4) | 24.208 | <0.001 |
| PTO | 1(0.4) | 1(0.6) | 0(0.0) | 0(0.0) | 0(0.0) | 18(3.9) | 20(1.3) | 34.796 | <0.001 |
| XDR | 4(1.8) | 3(1.8) | 5(4.7) | 10(5.3) | 4(1.2) | 7(1.5) | 33(2.2) | 11.580 | 0.041 |
| New cases | | | | | | | | | |
| Any drug-resistance | 35(28.0) | 22(25.0) | 13(33.3) | 29(30.9) | 47(24.0) | 67(23.7) | 213(25.8) | 3.759 | 0.585 |
| INH | 22(17.6) | 14(15.9) | 6(15.4) | 17(18.1) | 27(13.8) | 36(12.7) | 122(14.8) | 2.813 | 0.729 |
| RIF | 15(12.0) | 8(9.1) | 7(17.9) | 13(13.8) | 23(11.7) | 28(9.9) | 94(11.4) | 3.181 | 0.672 |
| EMB | 6(4.8) | 2(2.3) | 1(2.6) | 4(4.3) | 6(3.1) | 11(3.9) | 30(3.6) | 1.462 | 0.917 |
| STR | 17(13.6) | 9(10.2) | 6(15.4) | 13(13.8) | 25(12.8) | 31(11.0) | 101(12.2) | 1.604 | 0.901 |
| MDR | 14(11.2) | 4(4.5) | 5(12.8) | 8(8.5) | 15(7.7) | 25(8.8) | 71(8.6) | 4.210 | 0.520 |
| Any second-line drug resistance | 13(10.4) | 7(8.0) | 7(17.9) | 11(11.7) | 12(6.1) | 26(9.2) | 76(9.2) | 6.397 | 0.270 |
| CPM | 1(0.8) | 1(1.1) | 0(0.0) | 3(3.2) | 3(1.5) | 7(2.5) | 15(1.8) | 4.133 | 0.530 |
| KAR | 2(1.6) | 1(1.1) | 0(0.0) | 4(4.3) | 1(0.5) | 6(2.1) | 14(1.7) | 6.696 | 0.244 |
| OFX | 10(8.0) | 7(8.0) | 7(17.9) | 8(8.5) | 10(5.1) | 17(6.0) | 59(7.2) | 7.388 | 0.193 |
| PTO | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 2(0.7) | 2(0.2) | 4.289 | 0.509 |
| XDR | 0(0.0) | 1(1.1) | 0(0.0) | 1(1.1) | 0(0.0) | 1(0.4) | 3(0.4) | 4.383 | 0.496 |
| Retreatment cases | | | | | | | | | |

Running Title: Characteristics of drug-resistant *Mycobacterium tuberculosis*

| | | | | | | | | | |
|---------------------------------|----------|----------|----------|----------|----------|-----------|-----------|--------|--------|
| Any drug-resistance | 60(61.2) | 63(75.9) | 47(69.1) | 67(69.8) | 80(59.3) | 128(71.5) | 445(67.5) | 10.236 | 0.069 |
| INH | 49(50.0) | 53(63.9) | 36(52.9) | 51(53.1) | 58(43.0) | 96(53.6) | 343(52.0) | 9.512 | 0.090 |
| RIF | 45(45.9) | 55(66.3) | 41(60.3) | 55(57.3) | 63(46.7) | 108(60.3) | 367(55.7) | 14.257 | 0.014 |
| EMB | 17(17.3) | 10(12.0) | 19(27.9) | 22(22.9) | 20(14.8) | 30(16.8) | 118(17.9) | 9.294 | 0.098 |
| STR | 31(31.6) | 30(36.1) | 18(26.5) | 37(38.5) | 31(23.0) | 67(37.4) | 214(32.5) | 10.845 | 0.055 |
| MDR | 40(40.8) | 46(55.4) | 33(48.5) | 45(46.9) | 48(35.6) | 86(48.0) | 298(45.2) | 10.328 | 0.066 |
| Any second-line drug resistance | 29(29.6) | 36(43.4) | 27(39.7) | 35(36.5) | 35(25.9) | 70(39.1) | 232(35.2) | 10.741 | 0.057 |
| CPM | 1(1.0) | 2(2.4) | 3(4.4) | 4(4.2) | 3(2.2) | 14(7.8) | 27(4.1) | 10.541 | 0.061 |
| KAR | 4(4.1) | 4(4.8) | 7(10.3) | 9(9.4) | 5(3.7) | 8(4.5) | 37(5.6) | 6.544 | 0.257 |
| OFX | 28(28.6) | 34(41.0) | 26(38.2) | 34(35.4) | 33(24.4) | 59(33.0) | 214(32.5) | 8.806 | 0.117 |
| PTO | 1(1.0) | 1(1.2) | 0(0.0) | 0(0.0) | 0(0.0) | 16(8.9) | 18(2.7) | 35.332 | <0.001 |
| XDR | 4(4.1) | 2(2.4) | 5(7.4) | 9(9.4) | 4(3.0) | 6(3.4) | 30(4.6) | 7.670 | 0.175 |

Abbreviation: INH, isoniazid; RIF, rifampin; EMB, ethambutol; STR, streptomycin; first-line drug resistance, including isoniazid, rifampin, ethambutol and streptomycin; MDR, multi-drug resistant; CPM, capreomycin; KAR, kanamycin; OFX, ofloxacin; PTO, protionamide; XDR, extensively drug-resistant

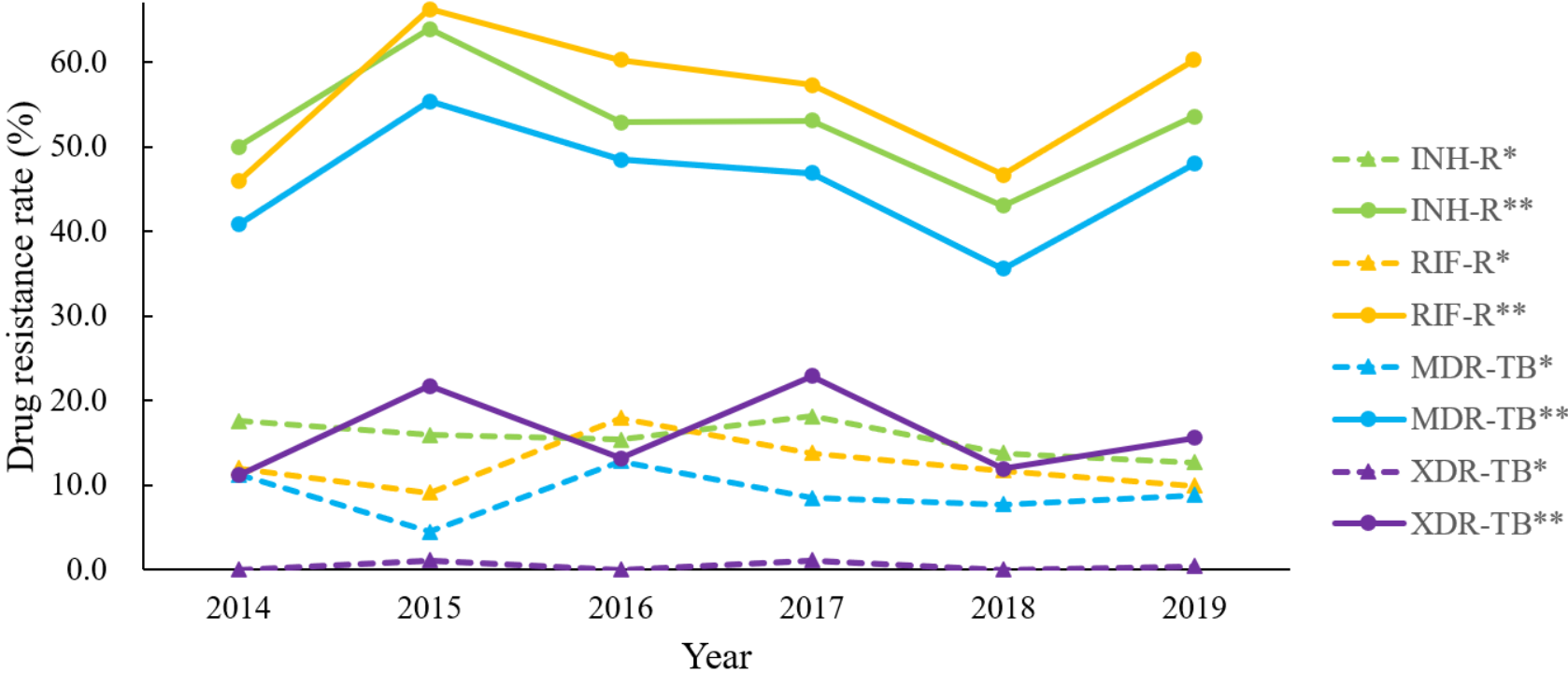


Figure 2 Trends of different drug-resistance patterns among 1484 culture-confirmed TB cases in Hainan, 2014 to 2019. In new cases, for INH resistance ($\chi^2=2.813$, $P=0.729$); for RIF resistance ($\chi^2=3.181$, $P=0.672$); for MDR-TB ($\chi^2=4.210$, $P=0.520$); for XDR-TB ($\chi^2=4.383$, $P=0.496$). In retreatment cases, for INH resistance ($\chi^2=9.512$, $P=0.090$); for RIF resistance ($\chi^2=14.257$, $P=0.014$); for MDR-TB ($\chi^2=10.328$, $P=0.066$); for XDR-TB ($\chi^2=7.670$, $P=0.175$).

Note: *new cases, **retreatment cases. Abbreviation: INH-R, isoniazid resistance; RIF-R, rifampin resistance; MDR-TB, multidrug resistant mycobacterium tuberculosis; XDR-TB, extensively drug resistant mycobacterium tuberculosis.

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Table 3Factors associated with drug-resistance TB

| Factors | Any drug resistant TB (n=658) | MDR-TB (n=369) | XDR-TB (n=123) | Pan-susceptible TB (n=826) | Any drug resistant TB VS Pan-susceptible TB | | | | MDR-TB VS Pan-susceptible TB | | | | XDR-TB VS Pan-susceptible TB | | | |
|-------------------|-------------------------------|----------------|----------------|----------------------------|---|--------|----------------------|--------|------------------------------|--------|-----------------------|--------|------------------------------|--------|-----------------------|--------|
| | | | | | OR (95% CI) | P | Adjusted OR (95% CI) | P | OR (95% CI) | P | Adjusted OR (95% CI) | P | OR (95% CI) | P | Adjusted OR (95% CI) | P |
| Gender | | | | | | | | | | | | | | | | |
| Male | 534(81.2) | 299(81.0) | 97(78.9) | 683(82.7) | Reference | | | | Reference | | | | Reference | | | |
| Female | 124(18.8) | 70(19.0) | 26(21.1) | 143(17.3) | 0.830 (0.614,1.121) | 0.225 | | | 0.820 (0.555,1.209) | 0.316 | | | 0.673 (0.387,1.172) | 0.162 | | |
| Age (years) | | | | | | | | | | | | | | | | |
| ~25 | 84(12.8) | 52(14.1) | 15(12.2) | 121(14.6) | Reference | | | | Reference | | | | Reference | | | |
| ~44 | 173(26.3) | 100(27.1) | 31(25.2) | 202(24.5) | 1.080 (0.737,1.582) | 0.694 | 1.052 (0.720,1.536) | 0.795 | 0.955 (0.588,1.550) | 0.852 | 0.917 (0.568,1.482) | 0.725 | 1.129 (0.540,2.363) | 0.747 | 1.062 (0.511,2.205) | 0.873 |
| ~64 | 311(47.3) | 183(49.6) | 67(54.5) | 324(39.2) | 1.079 (0.753,1.544) | 0.679 | 1.039 (0.730,1.479) | 0.833 | 0.941 (0.598,1.480) | 0.791 | 0.886 (0.568,1.328) | 0.593 | 1.329 (0.674,2.622) | 0.412 | 1.227 (0.629,2.390) | 0.549 |
| >64 | 90(13.7) | 34(9.2) | 10(8.1) | 179(21.7) | 0.626 (0.410,0.954) | 0.030 | 0.602 (0.397,0.913) | 0.017 | 0.300 (0.169,0.533) | <0.001 | 0.277 (0.157,0.486) | <0.001 | 0.317 (0.128,0.786) | 0.013 | 0.287 (0.117,0.702) | 0.006 |
| Treatment history | | | | | | | | | | | | | | | | |
| New cases | 213(32.4) | 71(19.2) | 19(15.4) | 612(74.1) | Reference | | | | Reference | | | | Reference | | | |
| Retreatment cases | 445(67.6) | 298(80.8) | 104(84.6) | 214(25.9) | 5.992 (4.763,7.538) | <0.001 | 5.958 (4.738,7.492) | <0.001 | 12.777 (9.356,17.448) | <0.001 | 12.753 (9.349,17.397) | <0.001 | 16.778 (9.959,28.268) | <0.001 | 16.498 (9.816,27.728) | <0.001 |

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Contact history

| | | | | | | | | | | | | |
|-----|---------------|-----------|-----------|-----------|--------------|-------|--------------|-------|---------------|-------|---------------|-------|
| No | 565(85.9) | 317(85.9) | 108(87.8) | 748(90.6) | Reference | | Reference | | Reference | | Reference | |
| | | | | | 1.408 | | 1.428 | | | | | |
| Yes | 93(14.1) | 52(14.1) | 15(12.2) | 78(9.4) | (0.985,2.014 | 0.060 | (1.000,2.039 | 0.050 | 1.247 | 0.338 | 1.049 | 0.889 |
| | | | | |) | |) | | (0.794,1.960) | | (0.532,2.069) | |

Note: * indicates that the difference is statistically significant compared with 2014

Table 4 Evolution of drug resistance mutation sites of *mycobacterium tuberculosis* to first-line anti *tuberculosis* drugs isoniazid and rifampin in 2014-2019

| Drug | Locus | Nucleic acid change | Codon mutation | 2014 n(%) | 2015 n(%) | 2016 n(%) | 2017 n(%) | 2018 n(%) | 2019 n(%) | Total |
|-------|-----------------------------|---------------------|------------------------|--------------|--------------|--------------|--------------|--------------|--------------|------------|
| INH | <i>KatG</i> | AGC→AAC | <i>Ser315Asn</i> | 4(10.0) | 0(0.0)* | 0(0.0)* | 1(2.7) | 3(6.3) | 5(5.2) | 13(4.5) |
| | | AGC→ACC | <i>Ser315Thr</i> | 30(75.0) | 5(11.1) | 10(47.6) | 34(91.9) | 40(83.3) | 72(74.2) | 191(66.3) |
| | <i>inhA</i> | C→T | <i>C(-15)→T</i> | 0(0.0) | 0(0.0) | 1(4.8) | 2(5.4) | 5(10.4) | 15(15.5)* | 23(8.0) |
| | <i>KatG+</i> <i>inhA</i> | AGC→ACC | <i>Ser315Thr+</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 3(3.1) | 3(1.0) |
| | | C→T | <i>C(-15)→T</i> | | | | | | | |
| | Other locus | Other mutations | Other substitutions | 6(15.0) | 40(88.9)* | 10(47.6)* | 0(0.0)* | 0(0.0)* | 2(2.1)* | 58(20.1) |
| Total | | | | 40(100.0) | 45(100.0) | 21(100.0) | 37(100.0) | 48(100.0) | 97(100.0) | 288(100.0) |
| RIF | <i>rpoB</i> | CTG→CCG | <i>Leu511Pro</i> | 8(17.0) | 1(1.8)* | 1(2.6)* | 3(6.4) | 4(5.8) | 10(8.1) | 27(7.1) |
| | | CAA→CCA | <i>Gln513Pro</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(0.8) | 1(0.3) |
| | | CAA→AAA | <i>Gln513Lys</i> | 2(4.3) | 0(0.0) | 1(2.6) | 1(2.1) | 1(1.5) | 0(0.0) | 5(1.3) |
| | | GAC→GGC | <i>Asp516Gly</i> | 0(0.0) | 1(1.8) | 0(0.0) | 0(0.0) | 1(1.5) | 2(1.6) | 4(1.0) |
| | | GAC→GTC | <i>Asp516Val</i> | 4(8.5) | 1(1.8) | 0(0.0) | 2(4.3) | 2(2.9) | 3(2.4) | 12(3.1) |
| | | GAC→TAC | <i>Asp516Tyr</i> | 0(0.0) | 0(0.0) | 0(0.0) | 2(4.3) | 1(1.5) | 2(1.6) | 5(1.3) |
| | | CAC→CGC | <i>His526Arg</i> | 1(2.1) | 0(0.0)* | 1(2.6) | 1(2.1) | 4(5.8) | 1(0.8) | 8(2.1) |
| | | CAC→CTC | <i>His526Leu</i> | 1(2.1) | 0(0.0) | 1(2.6) | 1(2.1) | 3(4.4) | 1(0.8) | 7(1.8) |

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|-------|-----------------------------|-----------------|---------------------|-----------|-----------|-----------|-----------|-----------|------------|------------|
| Total | | CAC→GAC | <i>His526Asp</i> | 4(8.5) | 1(1.8) | 0(0.0) | 4(8.5) | 4(5.8) | 10(8.1) | 23(6.0) |
| | | CAC→TAC | <i>His526Tyr</i> | 3(6.4) | 1(1.8) | 1(2.6) | 5(10.6) | 9(13.0) | 13(10.5) | 32(8.4) |
| | | TCG→TGG | <i>Ser531Trp</i> | 2(4.3) | 1(1.8)* | 0(0.0) | 1(2.1)* | 3(4.4)* | 1(0.8)* | 8(2.1) |
| | | TCG→TTG | <i>Ser531Leu</i> | 12(25.5) | 5(8.8) | 14(35.9) | 25(53.2) | 34(49.3) | 69(55.6) | 159(41.5) |
| | | CTG→CCG | <i>Leu533Pro</i> | 0(0.0) | 1(1.8) | 0(0.0) | 0(0.0) | 2(2.9) | 5(4.0) | 8(2.1) |
| | | CTG→CCG+ | <i>Leu511Pro+</i> | 1(2.1) | 0(0.0) | 0(0.0) | 1(2.1) | 0(0.0) | 0(0.0) | 2(0.5) |
| | | GAC→GGC | <i>Asp516Gly</i> | | | | | | | |
| | | CTG→CCG+ | <i>Leu511Pro+</i> | 1(2.1) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(0.3) |
| | | GAC→TAC | <i>Asp516Tyr</i> | | | | | | | |
| | | CTG→CCG+ | <i>Leu511Pro+</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(0.8) | 1(0.3) |
| | | GAC→GTC | <i>Asp516Val</i> | | | | | | | |
| | | CTG→CCG+ | <i>Leu511Pro+</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 2(1.6) | 2(0.5) |
| | | CAC→TAC | <i>His526Tyr</i> | | | | | | | |
| | | CAA→CCA+ | <i>Gln513Pro+</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(0.8) | 1(0.3) |
| | | GAC→GTC | <i>Asp516Val</i> | | | | | | | |
| | | GAC→GGC+ | <i>Asp516Gly+</i> | 2(4.3) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(0.8) | 3(0.8) |
| | | CTG→CCG | <i>Leu533Pro</i> | | | | | | | |
| | Other locus | Other mutations | Other substitutions | 6(12.8) | 45(79.0)* | 20(51.3)* | 1(2.1) | 1(1.5)* | 1(0.8)* | 74(19.3) |
| | | | | 47(100.0) | 57(100.0) | 39(100.0) | 47(100.0) | 69(100.0) | 124(100.0) | 383(100.0) |
| MDR | <i>rpoB+</i> <i>KatG</i> | CTG→CCG | <i>Leu511Pro+</i> | 8(21.6) | 0(0.0)* | 0(0.0)* | 0(0.0)* | 3(7.0) | 6(8.1) | 17(7.0) |
| | | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | | GAC→GGC | <i>Asp516Gly+</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(1.4) | 1(0.4) |
| | | AGC→AAC | <i>Ser315Asn</i> | | | | | | | |
| | | GAC→GGC | <i>Asp516Gly+</i> | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(1.4) | 1(0.4) |
| | | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | | GAC→GTC | <i>Asp516Val+</i> | 3(8.1) | 0(0.0) | 0(0.0) | 1(3.5) | 2(4.7) | 1(1.4) | 7(2.9) |
| | | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |

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|---------------|---------|--------------------|---------|---------|---------|----------|----------|----------|----------|
| <i>rpoB</i> + | GAC→TAC | <i>Asp516Tyr</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 1(3.5) | 1(2.3) | 1(1.4) | 3(1.2) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CAC→CGC | <i>His526Arg</i> + | 1(2.7) | 0(0.0)* | 1(5.0) | 1(3.5) | 3(7.0) | 0(0.0) | 6(2.5) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CAC→CTC | <i>His526Leu</i> + | 1(2.7) | 0(0.0) | 0(0.0) | 1(3.5) | 0(0.0) | 0(0.0) | 2(0.8) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CAC→GAC | <i>His526Asp</i> + | 1(2.7) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(0.4) |
| | AGC→AAC | <i>Ser315Asn</i> | | | | | | | |
| | CAC→GAC | <i>His526Asp</i> + | 3(8.1) | 1(2.6) | 0(0.0) | 3(10.3) | 3(7.0) | 5(6.8) | 15(6.2) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CAC→TAC | <i>His526Tyr</i> + | 1(2.7) | 0(0.0) | 1(5.0) | 5(17.2) | 5(11.6) | 7(9.5) | 19(7.9) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | TCG→TGG | <i>Ser531Trp</i> + | 0(0.0) | 0(0.0)* | 0(0.0) | 0(0.0) | 1(2.3)* | 0(0.0)* | 1(0.4) |
| | AGC→AAC | <i>Ser315Asn</i> | | | | | | | |
| | TCG→TGG | <i>Ser531Trp</i> + | 2(5.4) | 1(2.6) | 0(0.0) | 0(0.0) | 1(2.3) | 1(1.4) | 5(2.1) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | TCG→TTG | <i>Ser531Leu</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 2(4.7) | 4(5.4) | 6(2.5) |
| | AGC→AAC | <i>Ser315Asn</i> | | | | | | | |
| | TCG→TTG | <i>Ser531Leu</i> + | 8(21.6) | 2(5.1) | 8(40.0) | 14(48.3) | 18(41.9) | 34(46.0) | 84(34.7) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CTG→CCG | <i>Leu533Pro</i> + | 0(0.0) | 1(2.6) | 0(0.0) | 0(0.0) | 0(0.0) | 3(4.1) | 4(1.7) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | GAC→GGC | <i>Asp516Gly</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(2.3) | 0(0.0) | 1(0.4) |
| | C→T | <i>C(-15)→T</i> | | | | | | | |
| | CAC→CGC | <i>His526Arg</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(2.3) | 0(0.0) | 1(0.4) |
| | C→T | <i>C(-15)→T</i> | | | | | | | |
| | TCG→TGG | <i>Ser531Trp</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(2.3) | 0(0.0) | 1(0.4) |
| | C→T | <i>C(-15)→T</i> | | | | | | | |

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|---|-----------------|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
| <i>rpoB</i> + <i>rpoB</i> + <i>KatG</i> | TCG→TTG | <i>Ser531Leu</i> + | 0(0.0) | 0(0.0) | 1(5.0) | 2(6.9) | 1(2.3) | 1(1.4) | 5(2.1) |
| | C→T | <i>C(-15)→T</i> | | | | | | | |
| | CTG→CCG+ | <i>Leu511Pro</i> + | | | | | | | |
| | GAC→GGC+ | <i>Asp516Gly</i> | 1(2.7) | 0(0.0) | 0(0.0) | 1(3.5) | 0(0.0) | 0(0.0) | 2(0.8) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CTG→CCG+ | <i>Leu511Pro</i> + | | | | | | | |
| | GAC→GTC+ | <i>Asp516Val</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(1.4) | 1(0.4) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CTG→CCG+ | <i>Leu511Pro</i> + | | | | | | | |
| | CAC→TAC+ | <i>His526Tyr</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(1.4) | 1(0.4) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | CAA→CCA+ | <i>Gln513Pro</i> + | | | | | | | |
| | GAC→GTC+ | <i>Asp516Val</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(1.4) | 1(0.4) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| | GAC→GGC+ | <i>Asp516Gly</i> + | | | | | | | |
| | CTG→CCG+ | <i>Leu533Pro</i> + | 2(5.4) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 2(0.8) |
| | AGC→AAC | <i>Ser315Asn</i> | | | | | | | |
| | GAC→GGC+ | <i>Asp516Gly</i> + | | | | | | | |
| | CTG→CCG+ | <i>Leu533Pro</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(1.4) | 1(0.4) |
| | AGC→ACC | <i>Ser315Thr</i> | | | | | | | |
| <i>rpoB</i> + <i>KatG</i> + <i>inhA</i> | TCG→TTG+ | <i>Ser531Leu</i> + | | | | | | | |
| | AGC→ACC | <i>Ser315Thr</i> + | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 3(4.1) | 3(1.2) |
| | C→T | <i>C(-15)→T</i> | | | | | | | |
| Other locus | Other mutations | Other substitutions | 6(16.2) | 34(87.2)* | 9(45.0)* | 0(0.0)* | 0(0.0)* | 2(2.8)* | 51(21.1) |
| Total | | | 37(100.0) | 39(100.0) | 20(100.0) | 29(100.0) | 43(100.0) | 74(100.0) | 242(100.0) |

Note: * indicates that the difference is statistically significant compared with 2014

References

1. Krishna M, Gole SG: **Comparison of Conventional Ziehl-Neelsen Method of Acid Fast Bacilli with Modified Bleach Method in Tuberculous Lymphadenitis.** *J Cytol* 2017, **34**(4):188-192.
2. Vilchèze C, Jacobs WR, Jr.: **The Isoniazid Paradigm of Killing, Resistance, and Persistence in *Mycobacterium tuberculosis*.** *J Mol Biol* 2019, **431**(18):3450-3461.
3. Yang X, Yuan T, Ma R, Chacko KI, Smith M, Deikus G, Sebra R, Kasarskis A, van Bakel H, Franzblau SG *et al*: **Mce3R Stress-Resistance Pathway Is Vulnerable to Small-Molecule Targeting That Improves Tuberculosis Drug Activities.** *ACS Infect Dis* 2019, **5**(7):1239-1251.
4. Chen S, Guo L, Wang Z, Mao W, Ge Y, Ying X, Fang J, Long Q, Liu Q, Xiang H *et al*: **Current situation and progress toward the 2030 health-related Sustainable Development Goals in China: A systematic analysis.** *PLoS Med* 2019, **16**(11).
5. He XC, Zhang XX, Zhao JN, Liu Y, Yu CB, Yang GR, Li HC: **Epidemiological Trends of Drug-Resistant Tuberculosis in China From 2007 to 2014: A Retrospective Study.** *Medicine* 2016, **95**(15):0000000000003336.
6. Gebreweld FH, Kifle MM, Gebremicheal FE, Simel LL, Gezae MM, Ghebreyesus SS, Mengsteab YT, Wahd NG: **Factors influencing adherence to tuberculosis treatment in Asmara, Eritrea: a qualitative study.** *J Health Popul Nutr* 2018, **37**(1):017-0132.
7. Nguyen L: **Antibiotic resistance mechanisms in *M. tuberculosis*: an update.** *Arch Toxicol* 2016, **90**(7):1585-1604.
8. Zhao LL, Chen Y, Chen ZN, Liu HC, Hu PL, Sun Q, Zhao XQ, Jiang Y, Li GL, Tan YH *et al*: **Prevalence and molecular characteristics of drug-resistant *Mycobacterium tuberculosis* in Hunan, China.** (1098-6596 (Electronic)).
9. Yin QQ, Jiao WW, Li QJ, Xu F, Li JQ, Sun L, Li YJ, Huang HR, Shen AD: **Prevalence and molecular characteristics of drug-resistant *Mycobacterium tuberculosis* in Beijing, China: 2006 versus 2012.** *BMC Microbiol* 2016, **16**(85):016-0699.
10. Guo Y, Zhou Y, Wang C, Zhu L, Wang S, Li Q, Jiang G, Zhao B, Huang H, Yu H *et al*: **Rapid, accurate determination of multidrug resistance in *M. tuberculosis* isolates and sputum using a biochip system.** *Int J Tuberc Lung Dis* 2009, **13**(7):914-920.
11. Kigozi E, Kasule GW, Musisi K, Lukoye D, Kyobe S, Katabazi FA, Wampande EM, Joloba ML, Kateete DP: **Prevalence and patterns of rifampicin and isoniazid resistance conferring mutations in *Mycobacterium tuberculosis* isolates from Uganda.** *PLoS One* 2018, **13**(5).
12. Tadesse M, Aragaw D, Dimah B, Efa F, Abdella K, Kebede W, Abdissa K, Abebe G: **Drug resistance-conferring mutations in *Mycobacterium tuberculosis* from pulmonary tuberculosis patients in Southwest Ethiopia.** *Int J Mycobacteriol* 2016, **5**(2):185-191.
13. Chen L, Pang Y, Ma L, Yang H, Ru H, Yang X, Yan S, Jia M, Xu L: **First Insight into the Molecular Epidemiology of *Mycobacterium tuberculosis* Isolates from the Minority Enclaves of Southwestern China.** *Biomed Res Int* 2017, **2505172**(10):17.
14. Luo D, Chen Q, Xiong G, Peng Y, Liu T, Chen X, Zeng L, Chen K: **Prevalence and molecular characterization of multidrug-resistant *M. tuberculosis* in Jiangxi province, China.** *Sci Rep* 2019, **9**(1):019-43547.
15. Yu XL, Wen ZL, Chen GZ, Li R, Ding BB, Yao YF, Li Y, Wu H, Guo XK, Wang HH *et al*: **Molecular characterization of multidrug-resistant *Mycobacterium tuberculosis* isolated from south-central in China.** *J Antibiot* 2014, **67**(4):291-297.

16. Zhang Z, Liu M, Wang Y, Pang Y, Kam KM, Zhao Y: **Molecular and phenotypic characterization of multidrug-resistant *Mycobacterium tuberculosis* isolates resistant to kanamycin, amikacin, and capreomycin in China.** *Eur J Clin Microbiol Infect Dis* 2014, **33**(11):1959-1966.
17. Zhao LL, Chen Y, Chen ZN, Liu HC, Hu PL, Sun Q, Zhao XQ, Jiang Y, Li GL, Tan YH *et al*: **Prevalence and molecular characteristics of drug-resistant *Mycobacterium tuberculosis* in Hunan, China.** *Antimicrob Agents Chemother* 2014, **58**(6):3475-3480.
18. Zhao LL, Chen Y, Liu HC, Xia Q, Wu XC, Sun Q, Zhao XQ, Li GL, Liu ZG, Wan KL: **Molecular characterization of multidrug-resistant *Mycobacterium tuberculosis* isolates from China.** *Antimicrob Agents Chemother* 2014, **58**(4):1997-2005.
19. Rosser A, Marx FM, Pareek M: **Recurrent tuberculosis in the pre-elimination era.** *Int J Tuberc Lung Dis* 2018, **22**(2):139-150.
20. Liao S, Cai C, Huo FM, Wu JN, Kong CC, Huang HR, Xu SF, Sun ZG: **Trends in drug-resistant tuberculosis in China: data from a clinical tuberculosis centre.** (1815-7920 (Electronic)).
21. Chen MY, Lo YC, Chen WC, Wang KF, Chan PC: **Recurrence after Successful Treatment of Multidrug-Resistant Tuberculosis in Taiwan.** *PLoS One* 2017, **12**(1).
22. Timire C, Metcalfe JZ, Chirenda J, Scholten JN, Manyame-Murwira B, Ngwenya M, Matambo R, Charambira K, Mutunzi H, Kalisvaart N *et al*: **Prevalence of drug-resistant tuberculosis in Zimbabwe: A health facility-based cross-sectional survey.** *Int J Infect Dis* 2019, **87**:119-125.
23. Faustini A, Hall AJ, Fau - Perucci CA, Perucci CA, Harausz EA-O, Garcia-Prats AJ, Law S, Schaaf HS, Kredo T, Seddon JA-O, Menzies D *et al*: **Risk factors for multidrug resistant tuberculosis in Europe: a systematic review**
Treatment and outcomes in children with multidrug-resistant tuberculosis: A systematic review and individual patient data meta-analysis
Risk factors for tuberculosis in foreign-born people (FBP) in Italy: a systematic review and meta-analysis
Systematic review, meta-analysis and economic modelling of molecular diagnostic tests for antibiotic resistance in tuberculosis
Is HIV infection a risk factor for multi-drug resistant tuberculosis? A systematic review. (0040-6376 (Print)).
24. Yin QQ, Jiao WW, Li QJ, Xu F, Li JQ, Sun L, Li YJ, Huang HR, Shen AD: **Prevalence and molecular characteristics of drug-resistant *Mycobacterium tuberculosis* in Beijing, China: 2006 versus 2012.** (1471-2180 (Electronic)).
25. Lu Z, Jiang W, Zhang J, Lynn HS, Chen Y, Zhang S, Ma Z, Geng P, Guo X, Zhang H *et al*: **Drug resistance and epidemiology characteristics of multidrug-resistant tuberculosis patients in 17 provinces of China.** *PLoS One* 2019, **14**(11):e0225361.
26. Li Q, Wang Y, Li Y, Gao H, Zhang Z, Feng F, Dai E: **Characterisation of drug resistance-associated mutations among clinical multidrug-resistant *Mycobacterium tuberculosis* isolates from Hebei Province, China.** *Journal of Global Antimicrobial Resistance* 2019, **18**:168-176.
27. Liu L, Jiang F, Chen L, Zhao B, Dong J, Sun L, Zhu Y, Liu B, Zhou Y, Yang J *et al*: **The impact of combined gene mutations in *inhA* and *ahpC* genes on high levels of isoniazid resistance amongst *katG* non-315 in multidrug-resistant tuberculosis isolates from China.** *Emerg Microbes Infect* 2018, **7**(1):018-0184.
28. Llerena C, Medina R: **[Description of *Mycobacterium tuberculosis* mutations conferring**

- 522 **resistance to rifampicin and isoniazid detected by GenoType® MTBDRplus V.2 in Colombia].**
 523 *Biomedica* 2017, **37**(1):28-33.
- 524 29. Matsui T, Pinhata JMW, Rabello M, Brandão AP, Ferrazoli L, Leão SC, Viana-Niero C, Oliveira RS:
 525 **Frequency of first and second-line drug resistance-associated mutations among resistant**
 526 ***Mycobacterium tuberculosis* clinical isolates from São Paulo, Brazil.** (1678-8060
 527 (Electronic)).
- 528 30. Jagielski T, Bakula Z, Roeske K, Kamiński M, Napiórkowska A, Augustynowicz-Kopeć E, Zwolska
 529 Z, Bielecki J: **Mutation profiling for detection of isoniazid resistance in *Mycobacterium***
 530 ***tuberculosis* clinical isolates.** *J Antimicrob Chemother* 2015, **70**(12):3214-3221.
- 531 31. Li Q, Wang Y, Li Y, Gao H, Zhang Z, Feng F, Dai E: **Characterisation of drug**
 532 **resistance-associated mutations among clinical multidrug-resistant *Mycobacterium***
 533 ***tuberculosis* isolates from Hebei Province, China.** *J Glob Antimicrob Resist* 2019, **18**:168-176.
- 534 32. Isakova J, Sovkhozova N, Vinnikov D, Goncharova Z, Talaibekova E, Aldasheva N, Aldashev A:
 535 **Mutations of *rpoB*, *katG*, *inhA* and *ahp* genes in rifampicin and isoniazid-resistant**
 536 ***Mycobacterium tuberculosis* in Kyrgyz Republic.** *BMC Microbiol* 2018, **18**(1):018-1168.
- 537 33. Kanji A, Hasan R, Hasan Z: **Efflux pump as alternate mechanism for drug resistance in**
 538 ***Mycobacterium tuberculosis*.** (0019-5707 (Print)).
- 539