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Evaluation of Pyrosequencing for Detecting Extensively Drug-Resistant *Mycobacterium tuberculosis* among Clinical Isolates from Four High-Burden Countries

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Reliable molecular diagnostics, which detect specific mutations associated with drug resistance, are promising technologies for the rapid identification and monitoring of drug resistance in *Mycobacterium tuberculosis* isolates. Pyrosequencing (PSQ) has the ability to detect mutations associated with first- and second-line anti-tuberculosis (TB) drugs, with the additional advantage of being rapidly adaptable for the identification of new mutations. The aim of this project was to evaluate the performance of PSQ in predicting phenotypic drug resistance in multidrug- and extensively drug-resistant tuberculosis (M/XDR-TB) clinical isolates from India, South Africa, Moldova, and the Philippines. A total of 187 archived isolates were run through a PSQ assay in order to identify *M. tuberculosis* (via the IS*6110* marker), and to detect mutations associated with M/XDR-TB within small stretches of nucleotides in selected loci. The molecular targets included *katG*, the *inhA* promoter and the *ahpC-oxyR* intergenic region for isoniazid (INH) resistance; the *rpoB* core region for rifampin (RIF) resistance; *gyrA* for fluoroquinolone (FQ) resistance; and *rrs* for amikacin (AMK), capreomycin (CAP), and kanamycin (KAN) resistance. PSQ data were compared to phenotypic mycobacterial growth indicator tube (MGIT) 960 drug susceptibility testing results for performance analysis. The PSQ assay illustrated good sensitivity for the detection of resistance to INH (94%), RIF (96%), FQ (93%), AMK (84%), CAP (88%), and KAN (68%). The specificities of the assay were 96% for INH, 100% for RIF, FQ, AMK, and KAN, and 97% for CAP. PSQ is a highly efficient diagnostic tool that reveals specific nucleotide changes associated with resistance to the first- and second-line anti-TB drug medications. This methodology has the potential to be linked to mutation-specific clinical interpretation algorithms for rapid treatment decisions.

There is growing concern that the increase in the global prevalence of multidrug- and extensively drug-resistant tuberculosis (M/XDR-TB), as well as the emergence of what is being called "totally drug-resistant TB" (1–3), may compromise the recent successes seen by global TB control efforts (4). According to the World Health Organization (WHO), one of the greatest obstacles to the control and management of drug-resistant TB (DR-TB) is insufficient laboratory capacity and capability to diagnose resistance in *Mycobacterium tuberculosis* strains in a timely and cost-effective manner. Conventional TB diagnosis relies upon growth culture, which is slow and requires complex biosafety environments that are largely unattainable in developing nations. This is likely the primary reason why fewer than 25% of the estimated 450,000 new MDR-TB cases in 2012 were detected (5). Reliable molecular diagnostics, based upon the detection of specific mutations that confer drug resistance, are the most promising technologies for rapid identification of drug resistance in *M. tuberculosis* isolates (6). These technologies, most notably the Hain MTBDR Plus line probe assay and the GeneXpert MTB/RIF assay, are being implemented globally to screen for the most prevalent mutations associated with resistance to isoniazid (INH) and/or rifampin (RIF) (7, 8). However, there are limitations to both of these technologies, since they are unable to distinguish silent mutations from mutations associated with drug resistance, and there has been little progress toward the broad application of these molecular diagnostics for the detection of mutations associated with resistance to the second-line anti-tuberculous medications, such as the fluoroquinolones (FQs) and the so-called "injectable" medications: amikacin (AMK), kanamycin (KAN), and capreomycin (CAP) (9).

Pyrosequencing (PSQ), a real-time method for rapid sequencing of small segments of genomic DNA (10), is capable of reliably detecting mutations that confer first (11–13)- and second-line drug resistance (14, 15) in *M. tuberculosis*. PSQ not only deter-

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mines the presence or absence of these mutations, but also displays detailed sequence data, which enables users to distinguish mutations conferring resistance from silent mutations as well as from those conferring different levels of resistance (16, 17). Here, we report the findings of a study evaluating a PSQ assay (15) for detection of first- and second-line drug resistance in a well-characterized collection of archived clinical M/XDR-TB isolates collected from India, South Africa, Moldova, and the Philippines by the Global Consortium for Drug-Resistant TB Diagnostics (GCDD).

MATERIALS AND METHODS

Source of isolates. In the present study, 187 clinical *M. tuberculosis* isolates were selected to include at least one isolate of each strain type based on the results of mycobacterial interspersed repetitive unit (MIRU) typing. Of those selected, 56 were from Mumbai, India, 38 were from the national bank of strains from Moldova, 56 were from Manila, Philippines, and 37 were from a national collection of strains from South Africa. The full collection of isolates was described and characterized by Rodwell et al. (18). The 416 isolates were originally collected by the GCDD from different global regions with a high burden of M/XDR-TB in order to maximize the diversity of drug resistance phenotypes. Clinical sites were selected based upon a documented, high incidence of drug resistance (19) without consideration for the prevalence of specific genetic targets. Isolates were sent to the University of California, San Diego (UCSD), for standardized phenotypic drug susceptibility testing (DST), as well as genotyping, Sanger sequencing, and PSQ, to determine the genetic basis of drug resistance in these isolates. The GCDD isolate collection methods, phenotypic DST, and Sanger sequencing results of the entire collection of isolates are described in detail in a recent publication by Rodwell et al. (18). The present study was approved by the institutional review board of the UCSD.

Phenotypic MGIT drug susceptibility testing. We performed standardized DST to INH, RIF, MOX, OFX, AMK, KAN, and CAP at the UCSD using MGIT 960 (Becton Dickinson Diagnostic Systems, Sparks, MD) with EpiCenter software (Becton Dickinson, Franklin Lakes, NJ). The manufacturer protocols (20) were strictly followed, and the critical concentrations recommended by WHO (21) were utilized to determine phenotypic resistance patterns, with the exception of KAN, which was tested at 2.5 μg/ml, based upon previous findings (22). Detailed DST methods are available in a previous study by Rodwell et al. (18).

Pyrosequencing. The PSQ assay used in the present study was developed at the Microbial Diseases Laboratory (MDL) of the California Department of Public Health in collaboration with the GCDD. Details of the assay were described in a recent publication by Lin et al. (15). Briefly, the assay used crude DNA, extracted by a simple heating procedure (95°C, 25 min) and included eight PSQ subassays that were performed simultaneously. The molecular marker IS*6110* (23) was utilized to identify *M. tuberculosis*. The targeted loci for detection of DR-TB were (i) *katG*, the *inhA* promoter, and the *ahpC* promoter for INH resistance, (ii) the RIF resistance-determining region (RRDR) of *rpoB* for RIF resistance, (iii) the quinolone resistance-determining region (QRDR) of *gyrA* for FQ resistance, and (iv) the *rrs* gene for detection of AMK, KAN, and CAP resistance. Although *ahpC* promoter mutations are not common, when they occur they have been shown to compensate for the loss of *katG* catalase peroxidase activity, warranting their use in here as molecular markers of INH resistance (24, 25).

The raw sequences from PSQ were aligned against a GCDD-created sequence library containing wild-type and known mutant sequences using IdentiFire software (Qiagen, Valencia, CA). Sequences matching mutations previously associated with resistance were considered genotypically resistant, whereas wild-type sequences were considered genotypically susceptible, and those not matching resistance-associated mutations or wild-type sequences were considered genotypically indeterminate for the given gene regions (15). In addition, *rpoB* single-nucleotide polymorphisms (SNPs) documented to confer phenotypic resistance below the critical concentration of 1 μg/ml in liquid culture (16, 17) were considered genotypically indeterminate.

Data analysis. Standardized MGIT 960 DST results generated by the UCSD were compared to DSTs from each study site and, if there were discrepancies, the MGIT DST was retested at the MDL. PSQ results were then compared to MGIT 960 DST results. If discrepancies between the PSQ and MGIT DST results were found, PSQ was repeated. Remaining discrepancies between genotypic and/or phenotypic results were resolved using a consensus approach following an expert panel's review of all available data. In the few cases of RIF genetic resistance profiling where it was not possible to reach consensus due to inconsistencies in the data, the PSQ result was considered genotypically indeterminate. A discussion of this decision is presented here. After resolving all such discrepancies, we used Meta-DiSc software (v1.4) (26) to calculate the sensitivity and specificity of detected SNPs for predicting phenotypic resistance. The confidence intervals (CIs) for sensitivity and specificity were calculated using score/efficient-score method with continuity correction for sensitivities and specificities (27, 28). The agreement between PSQ and MGIT 960 results was also calculated for each drug.

RESULTS

Isolates. Of the 187 clinical isolates evaluated by PSQ, 56 were from Mumbai, India, 38 were from the national bank of strains from Moldova, 56 were from Manila, Philippines, and 37 were from a national collection of strains from South Africa.

Phenotypic DST results. The phenotypic MGIT DST results found 15/187 isolates to be MDR (with resistance to INH and RIF) with susceptibility to the FQs and injectables, 22/187 isolates to be MDR with additional resistance to the FQs, 16/189 isolates to be MDR with additional resistance to the injectables, 97/187 isolates to be XDR with resistance to KAN and OFX, 8/187 isolates to be INH monoresistant, and 2/187 isolates to be RIF monoresistant. Three isolates were found susceptible to RIF but resistant to all other drugs, and two isolates were shown to be susceptible to RIF and the injectables but resistant to all other drugs. A total of 22/187 isolates were phenotypically susceptible to all drugs tested.

Genotypic results of PSQ: *M. tuberculosis* detection. *M. tuberculosis* was accurately identified in all 187 (100%) isolates, since all yielded 100%-matched sequences with the *M. tuberculosis*-specific segment of IS*6110*.

INH resistance. Of the 187 isolates evaluated, 163 were phenotypically resistant to INH: 52 from India, 33 from Moldova, 47 from the Philippines, and 31 from South Africa. PSQ did not detect mutations within *katG*, *inhA*, or *ahpC* in 10 isolates that were phenotypically resistant to INH, yielding a sensitivity of 94% (95% CI = 89 to 97%) for the detection of INH resistance. A total of 12 unique mutations and combinations of mutations, found in 153 isolates, were detected in our study across these three loci. Forty-four isolates showed mutations in both *katG* and *inhA*, and two isolates were found to have mutations in both *katG* and *ahpC* (Table 1). Of the three genomic regions, the *katG* gene showed the highest frequency of mutations, with mutations appearing in 132 INH-resistant isolates (80.9%). Mutations in the *inhA* promoter only were found in 19 isolates (11.7%), and mutations in the *ahpC* promoter only were found in 1 isolate (0.6%). The diagnostic performance of the PSQ assay in comparison to phenotypic DST for the detection of INH resistance is summarized in Table 2. The overall concordance between PSQ and phenotypic DST findings for the detection of INH resistance was 94%.

RIF resistance. Of the 187 isolates studied, 152 were phenotypically resistant to RIF at the WHO critical concentration (1.0

TABLE 1 Mutations detected by pyrosequencing

Drug	Molecular locus	No. of isolates	Mutation(s)
INH	*katG*	86	315 ACC ($n = 85$); 315 ACC and 314 GCC ($n = 1$)
	inhA promoter	20	−15 T ($n = 19$); −17 T ($n = 1$)
	ahpC promoter	1	−48 T
	katG and *inhA* promoter	44	315 ACC and −17 T ($n = 3$); 315 ACC and −15 T ($n = 28$); 315 ACC and −8 A ($n = 8$); 315 ACC and −8 C ($n = 2$); 315 ACC and −8 G ($n = 2$); 315 ACA and −8 C ($n = 1$)
	katG and *ahpC* promoter	2	315 ACC and −48 T ($n = 1$); 315 ACC + insertion of A at −46 ($n = 1$)
RIF	*rpoB*	110	531 TTG ($n = 107$); 531 TGG ($n = 3$)
		14	526 TAC ($n = 7$); 526 GAC ($n = 3$); 526 CGC ($n = 2$); 526 TGC ($n = 2$)
		1	522 TTG
		10	516 GTC
		4	513 AAA ($n = 3$); 513 CCA ($n = 1$)
		5	516 GGC + 533 CCG
		1	509 CGC + 526 TAC
		1	511 CCG + 512 ACC + 516 TAC
Quinolones	*gyrA*	77	94 GGC ($n = 45$); 94 GCC ($n = 13$); 94 AAC ($n = 10$); 94 TAC ($n = 7$); 94 CAC ($n = 1$); 94 GTC ($n = 1$)
		5	91 CCG
		32	90 GTG
		1	88 TGC
Injectable drugs	*rrs*	79	1401 G

μg/ml). Among these, 52 were from India, 31 were from Moldova, 39 were from the Philippines, and 30 were from South Africa. A total of 13 unique mutations and combinations of mutations, found in 146 isolates, were detected in our study across the *rpoB* gene, yielding a sensitivity of 96% (95% CI = 92 to 98%). The most predominant SNP observed in *rpoB* was the 531 TCG-TTG mutation, which was detected in 107 (70.4%) of RIF-resistant isolates (Table 1).

Six isolates were phenotypically RIF sensitive but were found to have an SNP in the *rpoB* gene via PSQ. For these isolates, the observed mutations were as follows: 516 GAC-TAC ($n = 3$), 526 CAC-CTC ($n = 1$), 526 CAC-AGC ($n = 1$), and 515 ATG-ATA ($n = 1$). These SNPs have been well documented as "disputed" mutations conferring "low-level" RIF resistance (17). The association of these mutations with clinical relevance to RIF resistance subjects to further investigation; thus, we classified them as indeterminate and excluded them from data analysis The performance of the PSQ assay in comparison to phenotypic DST for the detection of RIF resistance is summarized in Table 2. The overall concordance between PSQ and phenotypic DST findings for the detection of RIF resistance was 97%.

Fluoroquinolone resistance. Among the 123 FQ-resistant isolates examined, 49 were from India, 25 from Moldova, 22 from Philippines, and 28 from South Africa. A total of 9 unique mutations, found in 115 isolates, were detected in our study across the *gyrA* gene, yielding a sensitivity of 93% (95% CI = 87 to 96%). Seventy-seven (61.6%) resistant isolates showed mutations in codon 94 of *gyrA*: 45 GAC-GGC, 13 GAC-GCC, 7 GAC-TAC, 1 GAC-GTC, and 1 GAC-CAC. Ten isolates contained a codon 94 GAC-AAC mutation. All isolates with these SNPs were phenotypically resistant to both OFX and MOX. Thirty-two isolates showed a codon 90 GCG-GTG mutation; of these, one isolate was susceptible to MOX but resistant to OFX. Five isolates had a codon 91 TCG-CCG mutation, and one isolate showed a codon 88 GGC-TGC mutation, and all were resistant to both OFX and MOX (Table 1). Based upon these findings, the diagnostic performance of the PSQ assay in comparison to phenotypic DST for the detection of both OFX and MOX resistance is summarized in Table 2. The overall concordance between the PSQ and phenotypic DST findings for detection of FQ-resistance was 95% for both OFX and MOX.

SLI drug resistance. Among the 187 isolates with phenotypic resistance to second-line injectable (SLI) drugs, 76 isolates were resistant to all three SLIs drugs (AMK, CAP, and KAN), and all had a codon 1401 A-G mutation (i.e., an A→G mutation at codon 1401) in the *rrs* gene (Table 1). Of the isolates with codon 1401 mutations, 39 were from India, 8 were from Moldova, 6 were from the Philippines, and 23 were from South Africa. Three isolates showed a codon 1401 A-G mutation and were resistant to AMK and KAN but susceptible to CAP. Twenty-one isolates that were resistant to KAN but susceptible to the other two SLI drugs showed no mutation in the codon 1400 region of the *rrs* gene. In summary, the sensitivities for the detection of resistance to AMK, CAP, and KAN were 84, 88, and 68%, respectively. The specificity for AMK and KAN was 100%, while that for CAP was 97% because three isolates with the A1401G mutation tested CAP susceptible. The concordances between the PSQ and the MGIT DST for AMK, CAP, and KAN were 92, 93, and 80%, respectively.

DISCUSSION

PSQ is a robust, rapid, and high-throughput diagnostic sequencing technique. Our assay, comprised of eight subassays, was capable of simultaneously detecting *M. tuberculosis* and the primary canonical mutations conferring phenotypic resistance to INH, RIF, MOX/OFX, AMK, KAN, and CAP. Although the assay was designed only to detect most known mutations, its adaptable nature also allowed for the detection of novel mutations within the targeted gene regions through the utilization of specific deoxy-

TABLE 2 Diagnostic performance of PSQ compared to MGIT 960 DST as the reference standard

	Phenotypic DST results (no. of isolates)		Accuracy analysis[c]		
Drug (*n*)	Resistant	Susceptible	% sensitivity	% specificity	% concordance
INH (187)					
Mutations detected	153	1[a]	94 (89-97)	96 (80-99)	94
No mutations	10	23			
RIF (181[b])					
Mutations detected	146	0	96 (92-98)	100 (88-100)	97
No mutations	6	29			
OFX (187)					
Mutations detected	115	0	93 (87-96)	100 (94-100)	95
No mutations	9	63			
MOX (187)					
Mutations detected	114	1	93 (86-96)	98 (92-100)	95
No mutations	9	63			
AMK (187)					
Mutations detected	79	0	84 (75-90)	100 (96-100)	92
No mutations	15	93			
CAP (187)					
Mutations detected	76	3	88 (80-94)	97 (92-99)	93
No mutations	10	98			
KAN (187)					
Mutations detected	79	0	68 (59-76)	100 (95-100)	80
No mutations	37	71			

[a] An isolate with an *inhA* −15T mutation tested as INH susceptible.
[b] For RIF the sensitivity and specificity was calculated after excluding the six isolates with SNPs known to confer only low-level resistance.
[c] The 95% CI is indicated in parentheses where applicable.

nucleoside triphosphate dispensation orders. PSQ also has an advantage over existing probe-based assays due to its provision of detailed sequence information, allowing users to interpret sequence results based on current and evolving knowledge about phenotypic expression.

The molecular target for identification of *M. tuberculosis* is IS*6110*. It worked very well in the present study, with 100% specificity and sensitivity. However, it is known that there are *M. tuberculosis* strains, particularly in South India, missing IS*6110* elements in their genome (23). For those strains, the IS*6110* target will yield no peaks despite having normal peaks as for *M. tuberculosis* strains in all other targets. It is advisable to design primers for sequencing another molecular locus as a backup for *M. tuberculosis* identification for strains lacking IS*6110* when testing clinical specimens or cultures whose *M. tuberculosis* identification has not been established.

The present study demonstrated that the PSQ assay was capable of reliable and robust detection of resistance-associated mutations in *M. tuberculosis* isolates. The sensitivity and specificity of the assay for detecting phenotypic INH resistance were 94 and 96%, respectively. INH sensitivity, while high, was <100%, since our PSQ assay did not detect any SNPs in 10/163 INH-resistant isolates. This could be due to mutations lying outside the genetic regions examined in our assay or to other resistance mechanisms not associated with the *katG*, *inhA*, or *ahpC* targeted gene regions (29). These discordant isolates are being further evaluated via whole-genome sequencing, but the results of that analysis are beyond the scope of the present study. In addition, we found a single isolate with mutations in the *inhA* promoter that tested INH susceptible via MGIT 960. These findings were possibly due to DST error, which highlights a complication of comparative diagnostic analyses that employ phenotypic DST as the "gold standard" when it is known that phenotypic DST has sensitivity and specificity limitations (16, 17, 30).

The sensitivity and specificity of the *rpoB* SNPs in predicting phenotypic RIF resistance in our study were 96 and 100%, respectively. Our assay did not detect mutations in six phenotypically RIF-resistant isolates, which could be due to the occurrence of mutations outside the RRDR or to other resistance-conferring mechanisms. In addition, PSQ detected SNPs in six isolates that were determined to be phenotypically "susceptible" at the critical concentration of 1.0 μg/ml on MGIT 960. However, the SNPs detected in these isolates are well documented to impart only low-level resistance (<1 μg/ml), which means that these isolates may appear RIF susceptible by MGIT 960 DST but could appear resistant on solid culture. The clinical relevance of these mutations is still unclear (17, 31, 32). For this reason, these isolates were not considered test discordant but rather "genotypically indeterminate" and were excluded from our specificity calculation. The ability to visualize and analyze the character of such mutations is actually a strength of PSQ diagnostic assays, since it is becoming clear that not all *rpoB* SNPs confer the same level of resistance.

Certain *rpoB* mutations, such as 516 GAC-TAC and 526 CAC-AAC, appear to confer "low-level resistance" as measured by the isolates' low MICs (33), while other SNPs, such as those within the *rpoB* 531 codon, result in high-level resistance (17, 34, 35). Rather than assuming that all *rpoB* SNPs confer a "resistant" or "susceptible" phenotype, sequence-based PSQ data allow for the prediction of the MIC of that isolate based on previous phenotypic studies of isolates with the same SNP. Although this interpretative functionality is still in its infancy due to the lack of published data on these low-MIC mutations, we expect that as molecular and phenotypic data accumulate, we will be able to develop more refined algorithms to aid treatment decisions in a clinical setting.

For the prediction of phenotypic FQ resistance, our assay had sensitivities of 93% for OFX and 92% for MOX and specificities of 100% for OFX and 98% for MOX. In the present study, the *gyrA* mutation 90 GCG-GTG was found in one MOX-susceptible but OFX-resistant isolate. Published research suggests that mutations found in codons 90 and 91 of *gyrA* may convey low to moderate levels of phenotypic resistance compared to mutations in codons 94 and 88, which confer higher levels of resistance to the FQs, and may additionally confer differential resistance to the different FQs (36–38). These findings may explain the phenotypic discordance that we observed between the two FQs for the isolate found to have a mutation at codon 90 (36–38). PSQ failed to detect QRDR mutations in nine phenotypically FQ-resistant isolates that could have harbored mutations outside the QRDR of *gyrA* examined with our PSQ assay. Despite this limitation, our findings of the performance of PSQ for the detection of FQ resistance are in agreement with previous PSQ studies (14, 15).

The sensitivities of the PSQ assay in detecting AMK, CAP, and KAN resistance were determined to be 84, 88, and 68%, respectively. The specificity was 100% for both AMK and KAN resistance detection but 97% for CAP resistance, which is comparable with a previously reported PSQ study evaluating the *rrs* 1401 A-G mutation as a marker for resistance to the SLIs (14). There were three isolates that were susceptible to CAP by phenotypic MGIT 960 DST but were found to have the 1401 A-G mutation by PSQ. A possible explanation of this discordance was described by Rodwell et al. (18) in their analysis of the full set of isolates from which ours were selected. In brief, "it has been demonstrated that [the WHO] critical concentration [for CAP] is "substantially higher" than the epidemiological cutoff (ECOFF) that separates wild-type *M. tuberculosis* from those with mutations conferring CAP resistance, which could result in non-wild-type isolates being be classified as CAP susceptible" (39). It is therefore possible that the WHO-recommended critical concentration that we used here resulted in the misclassification of CAP-resistant organisms as "susceptible" when, in fact, they contained the 1401 A-G mutation in the *rrs*, ultimately resulting in the reduced specificity of PSQ for predicting phenotypic CAP resistance. In addition, as discussed in Sirgel et al. (40), clinical isolates from South Africa with the 1401 A-G mutation in the *rrs* gene showed decreased phenotypic susceptibility to CAP. Furthermore, while the *rrs* 1401 A-G mutation is implicated in cross-resistance to all three injectable drugs, some studies have shown that mutations outside our considered genomic regions, most notably mutations in *eis* promoter and *tlyA*, may independently confer resistance to KAN and CAP, respectively (41–44). The sensitivity and specificity of PSQ for detecting phenotypic CAP and KAN resistance are likely to remain at <100% unless the CAP critical concentration issue is addressed independently of the PSQ platform, and other gene regions are added to the PSQ assay.

Twenty-one isolates tested phenotypically resistant to KAN but susceptible to CAP and AMK. It is likely the sensitivity of our PSQ assay for detecting isolates with KAN resistance and no CAP/AMK cross-resistance was low (68.1%) due to the fact that the *rrs* was the only gene region evaluated in our PSQ assay. Upon completion of the present study, Rodwell et al. determined through Sanger sequencing that the majority of the KAN isolates examined in their study that had no *rrs* 1401 SNPs had SNPs in the *eis* promoter (18). Undetected *eis* promoter mutations, along with recently reported mutations in *whiB7* gene regions (42–44), might be responsible for the observed KAN-resistant phenotypes that were not detected by our PSQ assay. Adding the *eis* promoter region as a PSQ target in a future version of the PSQ assay should considerably improve its sensitivity for detecting KAN resistance in any location where these SNPs are prevalent.

Conclusion. PSQ is a robust, rapid sequencing method that has been adapted to detect all of the primary canonical SNPs known to confer first- and second-line drug resistance in TB. Our assay can simultaneously confirm the presence of *M. tuberculosis* and detect the mutations associated with resistance to anti-TB drugs, thereby diagnosing XDR-TB with good sensitivity and specificity. Although our study was limited to testing clinical *M. tuberculosis* isolates grown from culture, the assay has also been used to test clinical specimens directly (15), which significantly shortens and simplifies the turnaround time for the detection of drug resistance in TB patients, and the performance of PSQ is comparable to that of commercially available line-probe assays (7, 9). Although there is room to improve the sensitivity and specificity of our assay, this and other published studies (14, 15) indicate that PSQ should be considered a supplemental method for obtaining rapid DST results before the availability of phenotypic DST results, especially when drug resistance is suspected. In addition, while strong evidence is mounting that specific SNPs confer particular MICs (45–47), more work is needed in order to develop the clinical treatment guidance algorithms needed for real-time interpretation of PSQ results.

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