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ORIGINAL ARTICLE

Development and validation of external quality assessment panels for mycobacterial culture testing to diagnose tuberculosis in China

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Abstract

Mycobacterial culture remains the gold standard for detection of *Mycobacterium tuberculosis* (MTB) in clinical samples. However, no external quality assessment (EQA) tools exist to validate results obtained using this sophisticated method. Therefore, we developed EQA panels to assess the quality of mycobacterial culture results produced by designated TB hospitals in China. Artificial sputum containing methylcellulose was used to supplement quantified mycobacterial solutions to simulate culture-negative and culture-positive clinical sputum samples of low or high mycobacterial concentration, respectively. After storage of the quantified simulated EQA panels for 4 weeks at 4 °C, experimental bacterial quantification of the panels was again conducted, with no impact of artificial sputum on mycobacterial culture results observed. Next, 47 tuberculosis (TB) hospitals were recruited for evaluation of the EQA panels. Overall, 29 hospitals (61.7%) produced mycobacterial culture test results matching expected results for the EQA panels, while the remaining 18 (38.3%) hospitals did not. False-negative results for the low mycobacterial concentration panel sample accounted for 33 (73.3%) diagnostic errors. Compared with hospitals using solid culture methods as a control group, hospitals using the liquid culture method were less likely to produce uncertified results (aOR 0.064, 95% CI 0.005–0.770). In conclusion, we first developed then evaluated EQA panels for validation of mycobacterial culture testing in China. Our data demonstrate that approximately one-third of TB hospitals failed to produce results that met criteria for classification as certified mycobacterial culture testing providers, emphasizing the importance of quality control and quality assurance in TB diagnostics.

Keywords Tuberculosis · Mycobacterial culture · External quality assurance · China

Introduction

Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* (MTB) complex, remains a major public health concern throughout the world [1, 2]. The World Health Organization (WHO) has estimated that in 2017, 10 million people developed tuberculosis and 1.6 million people died of this disease [2]. More importantly, the emergence of multidrug-resistant

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Main point We first developed then evaluated EQA panels for validation of mycobacterial culture testing in China. Approximately one-third of TB hospitals failed to produce results that met criteria for classification as certified mycobacterial culture testing providers.

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tuberculosis (MDR-TB) constitutes an increasing threat to TB control efforts [3]. Despite an estimated 480,000 new MDR-TB cases per year, just over a quarter of these cases have been diagnosed and reported to WHO [1]. This gap reflects insufficient diagnostic capacity for detection of MDR-TB cases, especially in settings with high TB burden [1]. Therefore, early detection of TB and rapid identification of drug resistance are important for improving individual patient outcomes and preventing transmission within the community [4].

Mycobacterial culture remains the gold standard for detection of MTB in clinical samples [5]. Positive cultures are also essential for subsequent drug susceptibility testing (DST) in order to diagnose drug-resistant TB, formulate effective therapeutic regimens [6], monitor MTB drug susceptibility, and assess TB transmission dynamics [5]. Unfortunately, sophisticated procedures for mycobacterial culture require skilled and experienced staff to obtain quality-assured culture results [7]. To meet these demands, an appropriate system of quality assurance must be in place to ensure consistently accurate results.

External quality assessment (EQA) is an important component of any quality assurance program [8]. In the past decade, a well-established EQA system has been widely used to test laboratory proficiency for the performance of smear microscopy and phenotypic DST [9–11]. In contrast, for performance of mycobacterial culture, only a limited number of internal quality control indicators exist for assessing reliability of results. This fact is due to rigorous requirements regarding the use of homogenized sputum samples containing live mycobacteria as EQA tools for validation of mycobacterial culture test results, the cornerstone of laboratory diagnosis of TB.

China has the second highest TB burden globally, with an estimated 0.9 million incident TB cases in 2017 [1, 12]. Despite the rapid expansion of molecular diagnostics, mycobacterial culture remains the MTB detection approach most accessible to resource-constrained laboratory settings in China. In fact, only approximately 30% of reported TB cases currently yield positive bacterial evidence, a percentage far below the global average [1]. This fact has hindered reform of TB control programs implemented by public health systems since 2010 to improve healthcare accessibility for TB patients through hospital-public health collaboration [13]. As a consequence, designated TB hospitals now play a greater role in the diagnosis and treatment of TB cases, but the lack of a quality control system for TB laboratories still poses a major challenge to ensuring high-quality results. To address these concerns, here we developed EQA panels for use in validation of mycobacterial culture test results. The panels were then submitted to laboratories for testing to evaluate the quality of mycobacterial culture results obtained by selected designated TB hospitals in China.

Materials and methods

Preparation of mycobacteria and *Escherichia coli*

The *Mycobacterium tuberculosis* H37Ra (ATCC25177) strain was subcultured on Löwenstein-Jensen (L-J) medium for 4 weeks at 37 °C. Freshly grown MTB colonies were scraped from the surface of L-J slants and then transferred to sterilized screw cap tubes containing glass beads. Turbidity of dispersed bacteria was adjusted to 1.0 using a McFarland turbidity standard (approximate 10^8 CFU/mL). Each filtrate was cultured on Middlebrook 7H10 medium for counting purposes. To obtain dead mycobacteria for artificial sputum, a concentrated quantified mycobacterial suspension was heated for 60 min at 85 °C and tested for complete inactivation by inoculation of heated bacteria onto Middlebrook 7H10 medium, as indicated by a lack of growth. In addition, we also prepared *E. coli* using the method described above for addition to artificial sputum (described below) to simulate potential bacterial contamination of sputum samples.

Preparation of artificial sputum

The methylcellulose method previously described by Yamada et al. was modified for preparation of artificial sputum for use in validation of smear microscopy test results [14]. Briefly, 20 g of methylcellulose was dispersed in 900 mL of distilled water and then heated at 121 °C for 15 min in an autoclave. After cooling to room temperature, 100 g of emulsified egg powder was mixed with the methylcellulose solution to generate artificial sputum. 1.8 mL of artificial sputum was pipetted into each centrifuge tube. Next, 0.2 mL of *E. coli* solution at a concentration of 10^6 CFU/mL was added to each tube. To these tubes, either 0.2 mL of a solution containing dead mycobacteria at 10^6 CFU/mL, 0.2 mL of a live mycobacterial solution at 10^4 CFU/mL, or 0.2 mL of a solution of live mycobacteria at 10^6 CFU/mL was added to generate simulated culture-negative sputum, sputum with low mycobacterial concentration, or sputum with high mycobacterial concentration, respectively.

External quality assurance (EQA) panel development

The external quality control panels consisted of 20 artificial sputum samples, including 8 samples without live mycobacteria, 6 samples of low mycobacterial concentration, and 6 samples of high mycobacterial concentration. To blind the study participants to panel specimen constituents, barcodes were generated to encode the panel matrix and the contents of each sample. Each panel also included EQA instructions for use provided with the panels supplied to each centralized laboratory.

Ten samples of each sputum type were randomly selected, and solid culture and BACTEC MGIT were performed to recheck the quality of artificial sputum samples. In addition, another 80 samples of each type were recruited to assess whether duration of storage at 4 °C had any effect on the results of mycobacterial culture testing after storage for 1 week, 2 weeks, 4 weeks, or 8 weeks.

Settings

A cross-sectional study was conducted from laboratories in designated TB hospitals in China between October 2018 and December 2018. The National Clinical Laboratory on TB (NCLT) of Beijing Chest Hospital supervised the implementation of this study. A total of 47 hospitals, which provide routine mycobacterial culture testing services, participated in the EQA program for assessment of mycobacterial culture results from each hospital (Fig. 1). Prior to transport of EQA panels to recipient hospitals, licenses for mycobacterial transport to hospital sites were approved by the National Health Commission of the People's Republic of China. All panels were packaged according to national safety protocols and then were transported under strict cold chain maintenance after passage no more than 2 days before transport to test laboratory recipients. Hospital sites participating in this study were required to complete culture procedures within 2 days after arrival of EQA samples and to use their routine mycobacterial culture methods for testing of the EQA panels. Solid culture methods on acid L-J medium or conventional L-J medium and liquid culture using BACTEC MGIT 960 tubes were recommended culture methods. In addition, the culture methods were divided into simple and concentration methods according to the decontamination procedures. For the simple method, the sputum was mixed with an equal amount of 4% NaOH solution. After incubation at 37 °C for 15 min with occasional shaking, 0.1 mL of the decontaminated sample was used as an inoculum to L-J medium. For the concentration method, the sputum was treated with an equal volume of N-acetyl-L-cysteine (NALC) plus 2% NaOH. After incubation at 37 °C for 15 min with occasional shaking, the volume was brought to 50 ml with 0.067 M phosphate buffer (pH 6.8). Then, the mixture was centrifugated at 3,000×*g* for 15 min, and the pellet was resuspended in 2 mL PBS buffer. A proportion of the decontaminated specimens were cultured on culture medium. NCLT staff provided technical assistance during the study period.

Data collection and analysis

Upon completion of testing by participating laboratories, we collected from each laboratory the mycobacterial culture method that was used, the manufacturing procedure used to prepare culture medium, mycobacterial culture experience of laboratory staff, and test results. All data were submitted via the website (http://chinaiatb.com/jiehe/certification/login.html). Performance of on-site mycobacterial culture testing methods was considered suitable if a laboratory met the following criteria: (i) a contamination rate no greater than 5% for

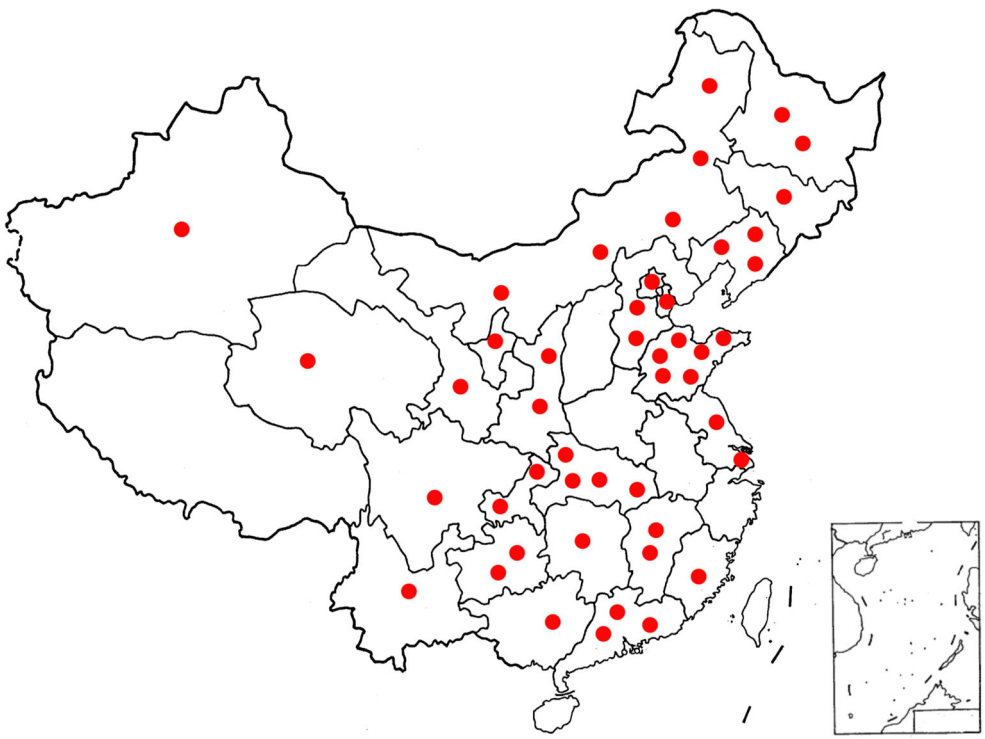

Fig. 1 Distribution of TB designated hospitals enrolled in this evaluation

solid culture or 10% for liquid culture and (a) a culture-positive rate for smear-positive sputum samples of no less than 90% [15]. As a consequence, the operational definition of a certified mycobacterial culture testing laboratory was one that produced results as follows: (i) no more than one sample with culture contamination for solid culture or two samples with culture contamination for liquid culture, (ii) no more than one high mycobacterial concentration sample with negative culture result, (iii) no more than two low mycobacterial concentration samples with negative culture result and no high mycobacterial concentration sample with a negative culture result, and (iv) no more than one negative sample with a positive culture result.

Statistical analysis was conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The chi-square test was used to evaluate associations among multiple categorical variables. Covariates that were statistically significant in the univariate analysis were introduced into the multivariate model to determine whether covariates were independently associated with the uncertified EQA results. Statistical differences were deemed significant if P values were less than 0.05.

Results

Influence of storage time on recovery of mycobacteria

We first evaluated the influence of storage time on recovery of mycobacteria in artificial sputum. Three hundred specimens were tested using two mycobacterial culture methods, solid and liquid, after different storage durations. As shown in Table 1, storage of artificial sputum had no effect on results obtained using the liquid culture method, even after 8 weeks of storage at 4 °C. For solid culture, both negative samples and positive samples with high mycobacterial load produced the expected results after 8 weeks of storage, although the proportion of positive cultures fell by 20% (2/10) after an 8-week storage period. Taken together, 4 weeks of storage of artificial sputum samples at 4 °C prior to experimental testing had no impact on results obtained using solid or liquid mycobacterial culture methods (Table 1).

EQA results for mycobacterial culture in China

EQA panels containing 20 artificial sputum samples were delivered to 47 TB hospitals via specific transportation pathways. Overall, 29 hospitals (61.7%) were confirmed as certified to provide reliable mycobacterial culture testing, while the remaining 18 hospitals (38.3%) were deemed uncertified after evaluation of their EQA test results. Upon further detailed analysis of EQA results (Table 2), of the 29 certified hospitals, 14 (48.3%, 14/29) obtained perfect scores for EQA testing. Among the 18 uncertified hospitals, a total of 45 errors were recorded, the most common of which were false-negative results for samples of low mycobacterial concentration that resulted in 33 (73.3%) errors. Other errors included false-negative results for samples of high mycobacterial concentration (22.2%, 10/45) and culture contamination (4.4%, 2/45) (Table 3).

Factors associated with uncertified EQA results of mycobacterial culture testing

After comparing characteristics of uncertified hospitals with those of certified hospitals, the distribution of uncertified hospitals showed no significant association with the geographic region ($P > 0.05$). However, comparison of hospital results overall with those obtained using a solid culture method as the control group demonstrated that hospitals using the liquid culture method were less likely to produce uncertified results (aOR 0.064, 95% CI 0.005–0.770). Although univariate analysis revealed that patient specimens tested using the simple culture method had significantly higher odds of having uncertified EQA results compared with those tested using the concentration

Table 1 Influence of storage time on recovery of mycobacteria in EQA specimens

Storage time	No. of specimens with expected results/no. of specimens tested					
	Liquid culture			Solid culture		
	Negative	High mycobacterial concentration	Low mycobacterial concentration	Negative	High mycobacterial concentration	Low mycobacterial concentration
0 week	10/10	10/10	10/10	10/10	10/10	10/10
1 week	10/10	10/10	10/10	10/10	10/10	10/10
2 weeks	10/10	10/10	10/10	10/10	10/10	10/10
4 weeks	10/10	10/10	10/10	10/10	10/10	10/10
8 weeks	10/10	10/10	10/10	10/10	10/10	8/10

Table 2 Overview of external quality assurance results for mycobacterial culture

No. of hospitals	Type of error				Results for external quality assurance
	False negative for samples with high mycobacterial concentration	False negative for samples with low mycobacterial concentration	False positive for samples without mycobacteria	Contamination	
14	0	0	0	0	Qualified
2	1	0	0	0	
6	0	1	0	0	
6	0	2	0	0	
1	0	2	0	1	
3	0	3	0	0	Unqualified
2	0	4	0	0	
8	0	5	0	0	
1	1	2	0	0	
1	1	3	0	0	
1	2	6	0	0	
1	4	6	0	0	
1	2	4	2	0	

method (OR 8.667, 95% CI 1.913–39.255), this difference disappeared during multivariate analysis (aOR 0.926, 95% CI 0.056–15.197). This result indicates that the sample decontamination method used by a hospital was a confounding factor with the culture method. In addition, hospital type and level had no significant association with certified and uncertified hospital status ($P > 0.05$) (Table 3).

Table 3 Factors associated with unqualified EQA results for mycobacterial culture

Characteristics[a]	Results of EQA results		Total (%)	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
	Qualified (%)	Unqualified (%)					
Region							
Eastern	13 (68.4)	6 (31.6)	19 (100.0)	1.000	Reference		
Middle	9 (81.8)	2 (18.2)	11 (100.0)	0.481 (0.079–2.948)	0.429		
Western	7 (41.2)	10 (58.8)	17 (100.0)	3.095 (0.789–12.144)	0.105		
Decontamination method							
Concentration method	26 (74.3)	9 (25.7)	35 (100.0)	1.000	Reference	1.000	Reference
Simple method	3 (25.0)	9 (75.0)	12 (100.0)	8.667 (1.913–39.255)	0.005	0.926 (0.056–15.197)	0.957
Culture method							
Solid culture	4 (23.5)	13 (76.5)	17 (100.0)	1.000	Reference	1.000	Reference
Liquid culture	25 (83.3)	5 (16.7)	30 (100.0)	0.062 (0.014–0.269)	< 0.001	0.064 (0.005–0.770)	0.030
Level of hospital							
First level	2 (22.2)	7 (77.8)	9 (100.0)	1.000	Reference	1.000	Reference
Second level	3 (60.0)	2 (40.0)	5 (100.0)	0.190 (0.018–2.061)	0.172	0.117 (0.006–2.268)	0.156
Third level	24 (72.7)	9 (27.3)	33 (100.0)	0.107 (0.019–0.615)	0.012	0.175 (0.022–1.365)	0.096
Type of hospital							
Prefectural	20 (58.8)	14 (41.2)	34 (100.0)	1.000	Reference		
Provincial	9 (69.2)	4 (30.8)	13 (100.0)	0.635 (0.163–2.477)	0.513		

[a] Eastern region includes Jiangsu, Liaoning, Hebei, Shandong, Shanghai, Fujian, Guangdong, Beijing, and Tianjin; middle region includes Hubei, Shanxi, Jilin, Heilongjiang, Hunan, and Jiangxi; western regions include Qinghai, Sichuan, Yunnan, Shanxi, Chongqing, Guizhou, Gansu, Guangxi Zhuang Autonomous Region, Inner Mongolia Autonomous Region, Xinjiang Uygur Autonomous Region, and Ningxia Hui Autonomous Region. In the calculation of odds ratios for extrapulmonary TB patients, patients with an OR of 1.00 served as a reference group. *EQA* external quality assurance, *OR* odds ratio, *CI* confidence interval.

Discussion

Sputum culture remains the gold standard diagnostic test for active TB and provides positive cultures that form the cornerstone of susceptibility testing and TB control [4]. Therefore, a quality-assured mycobacterial culture service is crucial for achieving national TB program (NTP) goals toward TB control. In this study, we first developed EQA panels for validation of mycobacterial culture test results. Next, we used the panels to evaluate mycobacterial culture testing performance of designated TB hospital laboratories in China. Our data demonstrated that the quality of mycobacterial culture test results was frequently unsatisfactory, with more than one-third of TB hospitals failing to meet criteria for classification as certified mycobacterial culture test providers. False-negative test results were the major type of error observed for testing of artificial specimens with low mycobacterial concentrations. This result suggests poor mycobacterial recovery rates from paucibacillary sputum specimens, as previously described in reports of numerous studies addressing interpretation of false-negative culture [15, 16]. Notably, poor recovery rates may result from bacterial overexposure to sodium hydroxide, the major reason cited for clinical false-negative mycobacterial culture test results [16]. Several plausible explanations have been debated to account for this phenomenon. On the one hand, according to estimates of a nationwide survey of TB hospitals in China [13], laboratory technicians in that country suffer routinely from high workloads. High workloads lead to higher numbers of sputum specimens decontaminated per batch, increasing the risk of specimen overtreatment that then leads to significantly decreased mycobacterial recovery rates from sputa. On the other hand, compared with a negative mycobacterial culture result, specimens that are undertreated with sodium hydroxide may harbor contamination that would require recollection of patient sputum specimens by already overworked clinicians. Weighing these considerations against one another ultimately tends to favor a bias toward overexposure of specimens to a decontamination reagent by overworked technicians. This concept is supported by the extremely low frequency of culture contamination observed in this study. In view of the challenges preventing effective mycobacterial culture testing, it is essential for uncertified hospitals to revise their routine operating procedures to produce more favorable MTB detection results.

Interestingly, the use of the solid culture method was identified as the sole independent risk of uncertified EQA results compared with the use of the liquid culture method. This aligns with previous studies showing that liquid culture exhibits superior performance for mycobacterial recovery from sputum specimens when compared with conventional solid culture, especially for smear-negative sputum samples [17]. Despite a lack of laboratory evidence, we hypothesize that liquid nutrient-rich medium was superior to the commercial solid medium used by most hospitals for recovery of tubercle bacilli with weakened viability due to prolonged exposure to sodium hydroxide. Therefore, culture medium quality is another potentially important factor that may affect mycobacterial culture results and should be considered while planning future studies.

EQA evaluation of mycobacterial culture results should facilitate implementation of national TB control programs in China for several reasons. First, recent WHO reports have revealed that only approximately 30% of diagnosed TB patient specimens tested positive via laboratory smear microscopy and/or mycobacterial culture testing, a rate significantly lower than the average rate worldwide (~ 50%) [1]. We speculate that unsatisfactory implementation of mycobacterial culture methods may be responsible for poor detection of bacteria-positive TB patients in China. Second, the hospitals participating in this study were all provincial- or prefectural-level hospitals. According to national TB control program statistics, more than 70% of county-level hospitals will possess capability to conduct mycobacterial culture services in China by the end of 2020. However, in view of the complicated procedures needed for successful mycobacterial culture method implementation, a worse situation should be expected in county-level hospitals compared with prefectural- and province-level hospitals. Consequently, more attention should be paid to ensure higher quality mycobacterial culture testing in resource-constrained settings. Third, as the use of a combination of liquid plus solid media is a widely accepted laboratory practice for improving the isolation of mycobacteria [18], extra costs and high contamination rates of liquid culture are major obstacles that preclude routine testing in clinical practice in county-level hospitals in China. Recently, the WHO endorsed the use of several molecular assays to diagnose active pulmonary TB [19, 20]. In order to achieve the NTP goal, the application of molecular diagnostics may serve as an alternative method to mycobacterial culture testing in settings where facilities lack capable certified facilities and personnel.

We also acknowledge several obvious limitations of this study. First, because the transport of specimens containing tubercle bacilli is a major biosafety issue that must follow a strict preapproval procedure in China, only 49 of a total of 202 prefectural hospitals were enrolled in this study. The small sample size may therefore limit the confidence power of statistical analysis. Second, despite the use of a differentiated standard to judge EQA results for both solid and liquid culture methods, the low certified EQA rate of the solid culture method here raises the issue

that a separate EQA interpretation standard may be needed for this type of testing. However, more experienced staff would be required to follow appropriate separate criteria for use with different culture methods. Nevertheless, this study provides a foundation for development of a feasible solution to assess laboratory reliability for performance of mycobacterial culture testing in high TB burden countries.

In conclusion, we have developed and evaluated external quality assessment panels suitable for use in validating mycobacterial culture test results in China. Our data demonstrate that approximately one-third of TB hospitals fail to meet quality criteria for consideration as certified mycobacterial culture testing laboratories, emphasizing the importance of quality control and quality assurance in TB diagnostics. Therefore, more attention should be paid toward solving this major problem, which hinders the achievement of future NTP goals to control TB in China.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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