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SHORT COMMUNICATION

Implementation of liquid culture for tuberculosis diagnosis in a remote setting: lessons learned

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SUMMARY

Although sputum smear microscopy is the primary method for tuberculosis (TB) diagnosis in low-resource settings, it has low sensitivity. The World Health Organization recommends the use of liquid culture techniques for TB diagnosis and drug susceptibility testing in low- and middle-income countries. An evaluation of samples from southern Sudan found that culture was able to detect cases of active pulmonary TB and extra-pulmonary TB missed by conventional smear microscopy. However, the long delays involved in obtaining culture results meant that they were usually not clinically useful, and high rates of non-tuberculous mycobacteria isolation made interpretation of results difficult. Improvements in diagnostic capacity and rapid speciation facilities, either on-site or through a local reference laboratory, are crucial.

KEY WORDS: tuberculosis; non-tuberculous mycobacteria; microscopy; liquid culture

DIAGNOSIS of pulmonary tuberculosis (PTB) in resource-poor settings has traditionally relied on sputum smear microscopy using the Ziehl-Neelsen (ZN) staining technique, which is inexpensive and not technically demanding. However, compared with culture, the method has low sensitivity,[1] particularly in patients co-infected with the human immunodeficiency virus (HIV).[2] ZN also has low sensitivity for diagnosing extra-pulmonary tuberculosis (EPTB).[3]

In 2007, the World Health Organization (WHO) recommended the implementation of liquid culture techniques for diagnosis and drug susceptibility testing of tuberculosis (TB) suspects in low- and middle-income countries.[4] The WHO observed that increased frequency of isolation of non-tuberculous mycobacteria (NTM) was a likely result of the highly sensitive culture system, and stated that a rapid method for differentiation of mycobacteria was essential.

Médecins Sans Frontières (MSF) has worked in Sudan since 1979. Southern Sudan is affected by high levels of poverty, along with a lack of basic infrastructure and services. The lack of roads and transportation prevents access to health care services and can result in loss to follow-up of patients with chronic conditions such as TB. HIV prevalence varies between 0.4% and 4.4%.[5] In 2008, 507 TB cases were admitted to MSF's southern Sudan TB programme: 30% of new cases were smear-positive, 5% were smear-negative and 65% were EPTB.

In March 2007, MSF opened a TB culture laboratory in Lokichoggio, Kenya, with the support of the Borstel Supranational Reference Laboratory, Borstel, Germany, to serve basic health care projects in four locations in southern Sudan: Lankien and Pieri in Jonglei State, Nasir in Upper Nile State and Leer in Unity State. The aim was to enable diagnosis of EPTB and smear-negative PTB, improve TB treatment management decisions, and investigate suspected *Mycobacterium bovis* infection as a potential explanation for the high EPTB prevalence.

In the present study, we describe our experience, outcomes, and lessons learned to help to guide others with implementing TB culture in remote and resource-poor settings.

METHODS

From March 2007 to April 2009, TB culture was performed on sputum samples from patients attending four MSF clinics in southern Sudan with clinical symptoms of smear-negative PTB or EPTB or with suspected treatment failure. Positive cultures were sent to Borstel Reference Laboratory for speciation and quality control.

Two sputum samples were processed per PTB suspect, and one extra-pulmonary sample (ascites or pleural fluid) was processed per EPTB suspect. Samples were collected at the clinics and refrigerated before

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being sent in batches to the Lokichoggio laboratory. No preservatives were added.

Sputum samples were decontaminated with *N*-acetyl-L-cysteine sodium hydroxide (NaOH; final NaOH concentration 1.5%). All samples were cultured using the manual Mycobacterial Growth Indicator Tube (MGIT) system (BD Diagnostics, Sparks, MD, USA), and were cultured on Löwenstein-Jensen (LJ) medium in parallel. Stonebrink medium was used for the first 60 samples to enhance detection of *M. bovis*. Smears were made from fluorescing MGIT tubes and examined for presence of acid-fast bacilli and contaminants. LJ and Stonebrink tubes were examined visually for growth, and smears were made from colonies. One positive tube per patient was sent to the Borstel laboratory for speciation using the Hain GenoType Mycobacteria (Hain Lifesciences, Nehren, Germany) series and sequencing as required. For quality control, the reagents used were cultured with each batch to ensure sterility. No positive growth control was used.

Ethics approval was not obtained for this programme description as all work described was done as part of routine programme activities.

RESULTS

Of 70 smear-negative diagnostic TB suspects, 23 (33%) were culture-positive (Table). All three smear-positive diagnostic patients were culture-positive. Patients on treatment had samples cultured to determine if viable bacteria could be isolated. Of 27 patients on treatment, seven were smear-positive, four of whom (57%) were culture-negative. Of 20 smear-negative patients on treatment, nine (45%) were culture-positive and 11 (55%) were culture-negative. Six of 20 smear-negative EPTB samples were culture-positive. Only 11 (29%) of 38 culture-positive PTB patients grew mycobacteria that could be identified as *M. tuberculosis* complex, while three (50%) of six culture-positive EPTB patients grew *M. tuberculosis* complex (Table).

The high proportion of NTM and unknown mycobacteria isolates could not be interpreted by clinicians, as it was not known whether the bacteria were disease-causing or environmental contaminants. That 50% of the NTM could not be identified suggests that they were environmental contaminants and not clinically significant. They may have been introduced to the sputum samples through dust during collection or introduced to the suspects' mouths through the water supply. Steps were taken to minimise environmental contamination—suspects rinsed their mouths with filtered water before expectoration, and expectorated directly into sterile Falcon tubes. EPTB samples were inoculated directly into prepared MGIT tubes, but their paucibacillary nature makes culture of these samples difficult. There was a long turnaround time (4–6 weeks) from sample production to speciation or a negative diagnosis, due to the shipment of samples from the projects to Lokichoggio, and of the positive cultures from Lokichoggio to Borstel. These delays led to concerns that TB suspects would be lost to follow-up, so smear-negative patients were put on treatment or had treatment adjusted according to clinical diagnosis rather than culture results. As a result, enrolment into the culture programme was suspended in February 2009.

DISCUSSION

Our experience demonstrates that smear microscopy is failing to detect cases of active PTB and EPTB, and that implementation of culture in remote and resource-limited settings is likely to face significant constraints. Because of the high recovery of NTM and lack of rapid on-site speciation facilities, a culture programme was of limited relevance for the diagnosis of smear-negative and follow-up TB patients. However, culture did identify some TB patients who had been negative on smear microscopy. Clarification is needed on the management of NTM infection and the relevance of NTM isolation in these settings, as existing guidelines are difficult to implement. The growth of NTM was unlikely to have resulted from laboratory contamination, owing to the variety of organisms isolated and the fact that we did not use a positive growth control.

Table Culture results by type of mycobacteria

	Diagnostic patients (n = 73)		Treatment patients (n = 27)		
	Smear-negative (n = 70) n (%)	Smear-positive (n = 3) n (%)	Smear-negative (n = 20) n (%)	Smear-positive (n = 7) n (%)	EPTB patients (n = 20) n (%)
M. tuberculosis complex	8 (11)	2 (67)	0	1 (14)*	3 (15)
Other identified mycobacteria	7 (10)†	1 (33)‡	5 (25)§	2 (29)¶	0
Other unknown mycobacteria	8 (11)	0	4 (20)	0	3 (15)
Culture-negative	47 (67)	0	11 (55)	4 (57)	14 (70)

* Plus *M. fortuitum*.
† *M. intracellulare* (n = 1), *M. asiaticum* (n = 1), *M. margaritense* (n = 1), *M. avium* complex (n = 2) and *M. fortuitum* complex (n = 2).
‡ *M. fortuitum*.
§ *M. fortuitum* complex (n = 4), *M. avium* complex (n = 1). ¶ *M. intracellulare* (n = 1), *M. fortuitum* complex (n = 1).
EPTB = extra-pulmonary tuberculosis.

NTM exist in the environment and may be present either in environmental dust or in the oral flora of patients with poor oral hygiene. Methods to minimise exogenous contamination such as rinsing of mouths and expectorating into sterile containers were clearly insufficient to completely prevent contamination.

The time involved in obtaining results meant they were not clinically useful. However, culture was able to detect 11% of PTB and 15% of EPTB patients missed by conventional smear microscopy, emphasising the need for improvements in diagnostic capacities in resource-limited settings. As clinical data were not linked to samples, patient outcomes cannot be linked to the culture results.

The WHO endorsed the use of line-probe assays for the rapid screening of multidrug resistance in 2008. Although molecular methods may have overcome the issues described here, they do not provide the growth control necessary for monitoring patients on treatment, as DNA from non-viable bacilli can be amplified. As line-probe assays can be used only on smear-positive sputum samples, they cannot be used for monitoring smear-negative patients on treatment.

Rapid speciation facilities, either on-site or through a local reference laboratory, are vital; however, until recently they were not readily available in many resource-limited settings. Until mid-2009, the only validated rapid immunochromatographic test for speciation (Tauns' Capilia TB Neo, Tauns, Shizuoka, Japan) was available for purchase exclusively through the Foundation for Innovative New Diagnostics (FIND, Geneva, Switzerland), which limited availability for implementation in routine culture programmes. New tests, such as the Becton Dickinson TBc Identification test (BD, USA), are now available on the market, and other tests are under evaluation.

When setting up a TB culture programme in a resource-limited setting, clear guidelines for the management of NTM results should be given to clinical staff. The recommendations of the American Thoracic Society and the Infectious Diseases Society of America[6] for management of NTM isolates state that, along with clinical and radiographic symptoms of infection, the same species must be isolated in at least two samples to be considered clinically significant. This recommendation implies that, once a patient's sample has been identified as growing NTM, the patient must submit a second sample for the same organism to be isolated again. This procedure was impractical in our setting because of the transport difficulties our patients commonly experienced when accessing our clinic, and the length of time involved in speciation. Chest X-ray facilities are uncommon in many resource-limited settings, which further prevents investigation of NTM infection according to current guidelines.

The problems we encountered with culture, despite our small sample size, are not exclusive to Sudan. A case-control study in Zambia[7] found high rates of NTM isolation (11% of TB patients and 6% of controls). The most commonly isolated species was *M. avium* complex, but 55 of 171 species were unknown and not previously identified. In 22 of 29 NTM-positive patients, only one of two consecutive cultures was positive. As liquid culture laboratories are further rolled out in low- and middle-income countries as per WHO recommendations, reaching more peripheral patients, the issues we encountered are likely to become more common.

CONCLUSION

Implementation of culture as recommended by the WHO in remote and resource-limited settings is likely to face significant constraints due to the isolation of NTM which are of questionable clinical significance. Clear guidelines are needed to help national TB programmes with the interpretation of NTM isolation.

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RÉSUMÉ

L'examen microscopique des frottis de crachats est la méthode principale de diagnostic de la tuberculose (TB) dans les contextes à faibles ressources, mais sa sensibilité est faible. Dans les pays à faibles ou moyens revenus, l'Organisation Mondiale de la Santé recommande l'utilisation des techniques de culture sur milieu liquide pour le diagnostic de la TB ainsi que pour les tests de sensibilité aux médicaments. Une évaluation des échantillons provenant du sud du Soudan a montré que la culture est capable de détecter des cas de TB pulmonaire active et de TB extrapulmonaire que l'examen microscopique conventionnel des frottis avait laissé échapper. Toutefois, les longs délais qu'implique l'obtention des résultats des cultures signifient qu'elles n'ont habituellement pas eu d'utilité sur le plan clinique ; d'autre part, les taux élevés d'isolement de mycobactéries non-tuberculeuses ont rendu l'interprétation des résultats difficile. Les améliorations des capacités de diagnostic et des services de détermination rapide de l'espèce, que ce soit sur le site ou dans un laboratoire local de référence, sont des éléments cruciaux.

RESUMEN

La baciloscopia del esputo constituye el principal método diagnóstico de la tuberculosis (TB) en los ámbitos con escasos recursos, pero ofrece una baja sensibilidad. La Organización Mundial de la Salud recomienda el uso de técnicas de cultivo en medio líquido para el diagnóstico de la enfermedad y para las pruebas de sensibilidad a los medicamentos antituberculosos en los países de bajos y medianos ingresos. En una evaluación de muestras provenientes del sur de Sudán, se observó que con el cultivo se pueden detectar los casos de TB activa, pulmonar y extrapulmonar, que pasaron desapercibidos con la baciloscopia convencional. Sin embargo, los plazos prolongados hasta la obtención de los resultados de los cultivos invalidaron su utilidad clínica y la alta tasa de aislamiento de micobacterias atípicas complicó la interpretación de sus resultados. Es primordial mejorar la capacidad diagnóstica y contar con medios rápidos de diferenciación de las especies en el terreno o por intermedio de laboratorios locales de referencia.