Memorial Lecture by the Imamura Award Winner

EXTERNAL QUALITY ASSESSMENT OF ANTI-TUBERCULOSIS DRUG SUSCEPTIBILITY TESTING FOR DIAGNOSING EXTENSIVELY DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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Abstract [Objective] The infectious disease control law in Japan was amended in May 2015, and the category definition of *Mycobacterium tuberculosis* as an infectious pathogen has been changed, following the definition of extensively drug-resistant *M. tuberculosis* (XDR-TB) by the World Health Organisation. To assess the diagnostic capacity of XDR-TB, we conducted an external quality assessment (EQA) for anti-tuberculosis drug susceptibility testing (DST). [Method] A total of 10 *M. tuberculosis* strains with known drug susceptibilities were sent to each participating laboratory. The drugs assessed were isoniazid (INH), rifampicin (RFP), streptomycin (SM), ethambutol (EB), levofloxacin (LVFX), and kanamycin (KM). DST was performed using each routine method(s), and the results were compared with the judicial diagnoses. The sensitivity, specificity, overall agreement (efficiency) and kappa coefficient were calculated for each drug tested. In addition, the diagnostic accuracy of multidrug-resistant *M. tuberculosis* (MDR-TB) and XDR-TB was assessed. [Results] A total of 88 institutes including 67 hospitals, 16 commercial laboratories, and 5 public health laboratories participated in the EQA. With two laboratories submitting two sets of results, a total of 90 independent data sets were analyzed. For INH, RFP and LVFX, the efficiency was over 95%, but we found two strains each for SM, EB and KM with efficiencies less than 95%. In particular, strain 1 and strain 2 showed efficiencies of 72.2% and 71.1% for SM, respectively. This error was mainly found with one particular test kit. If we consider a passing score as showing ≧ 95% sensitivity and specificity both to INH and RFP, the diagnostic accuracy of MDR-TB was 92.2% (83/90) in this study. With the same criteria for INH, RFP, LVFX and KM, that of XDR-TB was 79.7% (63/79). [Discussion] The diagnostic capacity for XDR-TB was not sufficient in the current study. Good case management and pathogen control requires higher accuracy. The government may need to conduct a constant EQA and apply relevant remedial actions.
Key words: *Mycobacterium tuberculosis*, Drug susceptibility testing, External quality assessment, Extensively drug-resistant *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis is still a major life-threatening disease in the world, especially when the bacteria have acquired antimicrobial drug resistance (AMR). The World Health Organisation (WHO) has defined that *Mycobacterium tuberculosis* strains that have acquired drug resistance to both isoniazid (INH) and rifampicin (RFP), the two major anti-tuberculosis drugs, as multidrug-resistant *M.tuberculosis* (MDR-TB). In 2007, a MDR-TB strain with injectable drug and fluoroquinolone resistances emerged and was designated as extensively drug-resistant *M.tuberculosis* (XDR-TB). Tuberculosis disease with such drug resistant strains is recognised as intractable, and remains life-threatening today.

To cope with MDR- and XDR-TB, a correct diagnosis is the key to an appropriate treatment. To correctly diagnose drug resistant tuberculosis, appropriate drug susceptibility testing (DST) is of great importance. Although quality assurance (QA) measures are quite important to secure the quality of DST, there has been no systematic QA in the era of anti-tuberculosis DST. Since 2002, the committee for the mycobacterial examinations in the Japanese Society for Tuberculosis has started external quality assessments (EQA) of anti-TB DST, and established a standard EQA method and associated

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(Received 11 Oct. 2018)

evaluation criteria in 2015[1]. The infectious disease control law was amended in May 2015, and the category definition of *Mycobacterium tuberculosis* as an infectious pathogen has been changed, following the definition of XDR-TB. To assess the diagnostic capacity of XDR-TB, based on the methods and evaluation criteria established in previous studies, we conducted an EQA for the anti-tuberculosis DST to identify XDR-TB.

MATERIALS AND METHODS

Participating laboratories

Laboratories that participated in the current EQA followed the facility and administrative conditions defined by the infectious disease control law for handling any *M.tuberculosis* strains other than XDR-TB. It was also clarified in the study protocol that the EQA study was not conducted by the Committee for the Mycobacterial Examinations in the Japanese Society for Tuberculosis to avoid the misunderstanding of the participants. A total of 88 laboratories participated in the study.

***Mycobacterium tuberculosis* strains**

A total of 10 *M.tuberculosis* strains with known drug susceptibilities were sent to each participating laboratory. The strains were selected from the stocks used in the internal control program of the TB Supranational Reference Laboratory Network (SRLN), a part of the Global Laboratory Initiative of WHO; chosen strains showed over 80% concordant results in SRLN. However, since streptomycin (SM) has been excluded from the panel since 2014, the judicial diagnosis (JUD) of SM was set as the concordant result of multiple DST methods, i.e., minimum inhibitory concentration (MIC) and conventional proportion, performed at the bacteriology division in the Department of Mycobacterium Reference and Research, Research Institute for Tuberculosis (RIT).

It was clearly mentioned in the protocol that the strains did not include XDR-TB due to the strict regulations for their transport in Japan, but precautions were clearly given to all participating laboratories because the strains contained MDR-TB.

Target drugs

The drugs assessed were INH, RFP, SM, ethambutol (EB), levofloxacin (LVFX), and kanamycin (KM). DST was performed using the routine method(s) used in each laboratory, and the results were compared with the judicial diagnoses (Table 1). The sensitivity, specificity, overall agreement (efficiency) and kappa coefficient were calculated for each drug tested. In addition, the diagnostic accuracy of MDR-TB and XDR-TB was assessed.

Statistical analysis

The data was stored in Microsoft Excel (Microsoft, Seattle, WA). Student-t tests were performed for the comparison of data with JMP 12.1 (SAS Institute Inc., CA), and p-value of <0.05 was considered statistically significant.

RESULTS

A total of 88 institutes including 67 hospitals, 16 commercial laboratories, and 5 public health laboratories participated in the EQA. All 88 laboratories submitted their results (100% recovery). The turn-around time was 63.2±20.9 (range: 21−109) days. With two laboratories submitting two sets of results, a total of 90 independent data sets were analysed. Among 880 strains sent to the laboratories, three strains in three facilities did not grow, so the loss rate was 0.34% (3/880). The judicial diagnoses in SRLN and the MICs of the strains used in this EQA are shown in Table 2.

For INH, RFP and LVFX, the efficiency was over 95%, but we found two strains each for SM, EB and KM with efficiencies less than 95%. In particular, strain 1 and strain 2 showed efficiencies of 72.2% and 71.1% for SM, respectively (Table 3). This error was mainly found when a specific test kit was used.

Table 4 shows the EQA results categorized by institutional types. The overall sensitivity, specificity, efficiency and kappa

Table 1 Proportion of diagnostic agreement among Supranational Reference Laboratory Network

Specimen ID	Proportion of agreement (%)					
	INH	RFP	SM	EB	LVFX	KM
1	100	100	NA	94.0	96.6	100
2	100	100	NA	90.0	96.6	100
3	100	97.0	NA	97.0	100	93.5
4	100	97.0	NA	100	96.6	100
5	100	97.0	NA	100	94.8	96.8
6	98.0	100	NA	94.0	96.6	100
7	98.0	98.0	NA	98.0	93.1	98.4
8	100	100	NA	98.3	100	100
9	98.3	100	NA	98.3	100	98.3
10	100	100	NA	100	100	100

ID: identification, NA: Not available, Agreement data was not available among Supranational Reference Laboratory Network, because streptomycin has been excluded from the programme since 2014.
INH: isoniazid, RFP: rifampicin, SM: streptomycin, EB: ethambutol,
LVFX: levofloxacin, KM: kanamycin

Table 2 Judicial diagnosis and MIC for each strain

Specimen ID	INH		RFP		SM		EB		LVFX		KM	
	JUD	MIC	JUD	MIC	JUD	MIC	JUD	MIC	JUD	MIC	JUD	MIC
1	R	16	R	≧32	S	4	R	32	R	4	S	4
2	R	16	R	≧32	S	4	R	16	R	4	S	4
3	R	16	R	≧32	R	32	S	2	S	0.5	R	64
4	S	0.125	S	≦0.03	R	128	S	2	R	4	S	1
5	S	0.125	S	≦0.03	S	2	S	1	S	0.5	R	≧128
6	R	16	R	≧32	R	≧128	R	16	S	0.5	R	≧128
7	S	0.25	S	≦0.03	S	1	S	2	R	8	S	2
8	R	16	R	≧32	S	1	R	16	S	0.5	S	4
9	S	0.125	S	≦0.03	S	1	S	1	S	0.5	R	≧128
10	S	0.125	S	≦0.03	R	128	S	2	R	4	S	1

JUD: Judicial diagnosis, R: Resistant, S: Susceptible, MIC: Minimum Inhibitory Concentration (μg/m*l*), measured by broth microdilution method (Middlebrook7H9 supplemented with OADC)

Table 3 Diagnostic agreements among the participants (n=90) and SRLN judicial diagnoses

ID	INH		RFP		SM		EB		LVFX		KM	
	JUD	EFFI	JUD	EFFI	JUD*	EFFI	JUD	EFFI	JUD	EFFI	JUD	EFFI
1	R	0.978	R	0.978	S	0.722	R	0.978	R	1.000	S	0.975
2	R	0.989	R	0.989	S	0.711	R	0.989	R	1.000	S	0.987
3	R	0.989	R	0.989	R	0.956	S	0.922	S	1.000	R	1.000
4	S	0.989	S	0.989	R	1.000	S	0.978	R	1.000	S	1.000
5	S	1.000	S	0.989	S	1.000	S	0.967	S	1.000	R	0.911
6	R	1.000	R	1.000	R	1.000	R	1.000	S	1.000	R	1.000
7	S	0.989	S	0.967	S	0.978	S	0.956	R	1.000	S	0.987
8	R	0.978	R	1.000	S	0.989	R	0.978	S	0.975	S	1.000
9	S	0.978	S	0.989	S	0.978	S	0.944	S	1.000	R	0.899
10	S	1.000	S	0.989	R	1.000	S	0.989	R	1.000	S	1.000

ID: identification, R: Resistant, S: Susceptible, JUD: judicial diagnosis by Supranational Reference Laboratory Network (*JUD of streptomycin was confirmed at the Research Institute of Tuberculosis.), EFFI: Efficiency (overall agreement of resistant and susceptible)

coefficient for INH were 99.1±5.1%, 99.1±4.1%, 99.1±3.9%, and 0.982±0.078, respectively. Similarly, those for the other drugs were 99.6±4.2%, 98.4±9.2%, 99.0±5.0%, and 0.980±0.100 for RFP, 98.9±5.2%, 89.6±15.2%, 93.3±9.0%, and 0.864±0.174 for SM, and 99.2±5.9%, 95.9±12.6%, 97.2±7.8%, and 0.942±0.141 for EB, respectively.

Testing of LVFX and KM was optional, so a total of 79 laboratories performed DST for those drugs. One laboratory performed DST for those drugs with MGIT AST. The overall sensitivity, specificity, efficiency and kappa coefficient for LVFX were 100%, 99.5±3.2%, 99.7±1.6%, and 0.995±0.032, respectively. Those for KM were 95.6±13.1%, 99.2±4.5%, 97.7±6.2%, and 0.952±0.136, respectively.

Four false susceptible (FS) and four false resistant (FR) results were observed for INH, and two and seven false results were observed for RFP, respectively. There was no significant difference between the proportion of FS and FR ($p=0.181$). The highest numbers of errors were observed for SM, i.e., four FS and 56 FR results ($p<0.001$). EB showed a similar trend as SM; FS and FR were 3 and 22, respectively ($p=0.007$). There were only two FR results for LVFX, and 14 FS and 4 FR results for KM ($p=0.002$).

Table 5 shows the performance of each DST kit for each drug tested. MGIT AST, Welpack S (Japan BCG Laboratory, Tokyo), Bitspectre SR (Kyokuto Pharmaceutical, Tokyo) and BrothMIC MTB-I (Kyokuto Pharmaceutical, Tokyo) were used in 12 (13.3%), 26 (28.9%), 30 (33.3%) and 22 (24.4%) of the participating laboratories, respectively. With INH, the sensitivity of MGIT AST was significantly lower than those of other kits, and the specificity of BrothMIC MTB-I was lower than those of Bitspectre SR and Welpack S ($p<0.05$). In particular with SM, Welpack S showed low specificity and kappa coefficients compared to other DST kits ($p<0.0001$).

Regarding the diagnostic capacity of M/XDR-TB, a total of 90 and 79 data sets were available. If we consider the passing score to be ≧95% sensitivity and specificity both to INH and RFP, the diagnostic accuracy of MDR-TB was 92.2% (83/90) in this study. With the same criteria for INH, RFP, LVFX and KM, that of XDR-TB was 79.7% (63/79). Statistically significant differences were observed between these indicators ($p<0.001$).

DISCUSSION

An EQA study of DST was implemented using MDR-TB

Table 4 Sensitivity, specificity, efficiency and kappa coefficient of each drug tested categorized by institutional types

Indicator	Hospitals (n = 68)			Commercial laboratory (n = 17)			Public health laboratory (n = 5)			All (n = 90)*		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
INH												
Sensitivity	0.988	1.000	0.600	1.000	1.000	1.000	1.000	1.000	1.000	0.991	1.000	0.600
Specificity	0.988	1.000	0.800	1.000	1.000	1.000	1.000	1.000	1.000	0.991	1.000	0.800
Efficiency	0.988	1.000	0.700	1.000	1.000	1.000	1.000	1.000	1.000	0.991	1.000	0.700
kappa*	0.976	1.000	0.400	1.000	1.000	1.000	1.000	1.000	1.000	0.982	1.000	0.400
RFP												
Sensitivity	0.994	1.000	0.600	1.000	1.000	1.000	1.000	1.000	1.000	0.996	1.000	0.600
Specificity	0.979	1.000	0.200	1.000	1.000	1.000	1.000	1.000	1.000	0.984	1.000	0.200
Efficiency	0.987	1.000	0.600	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000	0.600
kappa	0.973	1.000	0.200	1.000	1.000	1.000	1.000	1.000	1.000	0.980	1.000	0.200
SM												
Sensitivity	0.985	1.000	0.750	1.000	1.000	1.000	1.000	1.000	1.000	0.989	1.000	0.750
Specificity	0.918	1.000	0.500	0.814	1.000	0.667	0.867	1.000	0.667	0.896	1.000	0.500
Efficiency	0.945	1.000	0.700	0.888	1.000	0.800	0.920	1.000	0.800	0.933	1.000	0.700
kappa	0.888	1.000	0.444	0.778	1.000	0.615	0.839	1.000	0.615	0.864	1.000	0.444
EB												
Sensitivity	0.989	1.000	0.500	1.000	1.000	1.000	1.000	1.000	1.000	0.992	1.000	0.500
Specificity	0.953	1.000	0.000	0.980	1.000	0.833	0.967	1.000	0.833	0.959	1.000	0.000
Efficiency	0.968	1.000	0.400	0.988	1.000	0.900	0.980	1.000	0.900	0.972	1.000	0.400
kappa	0.933	1.000	0.000	0.976	1.000	0.800	0.959	1.000	0.800	0.942	1.000	0.000
LVFX												
Sensitivity	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Specificity	0.996	1.000	0.800	0.988	1.000	0.800	1.000	1.000	1.000	0.995	1.000	0.800
Efficiency	0.998	1.000	0.900	0.994	1.000	0.900	1.000	1.000	1.000	0.997	1.000	0.900
kappa	0.996	1.000	0.800	0.988	1.000	0.800	1.000	1.000	1.000	0.995	1.000	0.800
KM												
Sensitivity	0.947	1.000	0.500	0.971	1.000	0.500	1.000	1.000	1.000	0.956	1.000	0.500
Specificity	0.997	1.000	0.833	0.971	1.000	0.667	1.000	1.000	1.000	0.992	1.000	0.667
Efficiency	0.977	1.000	0.800	0.971	1.000	0.700	1.000	1.000	1.000	0.977	1.000	0.700
kappa	0.952	1.000	0.545	0.939	1.000	0.348	1.000	1.000	1.000	0.952	1.000	0.348

*n = 79 in LVFX and KM

strains in 2015. The amended infectious disease law allowed us to use MDR-TB for this EQA study. This might have affected the accuracy of the EQA evaluation, because no use of XDR-TB was clearly declared in the protocol. However, for INH and RFP, the EQA was completely blind, which is appropriate for this study.

In general, the sensitivity of MGIT AST for INH is higher than that of Ogawa medium[2]. However, in this study, the sensitivity of MGIT AST was lower than that of Ogawa medium based DST kits. This finding was somewhat unexpected, but such a discrepancy generally occurs for strains with MICs between 0.2−0.8 μg/m*l*[3]. However, the strains used in this study had relatively high MICs and only one strain fell into in this range. In addition, there were no laboratories that reported ≦80% of sensitivity with Ogawa based medium, and two laboratories using MGIT AST showed 60% and 80% sensitivity for INH. Technical difficulties may have affected the INH susceptibility testing results for MGIT AST.

Two strains showed low efficiencies (＜80%) for SM in this panel. The SRLN has already excluded SM from the testing panel due to low reproducibility; therefore, the JUD in this study was the consensus result of multiple DSTs done in RIT. Based on these results, it could be suggested that these two strains would be excluded from the panel due to their low efficiencies; however, low efficiencies were mainly found for the Welpack S kit in this study, while the other kits showed ≧95% efficiency. This result indicates that the false resistance errors observed for these two strains were mainly due to problems with the kit. Past EQA activities did not show the phenomenon[2], so the kit used in this EQA might have been defective.

This was the first EQA in Japan to evaluate the capacity to diagnose XDR-TB, and it included LVFX and KM as the tested drugs. It was considered that KM showed sufficient accuracy in this EQA, although BrothMIC MTB-I showed somewhat lower performance. However, the critical concentration of KM employed in Ogawa medium is 20 μg/m*l*[4], while that of Löwenstein-Jensen is 30 μg/m*l*[5]. Therefore, although more FR results were expected than FS, the actual results were opposite of this prediction (4 FR vs. 14 FS, p=0.002). Many FS results came from BrothMIC MTB-I and therefore, the MIC cut-off for KM should be reconsidered.

This study revealed that 79.7% of participating laboratories correctly identified INH, RFP, LVFX and KM resistances.

Table 5 Sensitivity, specificity, efficiency and kappa coefficient for each drug susceptibility testing kit

Indicator	MGIT AST (n=12)		Welpack S (n=26)		Bitspectre SR (n=30)		BrothMIC MTB-I (n=22)		Appendix
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
INH									
Sensitivity	0.950	0.947−0.953	1.000	−	1.000	−	0.991	0.990−0.992	wel, bit, bro>mgit, p<0.05
Specificity	0.983	0.982−0.985	1.000	−	1.000	−	0.973	0.971−0.974	bit, wel>bro, p<0.05
Efficiency	0.966	0.964−0.968	1.000	−	1.000	−	0.982	0.981−0.983	bit, wel>mgit, p<0.05
kappa*	0.932	0.928−0.936	1.000	−	1.000	−	0.964	0.962−0.965	bit, wel>mgit, p<0.05
RFP									
Sensitivity	0.967	0.964−0.969	1.000	−	1.000	−	1.000	−	wel, bit, bro>mgit, p<0.05
Specificity	0.979	0.977−0.981	1.000	−	1.000	−	0.945	0.943−0.948	bit, wel>bro, p<0.05
Efficiency	0.974	0.972−0.976	1.000	−	1.000	−	0.973	0.971−0.974	No significant difference
kappa	0.947	0.945−0.950	1.000	−	1.000	−	0.945	0.943−0.948	No significant difference
SM									
Sensitivity	0.979	0.977−0.981	1.000	−	0.975	0.974−0.976	1.000	−	No significant difference
Specificity	0.953	0.950−0.956	0.718	0.716−0.720	0.967	0.965−0.968	0.977	0.976−0.979	bro, bit mgit>wel, p<0.0001
Efficiency	0.965	0.963−0.967	0.831	0.829−0.832	0.970	0.969−0.971	0.986	0.985−0.987	bro, bit mgit>wel, p<0.0001
kappa	0.930	0.926−0.933	0.675	0.673−0.677	0.939	0.937−0.941	0.973	0.971−0.974	bro, bit mgit>wel, p<0.0001
EB									
Sensitivity	0.958	0.955−0.962	1.000	−	0.992	0.991−0.993	1.000	−	bro, wel>mgit, p<0.05
Specificity	0.983	0.982−0.985	0.987	0.986−0.988	0.956	0.954−0.957	0.917	0.913−0.92	No significant difference
Efficiency	0.974	0.972−0.976	0.992	0.991−0.993	0.970	0.969−0.971	0.950	0.948−0.952	No significant difference
kappa	0.944	0.941−0.947	0.985	0.983−0.986	0.940	0.939−0.942	0.909	0.906−0.913	No significant difference
LVFX									
Sensitivity	ND		1.000	−	1.000	−	1.000	−	No significant difference
Specificity	ND		0.992	0.991−0.993	0.993	0.992−0.994	1.000	−	No significant difference
Efficiency	ND		0.996	0.995−0.997	0.997	0.996−0.997	1.000	−	No significant difference
kappa	ND		0.992	0.991−0.993	0.993	0.992−0.994	1.000	−	No significant difference
KM									
Sensitivity	ND		1.000	−	0.992	0.991−0.993	0.864	0.860−0.867	bit, wel>bro, p<0.001
Specificity	ND		0.986	0.985−0.987	1.000	−	0.985	0.984−0.986	No significant difference
Efficiency	ND		0.992	0.991−0.993	0.997	0.996−0.997	0.936	0.935−0.938	bit, wel>bro, p<0.01
kappa	ND		0.984	0.982−0.985	0.993	0.992−0.994	0.859	0.855−0.862	bit, wel>bro, p<0.001

*kappa: kappa coefficient, wel: Welpack S, bit: Bitspectre SR, bro: BrothMIC MTB-I, mgit: MGIT AST, ND: Not done (no data)

This does not indicate correct diagnosis of XDR-TB, because XDR-TB were not included in the panel. Transportation of XDR-TB in Japan must follow strict regulations and is very costly, so the use of XDR-TB in EQA is technically impossible. Therefore, the EQA was not completely blinded for the identification of XDR-TB, which is the major limitation of this study.

Given the amended definition of pathogens in the infectious disease control law in Japan, the EQA for necessary drugs was performed. In the current study, the identification accuracy for MDR-TB was 92.2%, while that of XDR-TB was 79.7%. It was concluded that the accuracy of diagnosing XDR-TB was insufficient for the appropriate control of the pathogens. Therefore the quality assurance law may require appropriate remedial actions to ensure good DST performance. Good case management and pathogen control requires higher accuracy.

ACKNOWLEDGEMENT

The author would like to express sincere appreciation to Hiroyuki Yamada, Akio Aono, Kinuyo Chikamatsu, Takeshi Higuchi, Yuriko Igarashi and Akiko Takaki for their dedicated contributions to this study. The study was supported by the Japan Agency for Medical Research and Development (15fk0108017h0002).

COI declaration: The author has nothing to declare.

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