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Discordance between Xpert MTB/RIF Assay and Bactec MGIT 960 Culture System for Detection of Rifampin-Resistant *Mycobacterium tuberculosis* Isolates in a Country with a Low Tuberculosis (TB) Incidence

Eiman Mokaddas,[a,b] Suhail Ahmad,[a] Hanaa S. Eldeen,[b] Noura Al-Mutairi[a]

Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait[a]; Kuwait National TB Reference Laboratory, Shuwaikh, Kuwait[b]

Among 452 samples that were positive by the Xpert MTB/RIF (Xpert) assay and MGIT 960 system (MGIT), 440 and 10 *Mycobacterium tuberculosis* samples were detected as rifampin susceptible and rifampin resistant, respectively. Two isolates that were rifampin susceptible by the MGIT system were rifampin resistant by the Xpert assay. *rpoB* sequencing identified a silent (CTG521TTG) mutation in one isolate and a missense (GAC516TAC) mutation in another. The detection of rifampin resistance is imperfect with both the Xpert assay and MGIT system. Any discordant rifampin resistance results should be confirmed by sequencing of the *rpoB* gene.

Multidrug-resistant tuberculosis (MDR-TB) (defined as infection with a *Mycobacterium tuberculosis* strain resistant to at least the two most effective, rifampin and isoniazid, anti-TB drugs) is prevalent throughout the world, difficult to treat, and associated with higher rates of clinical failure and disease relapse (1, 2). The rapid and accurate laboratory diagnosis of MDR-TB is crucial for effective treatment, which will also limit the transmission of MDR-TB (2, 3). The resistance of *M. tuberculosis* to rifampin (RMP) in nearly 97% of isolates is due to mutations in an 81-bp rifampin resistance-determining region (RRDR) of the *rpoB* gene (4). Other RMP-resistant isolates contain mutations in either the N-terminal or cluster II region of the *rpoB* gene, or the resistance is due to other mechanisms (2, 4). Resistance to RMP is a key determinant in treatment failure and also correlates well with MDR-TB, since >85% of RMP-resistant *M. tuberculosis* isolates worldwide are also resistant to isoniazid (INH) (2–4). Molecular assays detect mutations in the RRDR of the *rpoB* gene for the rapid detection of RMP-resistant *M. tuberculosis* in clinical specimens and culture isolates (2, 3). The World Health Organization (WHO)-approved tests include two line probe assays, the INNO-LiPA Rif. TB (detecting resistance to RMP only) and the GenoType MTBDR*plus* (detecting resistance to RMP and INH), as well as the real-time PCR-based automated Xpert MTB/RIF (Xpert) assay (detecting resistance to RMP only) (3, 5). However, these tests are not specific, as silent mutations in the *rpoB* gene occasionally lead to the detection of false-positive RMP resistance (6–9). The current WHO recommendations are to use the Xpert assay as the initial diagnostic test and start treatment for MDR-TB if an RMP resistance result is expected, or, if unexpected, to repeat Xpert assay testing on another sputum sample, particularly in settings in which the prevalence of RMP-resistant TB is <15% (10). For those settings, treatment for MDR-TB should be initiated when the Xpert assay repeatedly detects RMP resistance. Treatment should be optimized by following susceptibility testing with other first-line and second-line drugs and confirmatory testing for RMP resistance by phenotypic or other genotypic methods; any discordant RMP susceptibility results can be resolved by sequencing of the *rpoB* gene (10).

Phenotypic drug susceptibility testing (DST) of *M. tuberculosis* for RMP and other first-line drugs, mainly employing the solid medium-based proportion and absolute concentration methods and the commercial liquid medium-based methods, such as the Mycobacteria Growth Indicator Tube (MGIT) 960 system, is considered the gold standard (11). The MGIT 960 system, although more rapid than are solid medium-based methods, has yielded highly discordant results for low-level but clinically significant RMP resistance in *M. tuberculosis* strains carrying specific *rpoB* mutations (9, 11, 12). In this study, we describe the results obtained with the Xpert assay and automated liquid culture (MGIT 960) system with regard to the concordance of RMP susceptibility results in a low-TB-incidence country.

A total of 452 clinical specimens collected from 452 different TB patients at the Kuwait National TB Reference Laboratory who tested positive for *M. tuberculosis* by the Xpert assay (Cepheid, Sunnyvale, CA) and yielded mycobacterial culture by MGIT 960 system (BD, Sparks, MD, USA) were tested. The clinical samples were collected from suspected TB patients as part of routine patient care and included 287 pulmonary and 165 extrapulmonary specimens. The extrapulmonary samples included fine needle aspirates ($n = 66$), pus ($n = 58$), pleural fluid ($n = 14$), tissue ($n = 10$), other sterile fluids ($n = 8$), urine ($n = 5$), cerebrospinal fluid ($n = 2$), and stool ($n = 2$). The samples were tested by smear microscopy and the Xpert assay and processed for culture on solid (Lo-

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Address correspondence to Suhail Ahmad, suhail_ah@hsc.edu.kw.

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TABLE 1 Initial drug susceptibility results for 452 clinical isolates of *M. tuberculosis*, as determined by the Xpert MTB/RIF assay and Bactec MGIT 960 system

Xpert MTB/RIF phenotype	No. of strains with rifampin susceptibility by MGIT 960 system in:							
	Pulmonary samples				Extrapulmonary samples			
	Smear positive[a]		Smear negative		Smear positive		Smear negative	
	S	R	S	R	S	R	S	R
Susceptible	147	0	132	0	28	0	133	0
Resistant	0	2	1	5	0	2	1	1

[a] S, susceptible; R, resistant.

wenstein-Jensen) and automated Bactec MGIT 960 system liquid media.

The smears for acid-fast bacilli (AFB) were prepared by Ziehl-Neelsen stain for direct microscopy. The Xpert assay was performed on clinical samples without prior extraction, and the results were interpreted according to the manufacturer's instructions. The nonsterile clinical specimens were processed by the standard *N*-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) method, while the sterile samples were processed directly (13). All specimens were cultured on solid (Lowenstein-Jensen) and MGIT 960 system media, according to the manufacturer's instructions and as described previously (13, 14). All samples with a positive growth reading on the MGIT 960 system were tested for acid-fast bacilli by Ziehl-Neelsen stain and for the presence of *M. tuberculosis* complex DNA using the AccuProbe DNA probe assay (13, 14). All MGIT 960 system cultures were subjected to DST against first-line drugs in the MGIT 960 system using the SIRE drug kit, which contains INH at 0.1 mg/liter, RMP at 1.0 mg/liter, streptomycin at 1.0 mg/liter, and ethambutol at 5.0 mg/liter (15). All isolates exhibiting RMP resistance by the Xpert assay and/or MGIT 960 system were tested by an in-house multiplex PCR assay specific for *M. tuberculosis* complex, the GenoType MDBDR*plus* assay, and direct DNA sequencing of three (N-terminal, RRDR, and cluster II) regions of the *rpoB* gene, performed as described previously (13, 16). Five drug-susceptible *M. tuberculosis* isolates were also tested for comparison.

Only 149 (52%) and 30 (18%) pulmonary and extrapulmonary samples, respectively, were smear positive for AFB. Both the MGIT 960 system and the Xpert assay detected RMP susceptibility and RMP resistance in 279 and 7 pulmonary and 161 and 3 extrapulmonary samples, respectively, while the Xpert assay additionally detected RMP resistance in one pulmonary and one extrapulmonary (both smear-negative) sample (Table 1). The GenoType MTBDR*plus* assay and DNA sequencing of the RRDR of the *rpoB* gene confirmed the RMP-resistant status of *M. tuberculosis* in all 10 samples showing RMP resistance by both the Xpert assay and the MGIT 960 system (data not shown). Although the GenoType MTBDR*plus* assay also indicated RMP resistance in the two (Kw5741-13 and Kw9101-13) discrepant *M. tuberculosis* isolates that were RMP resistant by the Xpert assay but RMP susceptible by the MGIT 960 system (by the lack of hybridization with one or more wild-type probes), no specific mutation was detected (Table 2). The sequencing of the RRDR of the *rpoB* gene identified a novel silent (CTG to TTG) mutation at codon 521 (Leu521Leu) in Kw5741-13 (isolated from an Egyptian patient) and a missense (GAC to TAC) mutation at codon 516 (Asp516Tyr) in Kw9101-13 (isolated from an Indian patient) (Table 2). No mutation was detected in the N-terminal or cluster II region of the *rpoB* gene in isolates Kw5741-13 and Kw9101-13. The isolates Kw5741-13 and Kw9101-13 were susceptible to all first-line drugs by phenotypic DST using the MGIT 960 system.

Only ~1.5% of all *M. tuberculosis* isolates in Kuwait (TB incidence, 24 cases per 100,000 populations) are detected as being MDR-TB strains (14). Since 2011, all clinical specimens from suspected TB patients are tested by the Xpert assay in addition to routine processing for smear microscopy and culture. Furthermore, the MGIT 960 system completely replaced the Bactec 460 TB system in 2011 for DST of *M. tuberculosis* isolates. The data presented here on 452 samples show that the Xpert assay correctly identified RMP susceptibility in 451 of 452 patients. This is excellent performance, considering that the pooled sensitivity and specificity of the Xpert assay for RMP resistance detection in pulmonary samples have been reported as 95% and 98%, respectively (17). The data also support the high (99%) negative predictive value of the Xpert assay for RMP resistance detection, as suggested by the WHO data for settings with a low prevalence of RMP resistance (10). Two *M. tuberculosis* isolates yielded discrepant RMP susceptibility results by the Xpert assay and the MGIT 960 system. The Xpert assay (and the GenoType MTBDR*plus* assay) accurately detected RMP resistance in Kw9101-13 containing Asp516Tyr in the *rpoB* gene, which was missed by the MGIT 960 system. The Asp516Tyr mutation in the *rpoB* gene causes low-level resistance to RMP, which is routinely missed by liquid (including MGIT

TABLE 2 Laboratory investigations performed on the two discrepant *M. tuberculosis* isolates

Laboratory investigations	Data for *M. tuberculosis* isolate[a]:	
	Kw5741-13	Kw9101-13
Clinical specimen	Pus	Sputum
Smear microscopy result	AFB negative	AFB negative
Xpert MTB/RIF assay result for specimen	Presence of RMP-resistant *M. tuberculosis*	Presence of RMP-resistant *M. tuberculosis*
MGIT 960 system culture result	Positive	Positive
RMP susceptibility (1 mg/liter)	Susceptible	Susceptible
Other resistance pattern	None	None
PCR for *M. tuberculosis* complex	Positive	Positive
GenoType MTBDR*plus* assay	RMP resistant (ΔWT5, no specific mutation)	RMP resistant (ΔWT3 & 4, no specific mutation)
Sequencing of *rpoB* gene	CTG521TTG (silent mutation [Leu521Leu])	GAC to TAC (missense mutation [Asp516Tyr])

[a] ΔWT, lack of hybridization with the wild-type probe.

960) culture systems (12, 18). Patients infected with low-level RMP-resistant *M. tuberculosis* strains raise a new therapeutic challenge, as the isolates are classified as RMP susceptible while the patients often fail treatment or relapse, and the Asp516Tyr mutation in the *rpoB* gene is one of the genetic alterations found in such strains (11, 12, 18–20). The exact frequencies of these disputed RMP resistance mutations in the *rpoB* gene in most countries remain unknown, since DST is usually carried out by liquid culture systems, which often fail to detect low-level RMP-resistant strains (11, 18). Recent WHO guidelines have also stated that the Xpert assay detects some RMP-resistant strains that are scored as RMP susceptible by phenotypic DST methods, and DNA sequencing of the *rpoB* gene usually resolves these discordant results in favor of the Xpert assay results (10). The Asp516Tyr mutation in Kw9101-13 would also have escaped detection if the sample was not tested simultaneously by the Xpert assay, further challenging the credibility of the current gold standard for RMP resistance detection (11).

The Xpert assay correctly identified RMP resistance in 11 of 12 patients, and retesting the samples yielded identical results. Given the low (~1.5%) prevalence of RMP resistance in Kuwait, the performance of the Xpert assay is better than was expected. The positive predictive value of 91.5% is better than the 70% expected for settings with an RMP resistance prevalence of <5% (10). The Xpert assay (and the GenoType MTBDR*plus* assay) detected RMP resistance in Kw5741-13 containing a silent (CTG to TTG [Leu521Leu]) mutation in the *rpoB* gene, which was scored as RMP susceptible by the MGIT 960 system. The Xpert assay result represents real false-positive RMP resistance, since silent mutations do not change the properties of encoded proteins, and this reinforces the recommendations of the WHO to perform a confirmatory DST by phenotypic or other genotypic methods and to resolve any discordant RMP susceptibility results by sequencing of the *rpoB* gene (10). Silent mutations in the *rpoB* gene have been reported at codon Thr508 in Haitian isolates (9), Gln510 in New Zealand isolates (7), Leu511 and Gln513 in South Korean isolates (21), Phe514 in Spanish and American isolates (6, 22), Thr525 in Chinese isolates (23), Ala532 in Indian isolates (24), and Leu533 in Indian and Belgian isolates (8, 24). The growing body of literature on silent mutations within the RRDR of the *rpoB* gene is alarming, since the Xpert assay was designed for the rapid diagnosis of MDR-TB for effective management of such patients, but it may actually result in overdiagnosis of MDR-TB in resource-poor settings due to limited access to confirmatory DST by phenotypic methods or *rpoB* sequencing.

In conclusion, the detection of RMP resistance is imperfect with both the Xpert assay and automated liquid culture systems. Phenotypic tests often fail to detect low-level but clinically significant RMP resistance, while the Xpert assay may report, albeit rarely, false-positive RMP resistance due to silent mutations in the *rpoB* gene. The detection of RMP resistance by the Xpert assay should be confirmed by phenotypic methods, particularly for settings where the prevalence of RMP resistance is <15%, and any discordant RMP susceptibility results should be confirmed by sequencing the *rpoB* gene.

Nucleotide sequence accession numbers. The nucleotide sequence data have been submitted to EMBL under accession no. LN651304 to LN651309.

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