Molecular characterization of multi drug-resistant tuberculosis isolated from Baghdad, Iraq

Ruqaya Mustafa Ali a,*, Amina N. Al-Thwani b, Daniela M. Cirillo c, Emanuele Borroni c, Ahmed A. Mankhi d

a Veterinary Directorate, Ministry of Agriculture, Baghdad, Iraq
b Genetic Engineering and Biotechnology Institute for Post-Graduate Studies, University of Baghdad, Iraq
c Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Italy
d Center of Chest and Respiratory Diseases, Ministry of Health, Iraq

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ABSTRACT

Background: Tuberculosis (TB) remains a major cause of mortality and morbidity all over the world. Multi drug-resistant tuberculosis (MDR-TB) is an important cause for mortality among TB patients, and management of MDR-TB remains a major challenge for national TB programs. Early detection of MDR-TB provides better treatment outcomes and reduces the transmission of MDR. Nucleic acid amplification test (NAAT) like Line Probe Assays have been recently endorsed for use in low-income settings and can be used to screen smear-positive sputum specimens for diagnosing of resistance to rifampicin and isoniazid in 1–2 days.

Objective: Overall objective of the study was to evaluate performances of Line Probe Assay GenoType MTBDRplus (Hain Life science, GmbH, Nehren, Germany) to move forward its introduction into routine diagnostics. Secondary objective was to determine the type of mutations in rpoB, katG and inhA genes from culture specimens isolated from Iraqi selected MDR-TB patients.

Materials and methods: The DNA was extracted by CTAB method from 51 clinical isolates which were previously characterized as MDR strains in reference laboratory/center of Chest and Respiratory Diseases obtained from patients living in Baghdad/Iraq (2010–2011). GenoType MTBDRplus ver. 2.0 (HainLife science, GmbH, Nehren, Germany) was performed at the Supra-national TB Reference Laboratory in Milan (Italy) as well as interpretation of mutations involved in drug resistance to rifampicin and isoniazide according to manufacturer’s instructions.

Results and conclusion: Line Probe Assays were performed on 51 MDR of Mycobacterium tuberculosis strains; 6 strains were excluded from the analysis due to un-interpretable results; 5 strains were identified as sensitive to both rifampicin (RIF) and isoniazid (INH) by MTBDR plus assay. The most common genetic mutation conferring RIF resistance was S531L of rpoB gene which was detected in 33 (82.5%) resistant strains. Other mutations in this gene were D516Vand H526Y which were detected in a single strain (2.5%) for each. Also, there were 5 (12.5%) RIF-resistant strains with mutations that could not be identified specifically in this assay.

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* Corresponding author.
E-mail address: rukia_mustafa19@yahoo.com (R.M. Ali).

Isoniazid (INH) resistance due to katG mutation S315T1 was found in 17 (42.5%) strains of INH-resistant M. tuberculosis. The second most common site of mutation was in the upstream promoter sequence of inhA, which was found in 15 (37.5%) strains, 14 (35%) strains of which carried a C → T transition at the –15 position, while single strains (2.5%) carried a T → A mutation at the –8 position. The result showed 8 (20%) strains missing a band on the wild-type region of these strains, but no hybridization with mutation probes on this gene suggested resistance due to mutations other than those included in this assay.

Regardless the type of mutations detected, GenoType® MTBDR plus ver. 2.0 showed an overall accuracy of 95.5% (C.I. 95% 89.9–98.1) in detecting MDR strains. Despite the relatively low number of MDR strains tested, they show very common mutations patterns being the majority mutated at the codon 531 and 315 of rpoB and katG gene. In such setting, GenoType® MTBDR plus ver. 2.0 could be a great tool to rapidly screen for MDR-TB and has the potential to substantially reduce the turnaround time of drug sensitivity test (DST) results.

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