Infection and Drug Resistance

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ORIGINAL RESEARCH

# Comparison of Nucleotide MALDI-TOF MS with Xpert MTB/RIF for Rifampicin Susceptibility Identification and Associated Risk Factors of Rifampicin Resistance Among Drug Resistant *Mycobacterium tuberculosis*

Song Song[1,*], Honghong Xu[1,*], Jiawei Cao[2,*], Guanghong Wu[2], Haiyan Sun[1], Xiaoqi Dai[1], Xuekui Li[1], Meng Chen[1], Menghan Zhang[1], Yueming Yan[1], Jingfeng Tong[3], Zhongdong Wang[1]

[1]Institute of Tuberculosis Control, Qingdao Preventive Medicine Research Institute, Qingdao Municipal Center for Disease Control and Prevention, Qingdao, People's Republic of China; [2]Department of Laboratory Medicine, Qingdao Chest Hospital, Qingdao, Shandong, People's Republic of China; [3]Department of Medical Affairs, Shanghai Conlight Medical Co., Ltd., Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhongdong Wang, Institute of Tuberculosis Control, Qingdao Preventive Medicine Research Institute, Qingdao Municipal Center for Disease Control and Prevention, No. 8, Fengxian Road, Jinhu Road Street, Shinan District, Qingdao City, Shandong Province, 266000, People's Republic of China, Email jkzxtb1@163.com

**Purpose:** Nucleotide-based matrix-assisted laser desorption ionization time-of-flight mass spectrometry (nucleotide MALDI-TOF MS) is an emerging molecular technology used for the diagnosis of tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) and its drug resistance. This study aimed to compare the ability of nucleotide MALDI-TOF MS to detect rifampicin (RIF) resistance in drug-resistant TB (DR-TB) patients with Xpert MTB/RIF and to analyze the disparate results individually. Additionally, potential factors associated with rifampicin resistance among DR-TB patients in Qingdao were investigated.

**Patients and Methods:** A retrospective study was conducted at Qingdao Chest Hospital, and patients with DR-TB were enrolled. Corresponding frozen isolates were recovered and subjected to nucleotide MALDI-TOF MS, Xpert MTB/RIF, and phenotypic drug susceptibility testing (pDST). Sanger sequencing was performed for the discordant results of nucleotide MALDI-TOF MS and Xpert MTB/RIF. Univariate and multivariate logistic regression analyses were used to identify potential factors associated with rifampicin resistance among patients with DR-TB.

**Results:** A total of 125 patients with DR-TB (18.8%, 125/668) were enrolled in this study from May 1 to July 31, 2023. Rifampicin-resistant (DR-TB/RR, 29) and rifampicin-sensitive (DR-TB/RS, 96) groups were divided according to the pDST results. Nucleotide MALDI-TOF MS performed better than Xpert MTB/RIF in terms of sensitivity, specificity, accuracy, and agreement with pDST. Only six cases had inconsistent results, and the sequencing results of five cases were identical to nucleotide MALDI-TOF MS. Furthermore, chest pain (aOR=12.84, 95% CI, 2.29–91.97, p=0.005), isoniazid sensitivity (aOR=0.14, 0.02–0.59, p=0.013), and ethambutol sensitivity (aOR=0.02, 0.00–0.10, p=0.000) were potential factors associated with rifampicin resistance among DR-TB patients in Qingdao.

**Conclusion:** The overall concordance between nucleotide MALDI-TOF MS and Xpert MTB/RIF was 95.2%, with the former performing better in determining rifampicin susceptibility among DR-TB cases in Qingdao. Chest pain, isoniazid, and ethambutol resistance might be factors associated with RIF resistance among patients with DR-TB in Qingdao.

**Keywords:** MTB, rifampicin, nucleotide MALDI-TOF MS, Xpert MTB/RIF, comparison, risk factors

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## Introduction

Tuberculosis (TB), one of the leading causes of death due to a single infectious agent -*Mycobacterium tuberculosis* (MTB), remains a global public health problem that poses a serious challenge to human health and social development, with 1.4 million deaths in 2022 according to the World Health Organization (WHO).[1] More than two thirds of global TB deaths occurred in eight countries, and China ranked third.[1] Furthermore, multidrug-resistant tuberculosis (MDR-TB) defined as TB resistant to at least isoniazid (INH) and rifampicin (RIF), has made the situation even worsened. China is the second-highest contributor to the global burden of MDR-TB, with a share of 14%.[2] Early diagnosis and treatment commencement of TB and MDR-TB are of great importance for achieving the global END-TB strategy targets.[3]

The Xpert MTB/RIF assay, the WHO-recommended molecular test for first-line diagnosis of TB helpful in achieving the targets,[4] has demonstrated high accuracy in numerous studies.[5–8]The assay is a cartridge-based fully automated nucleic acid amplification (real-time polymerase chain reaction) technology used in pulmonary and extra-pulmonary specimens for the rapid detection of MTB and to determine resistance of MTB to RIF. However, it has an obvious drawback in that the assay solely identifies RIF resistance associated with the *rpoB* mutation and depends largely on the bacterial loads of the samples in determining RIF resistance.[9] The Xpert MTB/RIF assay is available as an initial diagnostic method for all patients suspected with TB only in 2019 at Qingdao Chest Hospital.

The Nucleotide MALDI-TOF MS assay, a relatively novel technology, is a matrix-assisted laser desorption ionization time-of-flight mass spectrometry based on nucleotides, possessing the advantages of rapidity, high throughput, and high accuracy. By conducting multiple polymerase chain reaction (PCR) reactions targeting specific gene segments and single nucleotide extension reactions, the resulting product is combined with matrix, and a mass spectrum is generated finally, providing information on the species and drug resistance. The technology can be applied to clinical microbiological testing. Rybicka et al have demonstrated the superiority of nucleotide MALDI-TOF MS over real-time PCR for SARS-CoV-2 RNA detection.[10] Several studies have demonstrated the promising application of this technique in TB diagnosis and its drug resistance.[11–13] Recently, Gao et al published a study on the positive rates and drug resistance characteristics of MTB among presumed TB patients in Shandong Province based on the results of nucleotide MALDI-TOF MS,[14] indicating that this method is being increasingly recognized by clinicians. Moreover, an expert consensus for the clinical application of nucleotide MALDI-TOF MS for TB diagnosis was published in 2023.[15] Therefore, we here conducted a comparative analysis of the diagnostic determination of nucleotide MALDI-TOF MS and Xpert MTB/RIF for RIF susceptibility in MTB-cultured samples.

To this end, we retrospectively used the samples isolated from drug-resistant (DR) TB (DR-TB) patients admitted to Qingdao Chest Hospital between May 1 and July 31, 2023, using phenotypic drug susceptibility testing (pDST) results as the gold standard. Furthermore, we explored the potential risk factors associated with RIF resistance among DR-TB patients in Qingdao, providing references for prevention and targeted control policies in Qingdao.

## Materials and Methods

### Study Design

This study was conducted at Qingdao Chest Hospital, Qingdao, Shandong Province, eastern China. The hospital is known as a TB-designated hospital providing diagnosis and treatment services for almost all suspected TB patients in Qingdao, with over 200 beds, and is the only designated hospital in Qingdao with the capacity to test susceptibility to anti-TB drugs. We performed a retrospective review of the records of patients with confirmed TB between May 1, 2023, and July 31, 2023, to identify all drug-resistant (DR) cases. DR-TB cases in our study were defined as patients who were resistant to arbitrary anti-TB drugs, based on phenotypic drug susceptibility testing (pDST) results. Sociodemographic and clinical examination information for these cases was obtained from the case registration system. The variables included age, sex, history of tuberculosis, and comorbidities, etc. All MTB isolates were collected in 7H9 medium supplemented with 10% of oleic acid-albumin-dextrose-catalase (OADC) and 10% glycerol and stored at −80°C. Prior to testing, the corresponding frozen isolates were recovered and subcultured on the Löwenstein-Jensen (L-J) medium at 37°C for weeks until colony formation. The recovered isolates were scraped from the medium and suspended in sterile

saline and mixed thoroughly. The bacterial suspension was divided into three equal parts, subjected to Xpert MTB/RIF assay, nucleotide MALDI-TOF MS assay, and pDST.

## Ethics Statement

The study protocol was approved by the Ethics Committee of the Qingdao Municipal Center for Disease Control and Prevention (2023–05) and the requirement for individual consent was waived. This study was conducted in accordance with the principles of the Declaration of Helsinki of the World Medical Association and the Good Clinical Practice Guidelines. We had no access to information that could identify individual participants during or after data collection and we kept patient data confidentially.

## Phenotypic Drug Susceptibility Testing (pDST)

Phenotypic DST for cultured isolates was broth microdiluted using a Sensititre MYCOTB MIC plate (Baso, Guangzhou Province, China), according to the manufacturer's instructions. In brief, the bacterial suspension from the recovered isolates was vortexed and adjusted to 0.5 McFarland standards using a nephelometer. The prepared 100 μL bacterial suspension was added to each well of the MYCOTB plate, which was then covered with an adhesive seal and incubated at 37°C for 10 days. The critical concentrations for rifampin (RIF), isoniazid (INH), ethambutol (EMB), streptomycin (SM), fluoroquinolones (FQs) (moxifloxacin) were 1.0 mg/L, 0.2 mg/L, 5.0, 2.0, and 0.5 mg/L, respectively.

## Xpert MTB/RIF Assay

The Xpert MTB/RIF assay was performed in accordance with the manufacturer's instructions. In brief, cultured bacteria were scraped from the medium and collected into sterile screw-capped falcon tubes with a volume of 15 mL volume. The sample reagent was poured into the sample containing the tube (the ratio of sample reagent to sample was 2:1). The tubes were tightly screw-capped and vortexed vigorously before incubation at room temperature for 15 min. Then, 2 mL of the mixture was transferred to an Xpert MTB/RIF cartridge. The cartridge was then loaded into an Xpert instrument (Cepheid Inc., Sunnyvale, CA, USA) for DNA extraction and amplification of *rpoB*. Each probe indicates a different region of mutation, as the probes hybridize to the sequence of the *rpoB* gene: A (codons 507–511), B (codons 512–518), C (codons 518–523), D (codons 523–529) and E (codons 529–533), as illustrated in Figure 1.

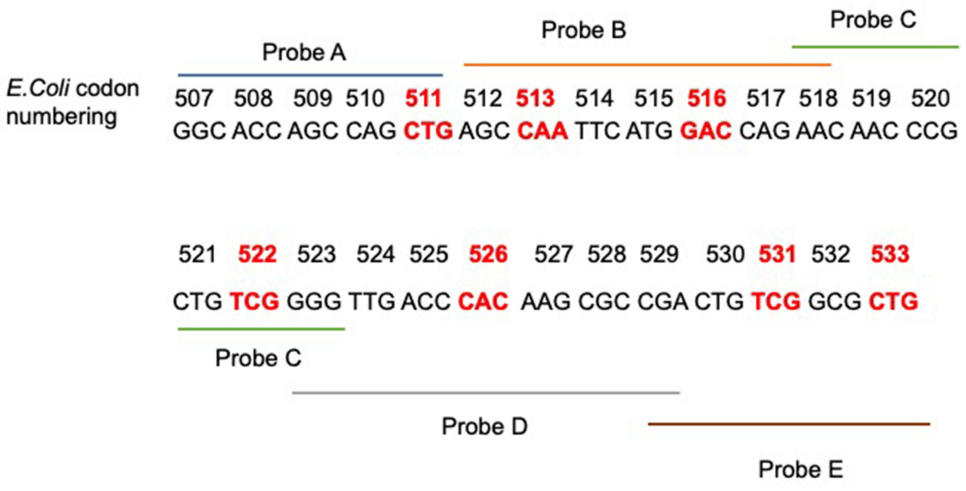


**Figure 1** An illustration of detection of *rpoB* RRDR by Xpert MTB/RIF and nucleotide MALDI-TOF MS. 81-bp rifampicin resistance determining region (RRDR) of MTB *rpoB* gene with corresponding *Escherichia coli* (*E. coli*) codon numbering is presented. The five lines represent the coverage areas of the five probes of Xpert MTB/RIF. The red labelled bold font is sites for nucleotide MALDI-TOF MS detection.

## Nucleotide MALDI-TOF MS Assay

The assay used the Conlight TB&DR®assay, which is well-established and conducted by Shanghai Conlight Medical Co., Ltd. This assay integrates the sensitivity of PCR technology with the high-throughput nature of mass spectrometry detection technology. One reaction can amplify up to 40 genes, which can be used for single nucleotide polymorphism analysis, mutant detection, and other research areas. The cultured bacteria were heat-inactivated and transported under appropriate cold chain conditions. Specific procedures can be found in the literature.[11,12] Briefly, the process included the following six steps: extraction of nucleic acids from samples, PCR to obtain target fragments, shrimp alkaline phosphatase (SAP) reaction, extension reaction, detection on the MS instrument, and automatic analysis of results using MassARRAY analysis software. The codons covered by the extension probes were 511CTG, 513CAA, 516GAC, 522TCG, 526CAC, 531TCG, and 533CTG, as shown in red in Figure 1.

## Sanger Sequencing

Sanger sequencing was performed for isolates with discordant results from Xpert MTB/RIF and nucleotide MALDI-TOF MS assays. The primers used for Sanger sequencing were *rpoB* forward primer, 5'-GATCACACCGCAGACGTTGA-3' and *rpoB* reverse primer, 5'-AACATCGGTCTGATCGGCTC-3'. The purified PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit on a 3730xl DNA Analyzer (Applied Biosystems). Sequence analysis was performed using BLAST, with the MTB H37Rv genome (NC_000962.3) as the reference sequence.

## Data Processing and Statistical Analysis

All patient information was recorded in EXCEL and analyzed using R 4.2.0. Descriptive statistics were calculated and presented as frequencies (percentages) for categorical variables and medians with interquartile ranges (IQR) for continuous variables. The Wilcoxon rank-sum and chi-squared tests were used to compare continuous and categorical variables, respectively. A univariate logistic regression analysis was conducted to determine the factors and variables associated with RIF resistance. Variables with a statistical significance of $p<0.200$ on univariate analysis were included in multivariate analysis. The sensitivity, specificity, accuracy, and consistency with 95% confidence interval (95% CI) of Xpert MTB/RIF and nucleotide MALDI-TOF MS were calculated using the pDST results as the reference criteria. The chi-square test was used to compare the indicators obtained using the various methods. Statistical significance was set at $p<0.05$.

# Results

## Basic Characteristics of DR-TB Cases in This Study

As shown in the flow chart (Figure 2), 668 cases were diagnosed with TB during the study period (May 1 to July 31, 2023), of which 125 cases (18.8%) were DR-TB based on the pDST results and were finally included in the study. The characteristics of these DR-TB patients with DR-TB are presented in Table 1. Of these patients, 96 (76.8%) were males and 29 (23.2%) were females, with a median age of 50.0 years (interquartile range [IQR]: 38.0–64.5) and a high concentration in the 26- to 65-year-old range. No history of smoking or alcohol consumption was present in 73 (58.4%) and 77 (61.6%) patients, respectively. Regarding comorbidities, diabetes mellitus (DM) were present in 26 (20.8%) patients and hypertension in 19 (15.2%) patients. The majority of cases included in the present study were primary patients (86.4%, 108/125). In terms of clinical symptoms, cough and sputum were the two most common symptoms, accounting for 77.6% and 74.4%, respectively, followed by fatigue (31.2%), fever (28.8%), and weight loss (27.2), with chest pain (12.8%) and hemoptysis (11.2%) accounting for the minority. As for drug resistance, more than half of the patients were resistant to streptomycin (SM) and isoniazid (INH), amounting to 64.0% and 51.2%, respectively, followed by rifampicin (RIF) and fluoroquinolones (FQs), which were closer to each other, at 23.2% and 22.4%, respectively (Table 1). We divided the patients into RIF-resistant (DR-TB/RR) and RIF-sensitive (DR-TB/RS) groups according to whether they were RIF-resistant based on the pDST. A comparison of demographic and clinical variables between the DR-TB/RR and DR-TB/RS groups is presented in Table 2. We found that the rates of resistance to INH and EMB in the DR-TB/RR group were much higher than those in the DS-TB/RS group (INH: 86.2% vs 40.6%, $p<0.001$; EMB: 58.6% vs 4.2%, $p<0.001$). Furthermore, serum aspartate aminotransferase (AST) levels were significantly higher in the DR-TB/RR group than in the DR-TB/RS group ($p=0.027$). The remaining variables were not significantly different between the groups (Table 2).

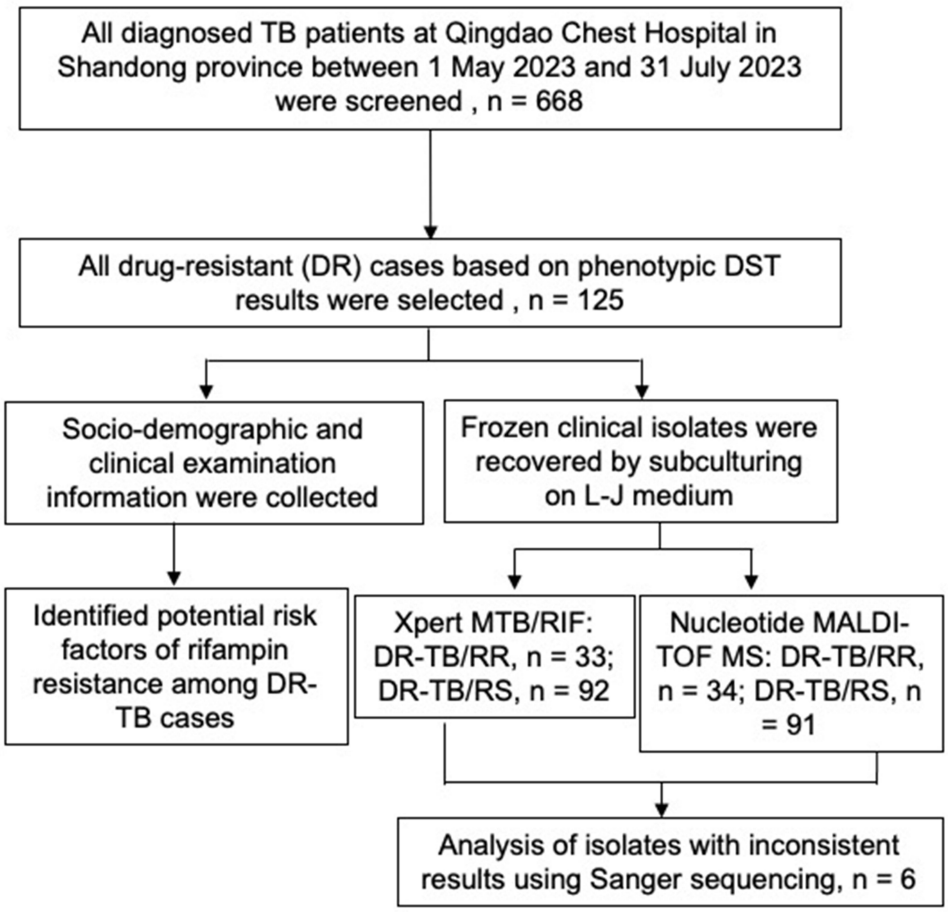


**Figure 2** Flow diagram of the study design.

## Diagnostic Performance of Nucleotide MALDI-TOF MS and Xpert MTB/RIF on Rifampicin Susceptibility Detection

According to nucleotide MALDI-TOF MS, 34 isolates (27.2%, 34/125) were identified as rifampicin-resistant, of which *rpoB* codon 531 TCG>TTG mutant was the most frequent, followed by *rpoB* codon 526, accounting for 61.8% (21/34)

**Table 1** Basic Information of Study Population

| Categories | Cases (n) | Percent (%) |
|---|---|---|
| **Gender** | | |
| Male | 96 | 76.8 |
| Female | 29 | 23.2 |
| **Age (median, IQR)** | 50.0 (38.0–64.5) | |
| ~25 | 13 | 10.4 |
| 26~45 | 39 | 31.2 |
| 46~65 | 46 | 36.8 |
| 66~ | 27 | 21.6 |

(*Continued*)

**Table 1** (Continued).

| Categories | Cases (n) | Percent (%) |
|---|---|---|
| **Smoking** | | |
| Yes | 52 | 41.6 |
| No | 73 | 58.4 |
| **Drinking** | | |
| Yes | 48 | 38.4 |
| No | 77 | 61.6 |
| **Hypertension** | | |
| Yes | 19 | 15.2 |
| No | 106 | 84.8 |
| **Diabetes mellitus** | | |
| Yes | 26 | 20.8 |
| No | 99 | 79.2 |
| **TB treatment history** | | |
| Yes | 17 | 13.6 |
| No | 108 | 86.4 |
| **Clinical symptoms** | | |
| Cough | 98 | 78.4 |
| Expectoration | 94 | 75.2 |
| Fatigue | 39 | 31.2 |
| Fever | 37 | 29.6 |
| Weight loss | 36 | 28.8 |
| Chest pain | 16 | 12.8 |
| Hemoptysis | 14 | 11.2 |
| **Anti-TB drug resistance rate** | | |
| RIF | 29 | 23.2 |
| INH | 64 | 51.2 |
| EMB | 21 | 16.8 |
| SM | 80 | 64.0 |
| FQs | 28 | 22.4 |

**Abbreviations**: TB, Tuberculosis; IQR, Interquartile range; RIF, rifampicin; INH, isoniazid; EMB, ethambutol; SM, streptomycin; FQs, fluoroquinolones.

and 20.6% (7/34), respectively (Figure 3a). Xpert MTB/RIF detected 33 isolates (26.4%, 33/125) to be resistant to rifampicin. Consistent with the nucleotide MALDI-TOF MS results, probe E, which covered *rpoB* codon 531, was the most predominant, followed by probe D, which covered *rpoB* codon 526 (Figure 3b).

**Table 2** Comparison of Demographic and Clinical Characteristics Between DR-TB/RR and DR-TB/RS Groups

| Characteristics | DR-TB/RR n=29 | DR-TB/RS n=96 | *p* Value |
|---|---|---|---|
| **Age (median, IQR)** | 48.0 (38.0–62.0) | 50.0 (38.5–64.3) | 0.907 |
| **Gender (n, %)** | | | >0.990 |
| Male | 22 (75.9) | 74 (77.1) | |
| Female | 7 (24.1) | 22 (22.9) | |
| **Smoking** | | | >0.990 |
| Yes | 12 (41.4) | 40 (41.7) | |
| No | 17 (58.6) | 56 (58.3) | |
| **Drinking** | | | 0.513 |
| Yes | 9 (31.0) | 38 (39.6) | |
| No | 20 (69.0) | 58 (60.4) | |
| **Hypertension** | | | >0.990 |
| Yes | 4 (13.8) | 15 (15.6) | |
| No | 25 (86.2) | 81 (84.4) | |
| **Diabetes mellitus (n, %)** | | | 0.190 |
| Yes | 3 (10.3) | 23 (24.0) | |
| No | 26 (89.7) | 73 (76.1) | |
| **TB treatment history (n, %)** | | | 0.541 |
| Yes | 5 (17.2) | 12 (12.5) | |
| No | 24 (82.8) | 84 (87.5) | |
| **Anti-TB drug resistance rate (n, %)** | | | |
| INH | 25 (86.2) | 39 (40.6) | **<0.001*** |
| EMB | 17 (58.6) | 4 (4.2) | **<0.001*** |
| SM | 23 (79.3) | 57 (59.4) | 0.076 |
| FQs | 10 (34.5) | 18 (18.8) | 0.082 |
| **Clinical symptoms (n, %)** | | | |
| Cough | 23 (79.3) | 75 (77.1) | >0.99 |
| Expectoration | 22 (75.9) | 72 (74.0) | >0.99 |
| Fatigue | 6 (20.7) | 33 (34.4) | 0.180 |
| Fever | 10 (34.5) | 27 (28.1) | 0.498 |
| Weight loss | 5 (17.2) | 31 (32.3) | 0.161 |
| Chest pain | 6 (20.7) | 10 (10.4) | 0.201 |
| Hemoptysis | 3 (10.3) | 11 (11.5) | >0.99 |
| **ALT (Median, IQR)** | 13.0 (8.0–23.5) | 14.0 (10.8–20.0) | 0.997 |
| **AST (Median, IQR)** | 23.0 (16.0–31.5) | 19.0 (14.0–24.0) | **0.027*** |

**Notes**: * Significant values are in bold.

**Abbreviations**: DR-TB, drug-resistant tuberculosis; RR, rifampicin-resistant; RS, rifampicin-sensitive; INH, isoniazid; EMB, ethambutol; SM, streptomycin; FQs, fluoroquinolones; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; IQR, Interquartile range.

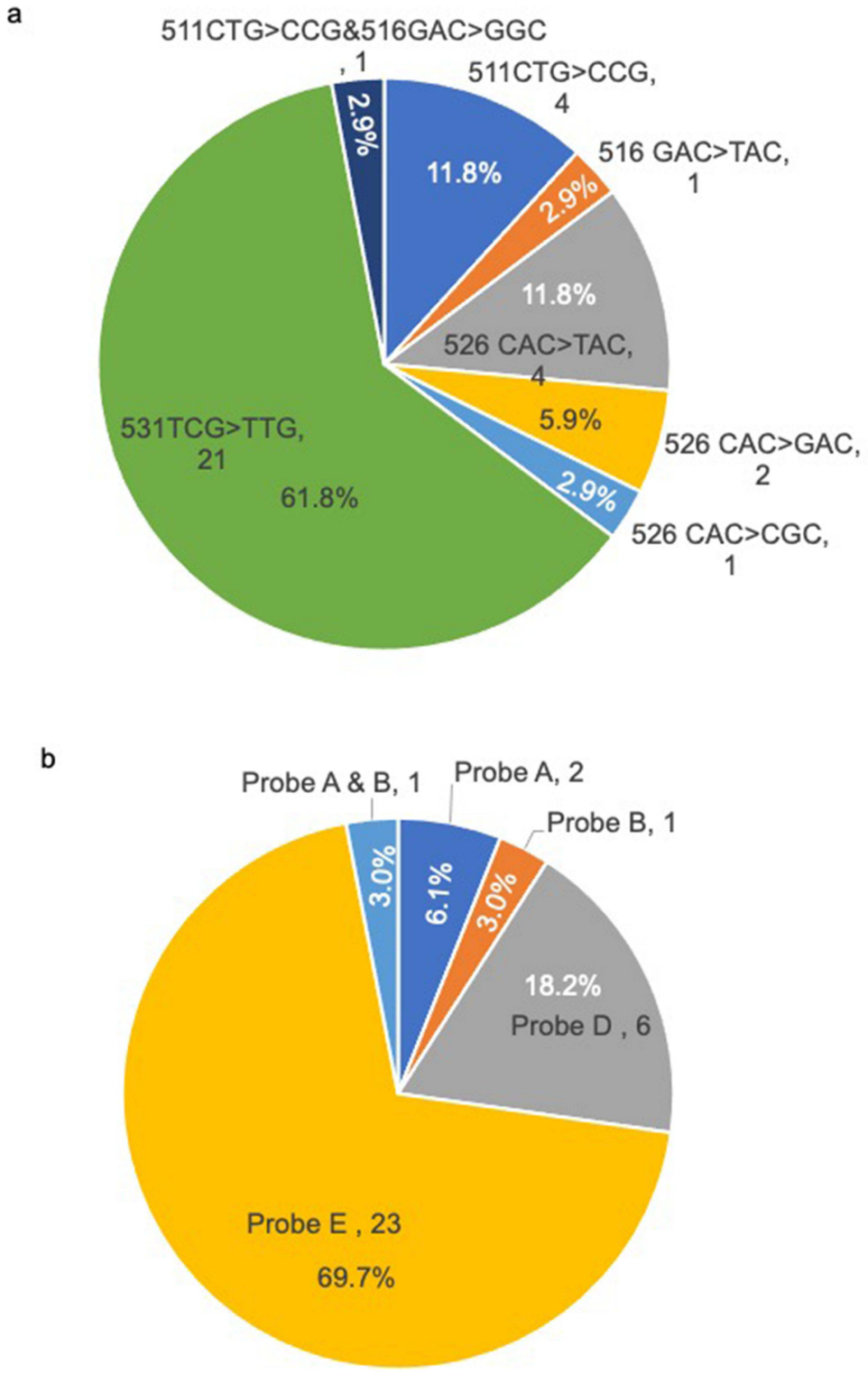


**Figure 3** Composition of *rpoB* mutation types detected by nucleotide MALDI-TOF MS and Xpert MTB/RIF. (**a**) Pie chart of the types, number and percentage of *rpoB* mutations detected by nucleotide MALDI-TOF MS. (**b**) Pie chart of the number and percentage of Xpert MTB/RIF mutant probes.

Using culture results as the gold standard, the sensitivity, specificity, and accuracy of nucleotide MALDI-TOF MS for RIF resistance detection were 93.1% (95% CI: 77.2–99.2%), 92.7% (85.6–97.0%) and 92.8% (86.8–96.7%), respectively, all higher than those of Xpert MTB/RIF to be 86.2% (68.3–96.1%), 91.7% (84.2–96.3%) and 90.4% (83.8–94.9%),

respectively. When kappa statistics were applied, the consistency of nucleotide MALDI-TOF MS with culture was 0.81 (0.69–0.93), slightly outperformed Xpert MTB/RIF, which was 0.74 (0.61–0.88) with culture (Table 3).

## Analyses for Inconsistent Results of Nucleotide MALDI-TOF MS and Xpert MTB/RIF

We further analyzed the discrepancies between the nucleotide MALDI-TOF MS and Xpert MTB/RIF results individually and found that six cases had inconsistent results, one of which (Case 96) was resistant by both of these two diagnostic methods but with different mutant types. However, this sample was sensitive to rifampicin, based on the pDST results. The remaining five cases showed different rifampicin susceptibility results using nucleotide MALDI-TOF MS and Xpert MTB/RIF. Case 10, 45, and 73 were rifampicin resistant by nucleotide MALDI-TOF MS and only case 45 was sensitive by pDST. Cases 117 and 120 were both rifampicin sensitive according to nucleotide MALDI-TOF MS and were consistent with pDST. Overall, among all discrepant results, 4/6 results identified by nucleotide MALDI-TOF MS were identical to those of pDST (Table 4).

Sanger sequencing of the *rpoB* core region (RRDR) was conducted to analyze inconsistent isolates between the two molecular diagnostic methods. We retrieved the results for all the cases, except for Case 96. Our sequencing results (Table 4 and Supplementary Table 1) are in agreement with those obtained using nucleotide MALDI-TOF MS.

## Identification of Associated Factors for Rifampicin Resistance Among DR-TB Patients

We next sought to identify the sociodemographic and clinical factors associated with rifampicin resistance in the included patients with DR-TB. Univariate regression analysis was initially performed to identify potential risk factors with $p<0.2$, which were consequently subjected to multivariable logistic regression analysis to control for potential confounders (Table 5). The results

**Table 3** Diagnostic Performance of Nucleotide MALDI-TOF MS and Xpert MTB/RIF for Rifampicin Susceptibility

| Test Method | Results of RIF Susceptibility | Culture | | Sensitivity (%, 95% CI) | Specificity (%, 95% CI) | Accuracy (%, 95% CI) | Kappa Value (95% CI) |
|---|---|---|---|---|---|---|---|
| | | RIF Resistant (n = 29) | RIF Sensitive (n = 96) | | | | |
| Nucleotide MALDI-TOF MS | Resistant | 27 | 7 | 93.1 (77.2–99.2) | 92.7 (85.6–97.0) | 92.8 (86.8–96.7) | 0.81 (0.69–0.93) |
| | Sensitive | 2 | 89 | | | | |
| Xpert MTB/RIF | Resistant | 25 | 8 | 86.2 (68.3–96.1) | 91.7 (84.2–96.3) | 90.4 (83.8–94.9) | 0.74 (0.61–0.88) |
| | Sensitive | 4 | 88 | | | | |

**Abbreviations**: RIF, rifampicin; CI, confidence interval.

**Table 4** Individual Analyses for Inconsistent Results of Rifampicin Susceptibility Identified by Nucleotide MALDI-TOF MS and Xpert MTB/RIF

| | Patient Number | Nucleotide MALDI-TOF MS | Xpert MTB/RIF | pDST | Sanger sequencing Results (*rpoB* RRDR) |
|---|---|---|---|---|---|
| Inconsistent Rifampicin susceptibility (n = 6, 4.8%) | 10 | Resistant (*rpoB* 511 CTG>CCG) | Sensitive | Resistant | 511 CCG |
| | 45 | Resistant (*rpoB* 526 CAC>GAC) | Sensitive | Sensitive | 526 GAC |
| | 73 | Resistant (*rpoB* 531 TCG>TTG) | Sensitive | Resistant | 531 TTG |
| | 96 | Resistant (*rpoB* 511 CTG>CCG) | Resistant (Probe E) | Sensitive | Failed |
| | 117 | Sensitive | Resistant (Probe E) | Sensitive | None |
| | 120 | Sensitive | Resistant (Probe E) | Sensitive | None |

**Abbreviations**: pDST, phenotypic drug susceptibility testing; RRDR, rifampicin resistance determining region.

**Table 5** Univariable and Multivariable Logistic Regression Analysis for Associated Factors of Rifampicin Resistant Among DR-TB Patients

| Variables | Univariable Analysis | | Multivariable Analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *p* Value | aOR (95% CI) | *p* Value |
| **Gender** | | 0.891 | | |
| Female | Ref. | | | |
| Male | 0.93 (0.36–2.62) | | | |
| **Age** | | | | |
| ~25 | Ref. | | | |
| 26~45 | 1.00 (0.24–5.16) | 1.000 | | |
| 46~65 | 0.93 (0.24–4.70) | 0.918 | | |
| 66~ | 1.17 (0.24–6.31) | 0.846 | | |
| **Smoking** | | 0.978 | | |
| No | Ref. | | | |
| Yes | 0.99 (0.42–2.28) | | | |
| **Drinking** | | 0.406 | | |
| No | Ref. | | | |
| Yes | 0.69 (0.27–1.63) | | | |
| **TB treatment history** | | 0.516 | | |
| Primary | Ref. | | | |
| Retreatment | 1.46 (0.43–4.37) | | | |
| **Diabetes mellitus** | | 0.125 | | 0.686 |
| No | Ref. | | Ref. | |
| Yes | 0.37 (0.08–1.17) | | 0.66 (0.06–4.47) | |
| **Hypertension** | | 0.810 | | |
| No | Ref. | | | |
| Yes | 0.86 (0.23–2.64) | | | |
| **Fever** | | 0.512 | | |
| No | Ref. | | | |
| Yes | 1.35 (0.54–3.22) | | | |
| **Cough** | | 0.892 | | |
| No | Ref. | | | |
| Yes | 1.07 (0.40–3.21) | | | |
| **Expectoration** | | 0.925 | | |
| No | Ref. | | | |
| **Yes** | 1.05 (0.41–2.92) | | | |

(*Continued*)

**Table 5** (Continued).

| Variables | Univariable Analysis | | Multivariable Analysis | |
|---|---|---|---|---|
| | **OR (95% CI)** | ***p* Value** | **aOR (95% CI)** | ***p* Value** |
| **Weight loss** | | 0.124 | | 0.651 |
| No | Ref. | | Ref. | |
| Yes | 0.44 (0.14–1.17) | | 0.67 (0.10–3.45) | |
| **Chest pain** | | 0.154 | | **0.005*** |
| No | Ref. | | Ref. | |
| Yes | 2.24 (0.70–6.72) | | **12.84 (2.29–91.97)*** | |
| **Fatigue** | | 0.169 | | 0.193 |
| No | Ref. | | Ref. | |
| Yes | 0.50 (0.17–1.28) | | 0.31 (0.04–1.57) | |
| **Hemoptysis** | | 0.868 | | |
| No | Ref. | | | |
| Yes | 0.87 (0.19–3.12) | | | |
| **ALT** | 1.01 (1.00–1.03) | 0.267 | | |
| **AST** | 1.03 (1.00–1.06) | 0.065 | 1.02 (0.99–1.07) | 0.549 |
| **INH** | | 0.000 | | **0.013*** |
| Resistant | Ref. | | Ref. | |
| Sensitive | 0.11 (0.03–0.31) | | **0.14 (0.02–0.59)*** | |
| **EMB** | | 0.000 | | **0.000*** |
| Resistant | Ref. | | Ref. | |
| Sensitive | 0.03 (0.01–0.10) | | **0.02 (0.00–0.10)*** | |
| **FQs** | | 0.079 | | 0.198 |
| Resistant | Ref. | | Ref. | |
| Sensitive | 0.44 (0.18–1.13) | | 0.32 (0.06–1.93) | |
| **SM** | | 0.055 | | 0.197 |
| Resistant | Ref. | | Ref. | |
| Sensitive | 0.38 (0.13–0.97) | | 0.36 (0.07–1.59) | |

**Notes**:* Significant values are in bold.
**Abbreviations**: INH, isoniazid; EMB, ethambutol; SM, streptomycin; FQs, fluoroquinolones; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CI, confidence interval; Ref, Reference; OR, odds ratio.

showed that the factors significantly associated with rifampicin resistance were chest pain (aOR=12.84, 95% CI, 2.29–91.97, p=0.005), INH sensitivity (aOR=0.14, 95% CI, 0.02–0.59, p=0.013), and EMB sensitivity (aOR=0.02, 95% CI, 0.00–0.10, p=0.000) (Table 5).

## Discussion

The main purpose of the present study was to compare two molecular diagnostic methods, namely nucleotide MALDI-TOF MS and Xpert MTB/RIF, for detecting rifampicin resistance of culture samples isolated from DR-TB patients. Our results showed that nucleotide MALDI-TOF MS performed better than Xpert MTB/RIF in determining RIF susceptibility in terms of sensitivity, specificity, accuracy, and consistency with the pDST. There was also excellent agreement (95.2%, 119/125) for detecting rifampicin susceptibility between these two molecular diagnostic methods. The secondary objective of our study was to explore the potential risk factors for rifampicin resistance among patients with DR-TB from Qingdao Chest Hospital. Chest pain, INH resistance, and EMB resistance were identified using a logistic regression analysis.

Nucleotide MALDI-TOF MS has emerged as a novel molecular detection technology in recent decades which does not require intermediate substances to mediate and directly detects nucleotides, and also serves as validation platform for sequencing technology.[16] In recent years, it has been increasingly used in the clinical identification of TB and DR-TB, with several studies proving that nucleotide MALDI-TOF MS has excellent clinical performance.[17,18] Xpert MTB/RIF has long been the first-line molecular diagnostic for identifying TB and RIF susceptibility, as it was at Qingdao Chest Hospital. Few studies have focused on direct comparison between nucleotide MALDI-TOF MS and Xpert MTB/RIF for the identification of MTB susceptibility to RIF. Although Liang et al recently performed a comparison between the two,[19] the Xpert MTB/RIF results were composite, that is, Xpert MTB/RIF tested more than one sample to obtain the result, which was equivalent to the result being a standard result, whereas nucleotide MALDI-TOF MS was only tested once, making it possible that there may be some bias. Nevertheless, the results of their study showed a good agreement (90%) between the two methods for the detection of RIF resistance. To avoid bias and to directly compare the diagnostic accuracy of these two methods for RIF susceptibility, the present study was carried out with cultured MTB isolates to compare the one-time results of nucleotide MALDI-TOF MS and Xpert MTB/RIF. Our results showed a high level of concordance (95.2%, 119/125) between nucleotide MALDI-TOF MS and Xpert MTB/RIF, with inconsistent results in only 6 patients. Sanger sequencing of *rpoB* RRDR was attempted for these disputed samples, and five of them were successfully generated, except for case 96, all of which were consistent with nucleotide MALDI-TOF MS. Both molecular assays for case 96 resulted in RIF resistance with different mutation types. Nucleotide MALDI-TOF MS showed a mutation at the *rpoB* 511 locus, whereas the Xpert MTB/RIF results showed a probe E mutation (*rpoB* 531). Considering that *rpoB* 511 mutation leads to RIF resistance as a low-level resistance,[20] which tends to cause pDST to be read as sensitive, the potential for nucleotide MALDI-TOF MS to be correct is higher. Financially, the cost of nucleotide MALDI-TOF MS in this study was at USD 60 per sample, being lower than Xpert MTB/RIF, with a cost of USD 100. In terms of turnaround time, it needs 2–3 days for nucleotide MALDI-TOF MS from sample collection to results interpretation whereas less than one day for Xpert MTB/RIF. Overall, the ability of nucleotide MALDI-TOF MS to identify RIF susceptibility was slightly higher than that of the Xpert MTB/RIF.

Qingdao Chest Hospital is the only designated hospital capable of testing anti-TB drug susceptibility in the city and is responsible for the diagnosis and treatment of almost all TB and DR-TB patients. As expected, all the patients included in this analysis were residents of Qingdao. The drug resistance rate of TB in Qingdao was 18.8% in this study, which is remarkably close to the global rate of DR-TB.[21] The highest resistance rate was observed for SM among the commonly prescribed anti-TB drugs included in the analysis, which might be related to the prescribing practices for treating TB patients in Qingdao. Physicians, particularly seniors, usually prefer SM for TB treatment. INH resistance ranked second, with a rate of up to 51.2%, much higher than the RIF resistance rate, suggesting a high prevalence of INH-mono-resistant TB in Qingdao. A systematic review and meta-analysis revealed that INH-mono-resistant TB have a higher likelihood of poor treatment outcomes,[22] highlighting the need to focus on timely diagnosis and targeted treatment for this group. In our DR-TB patients, cough and expectoration were among the most common symptoms, similar to the findings of other studies.[23]

In the present study, we also attempted to explore the risk factors associated with RIF resistance among DR-TB patients in Qingdao and unexpectedly identified chest pain as a high-risk factor for RIF resistance. The development of chest pain may portend a more severe disease course typically associated with pleuritic involvement. This may reflect late medical consultation, lack of TB awareness, or delayed TB diagnosis. TB awareness and education are of paramount importance in Qingdao. Our study found INH resistance to be one of the risk factors for RIF resistance, which is not

surprising given that it was shown that more than 80% of RIF-resistant strains were also resistant to INH.[24] This is also the reason that RIF resistance is to some extent a substitute for MDR-TB. In addition, our study also found that EMB resistance was also a risk factor of RIF resistance, and the underlying reasons remain to be investigated further.

This study had several limitations. First, we had no information on the subsequent treatment outcomes of the patients and could only use the pDST results, rather than the final clinical diagnosis, as the basis for grouping. Second, given the small number of RIF-resistant cases, the results of the analyses may be slightly biased and the time span could be increased in future studies. Finally, we did not perform sequencing of samples with discordant nucleotide MALDI-TOF MS and pDST results.

## Conclusion

In conclusion, our study describes the demographic and clinical characteristics of DR-TB cases in Qingdao. In terms of RIF susceptibility identification, compared with Xpert MTB/RIF, nucleotide MALDI-TOF MS exhibited better sensitivity and accuracy, and excellent consistency with pDST. Our results demonstrate that nucleotide MALDI-TOF MS is a promising and satisfactory method for detecting RR in Qingdao. Additionally, we identified chest pain and INH and EMB resistance as risk factors for RIF resistance in patients with DR-TB. Our study provides a reference for monitoring RIF resistance in TB patients in Qingdao, China.

## Ethical Approval and Informed Consent

This study was approved by the institutional review board and ethics committee of Qingdao Municipal Center for Disease Control and Prevention (2023-05). Written information consent was not required for this study because it is a retrospective study and consent was waived by the Institutional Review Board.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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