Tuberculosis diagnostics in Fiji: how reliable is culture?

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Settings: Acid-fast bacilli (AFB) smear microscopy and *Mycobacterium tuberculosis* culture are the first-line diagnostic tests for tuberculosis (TB). The contamination of TB cultures significantly reduces the reliability of TB diagnosis.

Objective: To investigate factors associated with TB culture contamination in Fiji, and the relative diagnostic performance of culture compared to microscopy.

Design: All tests performed at the Daulakao Mycobacterium Reference Laboratory (DMRL) in Fiji from 2010 to 2012 were reviewed. Study variables included AFB smear and TB culture results, age and type of specimen, referring TB testing centre and patient age.

Results: Of 5708 specimens reviewed, 70% had both AFB smear and culture results recorded; 421 specimens were contaminated; 2.7% of specimens were either degraded or had no result recorded. There was moderate agreement ($\kappa = 0.577$) between the two tests. Culture was more likely to be positive at higher AFB smear scores. Culture contamination was associated with distance from the DMRL, sample age and operator-associated factors.

Conclusion: Increases in the speed of referral from TB testing centres or the addition of preservatives to sputum specimens may result in less culture contamination. The planned introduction of liquid culture techniques in combination with culture on Ogawa media is likely to increase the sensitivity of TB diagnosis in Fiji.

Fiji is a low tuberculosis (TB) burden country, with an estimated incidence of 24 (21–27) cases per 100 000 population in 2012.¹ Sputum smear microscopy for acid-fast bacilli (AFB) using Ziehl-Neelsen (ZN) staining and sample culture using Ogawa medium are the current standard diagnostic methods in Fiji, where AFB microscopy is the routine screening method for all suspected cases of TB. Ogawa cultures require up to 8 weeks to yield a result. Sputum smear microscopy is a simple, inexpensive technique that enables the rapid detection of AFB. Culture is the reference standard in the TB diagnostic algorithm for evaluating patients with negative smears, as it increases sensitivity and enables earlier case detection. Culture also provides material for the identification of mycobacterial species and their drug susceptibility profiles, which is important in patients with suspected drug-resistant TB.² Recent reviews of TB diagnosis in Fiji identified issues affecting culture positivity rates, including culture degradation and contamination, which have a negative impact on the accuracy of TB diagnosis and effective TB control.^{3,4} The above issues have also been identified in other countries.⁵ A programme review in 2011 showed that up to 13–18% of

cultures were contaminated and that attempts to culture mycobacteria from 12% of smear-positive specimens had failed.⁴ The review recommended that the reasons for the high contamination and high negative culture rate among smear-positive samples should be investigated to enable corrective measures to be implemented.

The main objectives of the present study were to investigate the level of agreement between culture and AFB microscopy results, and to determine the culture contamination rate and associated factors.

METHODOLOGY

Setting

This retrospective study was conducted at the Daulakao Mycobacterium Reference Laboratory (DMRL), Suva, Fiji, the only TB reference laboratory in Fiji. TB treatment centres performing AFB microscopy are located in each of the three major divisions of Fiji, the Central, Northern and Western Divisions. Samples from four TB microscopy centres are processed for TB culture at the DMRL. An average of 2000 specimens are cultured for TB from the three divisions each year. Specimens referred for culture include sputum, ascitic fluid, pleural fluid, cerebral spinal fluid, pus, tissue, bronchial washing and lavage, stool and urine. All specimens undergo AFB microscopy and ZN staining before referral. Smear-positive samples and good quality smear-negative samples are referred for TB culture on Ogawa media at the DMRL.

Participants

All specimens referred from the three divisions of Fiji for TB culture from January 2010 to December 2012 were included in the study.

Data variables, data collection instrument

Data for each variable were extracted from the TB culture register held at the DMRL into a structured proforma. Exposure variables for each specimen included referring division, TB culture registration number, type of specimens submitted, AFB smear status and age of the sample based on the date of collection and date of processing. Outcome variables in this study are the agreement between culture and smear result and presence of contamination during the period of incubation.

Analysis and statistics

All data collected were entered into EpiData Version 3.1 (EpiData Association, Odense, Denmark). The proportion and 95% confidence interval (CI) for each determinant associated with each culture outcome category was calculated. The statistical signifi-

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FIGURE 1 Flow diagram of the analysis of the results of smear microscopy and Ogawa culture in the detection of *Mycobacterium tuberculosis* for all samples submitted to the Daulako Mycobacterium Reference Laboratory, Suva, Fiji, 2010–2012.

cance of any difference was determined using the χ^2 test, with 95%CI. The sensitivity and specificity of AFB microscopy as regards culture and their binomial 95%CI were calculated using WinEpiscope 2.0 (Agricultural University, Wageningen, The Netherlands; www.clive.ed.ac.uk/winepiscope/) using the culture results as the gold standard. The agreement between culture and AFB microscopy was also calculated using WinEpiscope.

Ethics

Ethics approval was obtained from the Fiji National Research Ethics Committee, Suva, Fiji, and the International Union Against Tuberculosis and Lung Disease Ethics Advisory Group, Paris, France.

RESULTS

AFB microscopy and culture results were available for 3998 of the 5708 samples tested between 2010 and 2012 (Figure 1); 630 (15.8%) specimens were smear-positive and 434 (10.8%) were culture-positive. However, 98/3368 (2.9%) smear-negative specimens were culture-positive, and 294/630 (46.7%) smear-positive specimens were culture-negative. There was moderate agreement ($\kappa = 0.577$) between the two diagnostic methods. The overall sensitivity and specificity of AFB smear microscopy using culture as the reference standard was respectively 77% (95%CI 73–81) and 92% (95%CI 90–93).

The culture contamination rate was significantly lower in samples from the Western Division compared to the Central and Northern Divisions, and significantly higher in the Northern than in the Central Division ($P < 0.05$) (Table 1). Samples were more likely to be contaminated >30 days after collection ($P = 0.006$). Finally, there were significantly higher rates of contamination in samples collected in 2011 and 2012 than in 2010 ($P < 0.05$) (Figure 2). The culture con-

tamination rate of AFB smear-positive samples was significantly higher than AFB smear-negative samples ($P < 0.05$), and was significantly higher in AFB 3+ samples than in other positivity categories ($P < 0.05$) (Figure 3). There was no significant difference in contamination rate in samples from patients in different age groups and in the different types of specimens.

DISCUSSION

This is the first study to evaluate the rate and factors associated with contamination of diagnostic TB cultures in Fiji. Only 70% of the records from specimens submitted to the DMRL for TB culture were valid for use in this study; the most significant reason for exclusion was the absence of AFB smear results. This is an important omission from the laboratory records, as AFB smear microscopy is the first-line diagnostic procedure for TB in Fiji. These omissions most likely occur when specimens are initially submitted to or during referral from the divisional laboratories that perform AFB smear microscopy. This suggests that a review of the implementation of specimen submission and/or referral protocols is required to ensure that data recording is standardised. It may also be worth considering introducing microscopy on sediment before culture inoculation, which may provide an alternative mechanism for capturing AFB smear results. Results show that a significant number of samples are AFB smear-positive but culture-negative. The bacterial load in a specimen is an important factor in determining the culture positivity rate. This is illustrated in this study by the increasing positivity rate in AFB smear grades of more than 3+. A recent study revealed that most culture failures from smear-positive samples were from patients in the intensive phase of treatment for TB.⁶

Culture quality cannot only be determined by eval-

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TABLE 1 Factors associated with the contamination of Ogawa cultures in the detection of *Mycobacterium tuberculosis*, Fiji, 2010–2012

Variable	Total samples (<i>n</i> = 5708) <i>n</i>	Samples contaminated (<i>n</i> = 421) <i>n</i> (%)
Referral centre		
Central/Eastern	4517	318 (7.0)
Northern	835	97 (11.6)
Western	356	6 (1.7)
Age group, years		
0–14	435	31 (7.1)
15–24	682	62 (9.1)
25–34	867	64 (7.4)
34–44	754	54 (7.2)
45–54	875	56 (6.4)
55–64	852	55 (6.5)
≥65	825	76 (9.2)
Unknown	419	23 (5.5)
Specimen type		
Sputum	5346	386 (7.2)
Ascitic fluid	43	2 (4.7)
Pleural fluid	143	13 (9.1)
CSF	20	0
Pus	31	3 (9.7)
Tissue	2	0
Lavage/washing	123	17 (13.8)
AFB smear		
Positive	706	51 (7.2)
Negative	3698	217 (5.9)
Status unknown	1304	153 (11.7)
Sample age, days*		
0–9	397	24 (6)
10–20	553	33 (6)
21–30	311	24 (8)
>30	305	37 (12)

*Calculated only for 1566 specimens that had a recorded date of collection.
CSF = cerebrospinal fluid; AFB = acid-fast bacilli.

uating culture contamination. Factors such as samples from patients on treatment, reporting of false-positive smears and excessive decontamination of specimens while processing should also be considered; however, as it could not be evaluated in this study due to lack of data, this is a major study limitation. The sensitivity of AFB smear microscopy is also dependent on the type and quality of specimen.⁷ The sensitivity of smear in comparison with the reference standard in this study was a little lower than observed in other studies.^{8–10} It is difficult to interpret this observation due to the different culture techniques used in each study. The World Health Organization (WHO) now recommends expanded use of liquid culture systems in resource-constrained settings.¹¹ Studies have shown that the recovery rate of mycobacteria and overall TB case detection rates can be improved if a solid culture medium, such as Ogawa, is used in combination with Löwenstein-Jensen (LJ) liquid culture medium and/or the Mycobacterium Growth Indicator Tube (MGIT™; BD, Sparks, MD, USA).^{12–14}

The rate of culture contamination differed depending on the origin of the specimen in Fiji. The highest contamination rate was observed in specimens from the Northern Division, which is a separate island some distance from Suva, where the DMRL is located, requiring transport by air freight. Samples are routinely

FIGURE 2 Proportion of contaminated Ogawa cultures for *Mycobacterium tuberculosis*, Fiji, 2010–2012. **FIGURE 3** Proportion of contaminated Ogawa cultures for *Mycobacterium tuberculosis* categorised based on positive AFB smear results, Fiji, 2010–2012. AFB = acid-fast bacilli.

batched and transported each week (or less frequently) to reduce costs associated with transportation of hazardous materials. This results in a higher sample age than that of samples from the Central and Western Divisions, which are on the main island and connected to Suva by road. The introduction of a cold chain for sample transportation could reduce the contamination rate.

The observed change in contamination rate over different years is most likely due to the rotation of inexperienced laboratory staff to perform TB culture at the DMRL, when workloads were high or when regular staff were absent. Care must be taken to ensure that staff in the DMRL are adequately trained before they perform culture; this includes instruction in culture practices and standard operating procedures with regard to sample handling and data entry. Any changes to these protocols should be communicated directly to staff involved in microbiological culture in general, and more specifically to those deployed to perform TB culture. Culture contamination reduces the proportion of interpretable results and diminishes the diagnostic value of culture systems.

Studies have shown varying contamination rates in different TB culture systems.^{15–18} Recent studies showed that an increase in storage time of up to 7 days at room temperature resulted in a reduced rate of recovery of *Mycobacterium tuberculosis* and rising contamination rates.^{19–21} This is also reflected in the present study, where the contamination rate gradually increased with sample age. Detection of growth of *M. tuberculosis* from samples inoculated onto solid medium such as Ogawa may take 6–8 weeks, leading to delays in treatment. The WHO recommends that specimens be collected from remote areas and transported to reference laboratory without delay.²² It is also recommended that when specimens are likely to be exposed to room temperature for >48 h, an equal volume of either 0.6% cetylpyridinium bromide or 1% cetylpyridinium chloride be added to homogenise and de-

contaminate samples before they are sent to the reference laboratory.^{21,23} A recent study showed that culture contamination rates are significantly reduced if preservative is added to specimens before shipping.¹⁹

The contamination of culture due to oral-pharyngeal flora is another possible interpretation of our study findings. It was observed that contamination was more likely in smear-positive than in smear-negative specimens. Recent studies have shown that the use of an oral antimicrobial mouth rinse before sample collection significantly reduces TB culture contamination.^{24,25} The contamination rates in Fiji might thus be reduced if the use of an inexpensive oral mouth wash such as chlorohexidine was incorporated into standard sputum collection protocols.

An important limitation of this study was that it was a retrospective review of laboratory records, and there was thus no additional means of validating the data used in the study. Future studies could be improved by collecting additional data on samples from patients on anti-tuberculosis treatment from the registers of the referring laboratories. The information available did not permit an evaluation of the association between individual operators and culture contamination.

CONCLUSION

Sample referral systems should be improved to enhance data collection and improve specimen collection and handling to maintain the viability of *M. tuberculosis* while reducing contamination. Adoption of liquid culture systems by the Fiji Ministry of Health is planned in the context of a comprehensive and detailed country plan for TB laboratory capacity strengthening. A review of TB culture techniques and the incorporation of methods such as LJ and MGIT, together with current practices, will boost the sensitivity of culture. However, the current issues with aged samples for culture may become more challenging with the sensitivity of culture on MGIT. A further study will be required to compare contamination rates and culture sensitivity after implementation of liquid culture methods. Improved management and increases in the number of trained staff available to perform TB culture may also reduce the rate of contamination. Adopting the use of preservatives to decontaminate referred specimens for TB culture and the use of mouth wash before sputum collection may reduce the risk of contamination due to the presence of normal flora.

References

- World Health Organization. Global tuberculosis report 2013. WHO/HTM/TB/2013.11. Geneva, Switzerland: WHO, 2013.
- World Health Organization. Policy framework for implementing new tuberculosis diagnostics. Geneva, Switzerland: WHO, 2010.
- Raheva K M M, Hovsepian L, Nguyen L. External review of the National TB Programme, Republic of Fiji Islands: 21st November-2nd December 2011. Suva, Fiji: Ministry of Health, 2011.
- Pandey S. DMRL Assessment and GeneXpert® training. Suva, Fiji: Ministry of Health, Republic of Fiji Islands, 2012.
- Swai H, Mugusi F, Mbwanbo J. Sputum smear negative pulmonary tuberculosis: sensitivity and specificity of diagnostic algorithm. BMC Res Notes 2011; 4: 475.
- Lim C-S, Lee C-H, Chien Y-J, et al. Culture result of smear-positive sputum samples after 2 months of antituberculous treatment. Eur Respir J 2010; 35: 218–220.
- Bell D J, Dacombe R, Graham S M, et al. Simple measures are as effective as invasive techniques in the diagnosis of pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis 2009; 13: 99–104.
- Angeby K A, Alvarado-Galvez C, Pineda-Garcia L, Hoffner S E. Improved sputum microscopy for a more sensitive diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis 2000; 4: 684–687.
- Aber V R, Allen B W, Mitchison D A, Ayuma P, Edwards E A, Keyes A B. Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle 1980; 61: 123–133.
- Rickman T W, Moyet N P. Increased sensitivity of acid fast smears. J Clin Microbiol 1980; 11: 618.
- World Health Organization. Use of liquid medium for culture and drug susceptibility testing (DST) in low- and medium-income settings. Summary of the expert group meeting on the use of liquid culture systems. Geneva, Switzerland: WHO, 2007.
- Alp A, Hasçelik G. Comparison of BBL mycobacteria growth indicator tube (MGIT) method, BACTEC radiometric system and Löwenstein-Jensen culture media for the detection of mycobacteria in clinical specimens. Mikrobiyol Bul 2002; 36: 229–235. [Turkish]
- Dirraa O, Fdany K, Boudouma M, Elmdaghri N, Benbachir M. Assessment of the mycobacteria growth indicator tube for the bacteriological diagnosis of tuberculosis. Int J Tuberc Lung Dis 2003; 7: 1010–1012.
- Pauwels P, Kalonga M B, Sykalon S K, Willame J C, Carpels G. Comparison of 2 culture media for isolation of *Mycobacterium tuberculosis*. Ann Soc Belg Med Trop 1990; 70: 237–241.
- Chien H P, Yu M C, Wu M H, Lin T P, Luh K T. Comparison of the BACTEC™ MGIT™ 960 with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. Int J Tuberc Lung Dis 2000; 4: 866–870.
- Lee J J, Suo J, Lin C B, Wang J D, Lin T Y, Tsai Y C. Comparative evaluation of the BACTEC™ MGIT™ 960 system with solid medium for isolation of mycobacteria. Int J Tuberc Lung Dis 2003; 7: 569–574.
- Srisuwanvilai L O, Monkongdee P, Podewils L J, et al. Performance of the BACTEC™ MGIT™ 960 compared with solid media for detection of *Mycobacterium* in Bangkok. Diagn Microbiol Infect Dis 2008; 61: 402–407.
- Tortoli E, Cichero P, Piersimoni C, Simonetti M T, Gesu G, Nista D. Use of BACTEC™ MGIT™ 960 for recovery of mycobacteria from clinical specimens: multicenter study. J Clin Microbiol 1999; 37: 3578–3582.
- Paramasivan C N, Narayana A S, Prabhakar R, Rajagopal M S, Somasundaram P R, Tripathy S P. Effect of storage of sputum specimens at room temperature on smear and culture results. Tubercle 1983; 64: 119–124.
- Lumb R, Ardian M, Waramori G, et al. An alternative method for sputum storage and transport for *Mycobacterium tuberculosis* drug resistance surveys. Int J Tuberc Lung Dis 2006; 10: 172–177.
- Pardini M, Varaine E, Iona E, et al. Cetyl-pyridinium chloride is useful for isolation of *Mycobacterium tuberculosis* from sputa subjected to long-term storage. J Clin Microbiol 2005; 43: 442–444.
- World Health Organization. Guidelines for surveillance of drug resistance. WHO/CDS/TB/2003.320. Geneva, Switzerland: WHO, 2003.
- World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. WHO/TB/96.216. Geneva, Switzerland: WHO, 1997.
- Muzanye G, Morgan K, Johnson J, Mayanja-Kizza H. Impact of mouth rinsing before sputum collection on culture contamination. Afri Health Sci 2009; 9: 1024–1031.
- Kalema N, Boon S D, Cattamanchi A, et al. Oral Antimicrobial rinse to reduce mycobacterial culture contamination among tuberculosis suspects in Uganda: a prospective study. PLOS ONE 2012; 7: e38888.

Contexte : L'examen microscopique de crachats à la recherche de bacilles acido-alcool résistants (AFB) et la culture de *Mycobacterium tuberculosis* sont des examens de première intention dans le diagnostic de la tuberculose (TB). La contamination des cultures de TB réduit la fiabilité du diagnostic.

Objectif : Examiner les facteurs associés à la contamination des cultures de TB aux Fidji et les performances relatives de la culture et de l'AFB.

Méthodes : Tous les examens réalisés au Laboratoire de Référence des *Mycobacterium* de Daulakao aux Fidji de 2010 à 2012 ont été inclus. Les variables comprenaient les résultats de l'AFB et de la culture, l'âge et le type de spécimen, le centre d'examen ayant envoyé l'échantillon et l'âge du patient.

Résultats : Des 5708 spécimens enregistrés, 70% comprenaient à la

fois les résultats du frottis AFB et de la culture. Une contamination est survenue dans 421 spécimens et 2,7% d'entre eux étaient soit dégradés, soit sans résultats. L'accord entre les deux tests était modéré ($\kappa = 0,577$). La culture avait plus de chances d'être positive quand le score du frottis AFB était plus élevé. La contamination de la culture était associée à la distance par rapport au laboratoire de référence, à l'âge de l'échantillon et à des facteurs liés à l'opérateur.

Conclusion : Une plus grande rapidité de transmission des échantillons de crachats depuis le centre d'examen anti-tuberculeux ou l'adjonction de conservateurs aux spécimens de crachats pourrait amener une diminution du taux de contamination des cultures. L'introduction de techniques de cultures liquides combinée à la culture sur milieu Ogawa augmentera la sensibilité du diagnostic de la TB aux Fidji.

Marco de Referencia: El examen microscópico en busca de bacilos acidorresistentes (AFB) y el cultivo de *Mycobacterium tuberculosis* constituyen las pruebas de elección en el diagnóstico de la tuberculosis (TB). Sin embargo, la contaminación de los cultivos disminuye la fiabilidad del diagnóstico.

Objetivo: Investigar los factores que se asocian con la contaminación de los cultivos de *M. tuberculosis* en Fiji y el desempeño relativo del cultivo y la AFB.

Metodos: Se incluyeron en el estudio todas las pruebas realizadas en el Daulako *Mycobacterium* Reference Laboratory (DMRL) en Fiji del 2010 al 2012. Las variables examinadas fueron el resultado de la AFB y del cultivo, el tipo de muestra, el lapso transcurrido desde la recogida de la muestra hasta la prueba, el centro de TB que la remitió y la edad del paciente.

Resultados: Se registraron 5708 muestras; el 70% de los casos

comportaba el resultado de la AFB y el cultivo. Se contaminaron 421 muestras y 2,7% de las muestras se degradaron o carecían de resultado. Se observó una concordancia moderada entre ambas pruebas ($\kappa = 0,577$). El cultivo fue con mayor frecuencia positivo en las muestras que presentaban una alta puntuación de AFB. La contaminación del cultivo se asoció con la distancia entre el centro de origen y el DMRL, el lapso transcurrido desde la recogida de la muestra y otros factores propios del operador.

Conclusión: Al aumentar la velocidad de remisión de las muestras desde el centro de pruebas de TB o agregar conservantes a las muestras de esputo se puede disminuir la tasa de contaminación de los cultivos. La introducción de las técnicas de cultivo en medio líquido, además de la utilización del medio de Ogawa, permitirá mejorar la sensibilidad del diagnóstico de la TB en Fiji.