Case Studies of Rifampin Resistance MTBC in Contra Costa County

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Abstract

Rapid detection of *Mycobacterium tuberculosis* complex (MTBC) is crucial in minimizing the spread of disease and improving patient outcomes. The Cepheid GeneXpert MTB/rif assay detects MTBC and potential rifampin resistance. Previous studies have shown that there are a multitude of reasons in which false-positive rifampin resistance may occur. We retrospectively analyzed five MTBC rifampin resistant results, including three falsely reported as resistant.

Introduction

The GeneXpert MTB/rif assay provides MTBC results, as well as the detection of mutations in the *rpoB* gene, indicating potential rifampin resistance. Drug regimens prescribed as a result of interpretation of erroneous lab results may result in worse patient outcomes such as increased costs, rates of drug failure, toxicities and death.

We investigated five cases of reported rifampin resistance.

The rifampin resistance detection works in the "opposite" fashion of a traditional PCR assay. When a mutation is present in the *rpoB gene*, no binding occurs, and rifampin "resistance" is detected (Fig. 1). As a result, a false-resistant result may occur due to in specimens with silent mutations or low bacillary load.

Methods

We retrospectively analyzed all Contra Costa County PHL AFB sputum specimens from 2017 to 2021. Specimens were included if:

- MTB/*rif* PCR ordered
- Were positive for MTB
- Were positive for rifampin resistance

Four specimens were found to match the selection criteria and an additional specimen was submitted by the San Francisco Department of Public Health (SFPHL) (Sample E).

Confirmatory testing was performed using either the BD MGIT DST protocol or by CDPH's Microbial Diseases Laboratory pyrosequencing assay.

Results

Five cases of rifampin resistance were identified (Table 1).
3 cases of false-rifampin resistance
2 cases of true-rifampin resistance.

Sample A
Patient A's initial sample was identified by Xpert MTB/*rif* as rifampin susceptible, but a second specimen (Sample A) collected 11 days later resulted as rifampin resistant. PSQ and DST were also ordered to confirm rifampin resistance. Both showed the 2nd specimen was rifampin susceptible.

Fig. 1 Location of GeneXpert probes A-E along the *rpoB* gene.

Results (Cont.)

We suspect that the discrepant 2nd result was due to the treatment for active MTBC at the time of specimen collection, it is possible that ongoing medical therapy may have contributed to the discrepant results.

Sample B
Sample B was identified by Xpert MTB/*rif* as MTBC positive with rifampin resistance. Subsequent PSQ revealed a silent mutation at MTBC codon 514, TTC > TTT, with no change from the original amino acid phenylalanine. Because MTBC codon 514 is located in the middle of Probe B, no amplification occurred which ultimately resulted in the reporting of a rifampin resistant result.

Sample C
Sample C was identified as MTBC positive with rifampin resistance by Xpert MTB/rif, followed by PSQ which showed a missense mutation at MTBC codon 450, TCG > TTG, and resulted in a change from serine to leucine. The MTBC codon 450 is also located in the middle of probe E and likely contributed to the lack of amplification. DST confirmed that the isolate was resistance to rifampin at 1.0 µg/mL. Of note, this mutation has previously been associated with rifampin and rifabutin resistance.

Sample D
A sample from Patient D was identified as MTBC and rifampin resistance positive. PSQ results showed the same mutation as in sample C.

Definitions

- False-resistance: Contradictory Xpert rifampin resistance with confirmatory genotypic or phenotypic methods
- True-resistance: Confirmed Xpert rifampin resistance confirmed with genotypic or phenotypic methods

	Probe A	Probe B	Probe C	Probe D	Probe E	Reason for Resistance	DST/PSQ Performed?	True Resistance (Y/N)
Sample A	30.6	29.4	29.0	34.7	34.0	Probe Delay	DST/PSQ	No
Sample B	21.6	0.0	21.7	22.9	23.2	Probe Drop-off	DST/PSQ	No
Sample C	24.2	25.1	24.7	25.3	0.0	Probe Drop-off	DST/PSQ	Yes
Sample D	16.9	17.9	17.1	18.2	0.0	Probe Drop-off	PSQ	Yes
Sample E	31.3	30.1	30.0	34.3	34.3	Probe Delay	DST/PSQ	No

Table. 1 Location of GeneXpert probes A-E along the *rpoB* gene.

Results (Cont.)

Specimen E
A fifth case of false rifampin resistance was contributed by the SFPHL. The specimen was positive for MTBC with rifampin resistance detected. There were no previous indications or suspicion that the patient had MDR-TB. The isolate was tested for DSTs at the SFDPHL and was pansensitive to RIPE. PSQ detected no mutations in the *rpoB* core region associated with rifampin resistance. Contrary to the Xpert MTB/*rif* results, the isolate was shown to be both phenotypically and genotypically susceptible to rifampin. The original PSQ was unavailable, so it is not possible to determine if this was due to a silent mutation.

Conclusion

The GeneXpert MTB/*rif* assay is a very valuable tool for providers to rapidly diagnose TB and rifampin resistance in a highly contagious disease. Its limitations must be understood by the end user/clinician. Laboratories should implement algorithms, such as those recommended by the APHL and WHO, to confirm the initial resistant result to reduce the risk of using inappropriate treatment due to the detection of false rifampin resistance. Such algorithms may involve utilizing confirmatory molecular methods and DST on sputa from patients with initial positive rifampin resistant GenXpert results.

References

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