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Investigating the quality of expectorated sputum for tuberculosis diagnosis in Bolivia

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SUMMARY

A low-power microscope-based cytological system to assess the quality of expectorated sputum provided for tuberculosis (TB) diagnosis was piloted in Bolivia. A total of 3688 samples were subjected to visual and cytological examination in nine laboratories: of these, 591 (16%) were misclassified by visual examination and 294 (8%) were found to be degraded. The degree of discordance varied between locations, and laboratories received a higher number of degraded specimens from isolated health clinics. Cytological assessment of sputum was found to be feasible and identified areas for improvement in the Bolivian diagnostic system for TB.
KEY WORDS: diagnosis; smear microscopy; cytology

BOLIVIA HAS THE SECOND HIGHEST tuberculosis (TB) incidence in the Americas region after Haiti, with 127 cases per 100 000 population.[1] The case detection rate is reported to be 62% for all new cases and 71% for all smear-positive cases. The National TB Control Programme (NTCP) currently follows the Strategic Plan for Tuberculosis Control in Bolivia 2008–2015, which aims to increase detection and successfully treat 86% of newly detected cases with DOTS by 2015.[2] Together with clinical symptoms and, in some cases, radiological evidence, the standard method for diagnosing pulmonary TB is bacilloscopy. The sensitivity of smear microscopy is dependent on the quality of the expectorated sputum sample.[3] In Bolivia, the quality of expectorated sputum samples is only assessed visually. Samples classed as saliva or bloody are considered unsuitable for TB diagnosis. When possible, a new sample is requested from patients and poor quality samples are discarded, but often poor quality samples are accepted to avoid losing the patient to follow-up. To investigate the effectiveness of the visual classification of sample quality, a cytological classification system using low-power microscopy[4] was introduced. A 6-week pilot in four urban laboratories was expanded to five laboratories in various geographic locations.

METHODS

The studies were conducted in cooperation with the Tuberculosis Departmental Laboratory Servicio Departamental de Salud, La Paz, Bolivia. Five urban hospitals participated in the initial pilot study: the Instituto Nacional de Tórax and Hospital Luis Uría de la Oliva in La Paz, and Hospital Boliviano Holandés, Hospital Corea and the maternal centre Lotes y Servicios in El Alto. For the extended study, hospitals in the municipalities of Achacachi and Patacamaya in the Altiplano, and Coroico, Caranavi and La Asunta in the subtropical Yungas region of the Department of La Paz took part. Bolivian guidelines indicate the methodology for bacilloscopy to be followed in the country and classify macroscopic samples as saliva, purulent, mucopurulent or bloody.[5] In general, on visual examination, macroscopic purulent and mucopurulent samples are considered good quality for diagnosis, whereas saliva and bloody samples are classified as unsuitable.

The Sputum Cytology Analysis System (SCAS) developed by Médecins Sans Frontières (MSF, London, UK) was adapted to the established macroscopic system in Bolivia to create a classification based on both microscopic and macroscopic categories. According to SCAS, after examination with a 10x magnifying objective, samples are divided into five categories:[6] saliva, mucus, degraded sputum, insufficient sputum and real sputum. Real sputum and insufficient sputum are considered appropriate for diagnosis, although insufficient sputum samples should be improved. Mucus, saliva and degraded sputum are considered poor quality samples. For SCAS implementation, training and kits were provided for each hospital, including sample slides with

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Table Results comparing visual classification with microscopy

Sputum classification	Boliviano Holandés *n* (%)	Corea *n* (%)	Centro Materno Lotes y Servicios *n* (%)	Luís Uría de la Oliva *n* (%)	Achacachi *n* (%)	Patacamaya *n* (%)	Coroico *n* (%)	Caranavi *n* (%)	La Asunta *n* (%)	Total *n* (%)
Purulent										
Real	3 (4)	26 (19.4)	9 (5.1)	9 (3.6)	91 (27.9)	60 (27.9)	5 (1)	336 (25.8)	28 (3.8)	567 (4.3)
Insufficient	11 (14.5)	3 (2.2)	3 (1.7)	0	31 (9.5)	37 (17.2)	58 (12.1)	311 (23)	39 (5.3)	493 (13.4)
Degraded	2 (2.6)	4 (3)	2 (1.1)	0	1 (0.3)	0	7 (1.5)	43 (3.2)	12 (1.6)	71 (2)
Mucus	4 (5.3)	20 (15)	3 (1.7)	0	0	2 (0.9)	5 (1)	36 (2.8)	2 (0.3)	72 (2)
Saliva	6 (8)	2 (1.5)	2 (1.1)	0	9 (2.8)	0	1 (0.2)	48 (4.2)	18 (2.4)	86 (2.3)
Mucopurulent										
Real	18 (23.7)	26 (19.4)	12 (6.7)	141 (56.4)	26 (8)	0	174 (38.8)	77 (6.5)	397 (53.9)	871 (23.6)
Insufficient	8 (10.5)	0	60 (33.7)	0	2 (0.6)	2 (0.9)	144 (32.2)	17 (1.3)	113 (15.4)	346 (9.4)
Degraded	0	0	8 (4.5)	0	0	0	4 (0.8)	3 (0.3)	15 (2)	30 (0.8)
Mucus	4 (5.3)	2 (1.5)	10 (5.6)	0	0	2 (0.9)	2 (0.4)	23 (2.1)	10 (1.4)	53 (1.4)
Saliva	0	0	1 (0.6)	0	0	0	4 (0.8)	3 (0.2)	5 (0.7)	13 (0.4)
Saliva										
Real	0	1 (0.7)	0	0	11 (3.4)	0	0 (0.2)	10 (0.7)	2 (0.3)	24 (0.7)
Insufficient	4 (5.3)	9 (6.7)	7 (4)	3 (1.2)	17 (5.2)	0	0	71 (5.3)	21 (2.9)	132 (3.6)
Degraded	1 (1.3)	4 (3)	12 (6.7)	0	24 (7.4)	42 (19.5)	12 (2.5)	65 (4.5)	7 (1)	167 (4.5)
Mucus	1 (1.3)	8 (6)	1 (0.6)	0	3 (0.9)	0	0	3 (0.3)	1 (0.1)	17 (0.5)
Saliva	6 (8)	15 (11.2)	41 (23)	86 (35)	98 (30.1)	66 (30.7)	9 (2.1)	209 (15.5)	51 (6.9)	581 (15.8)
Bloody										
Real	4 (5.3)	10 (7.5)	0	0	6 (1.8)	1 (0.5)	16 (3.3)	20 (1.3)	6 (0.8)	63 (1.7)
Insufficient	1 (1.3)	1 (0.7)	4 (2.2)	2 (0.8)	0	3 (1.4)	8 (2.3)	20 (1.4)	8 (1.1)	47 (1.3)
Degraded	0	0	3 (1.7)	5 (2)	0	0	0	18 (1.4)	0	26 (0.7)
Mucus	1 (1.3)	3 (2.2)	0	3 (1.2)	0	0	2 (0.4)	5 (0.2)	0	14 (0.4)
Saliva	2 (2.6)	0	0	1 (0.4)	7 (2.1)	0	1 (0.2)	3 (0.1)	1 (0.1)	15 (0.4)
Total	76	134	178	250	326	215	452	1321	736	3688

written and pictorial guidance for technical personnel. In the initial pilot study, the microscopic (cytology) classification of patient samples was performed by the laboratory staff in parallel to the usual macroscopic system for 6 weeks. In the second study, the same strategy was performed for 6 months in five health facilities in rural areas.

No ethics approval was required for the study, as the sample classification was performed in parallel to routine classification in the laboratories.

RESULTS AND DISCUSSION

Of the 3688 samples examined across the nine sites, 2543 (69%) were judged adequate for diagnosis following microscopic assessment, 294 (8%) were classed as degraded sputum and the remainder were mucus ($n = 156$, 4.2%) or saliva ($n = 188$, 18.8%). Results comparing visual classification with microscopy are presented in the Table. The proportion of samples misclassified by the visual classification, or found degraded, are presented in the Figure. Of samples classed as unsuitable for diagnosis on visual examination, 266 (7.2%) were found to correspond to the 'sputum' or 'insufficient sputum' categories by cytology. A total of 325 (8.8%) mucus, saliva or degraded samples were misclassified as purulent or mucopurulent samples on visual examination.

The degree of concurrence between the two quality assessment methods across the sites varied. High consensus was observed at the Hospital Luís Uría de la Oliva, a reference centre where patients with advanced disease are referred, as no sputum samples were misclassified as saliva. The highest proportion of misclassification was observed at the Hospital Boliviano Holandés in the Altiplano and Hospital Corea. The high proportion of degraded sputum samples observed at Patacamaya ($n = 42$, 19.5%) and the Lotes y Servicios maternal centre ($n = 25$, 14%) may be due to the receipt of specimens from peripheral health centres, as deliveries are not made daily, resulting in sample degradation. The high numbers of saliva samples observed, particularly at Hospital Luis Uría de la Oliva ($n = 87$, 35.4%), Achacachi ($n = 114$, 35%) and Patacamaya ($n = 66$, 30.7%), suggest that improved instructions to patients would be beneficial.[7,8] A limitation of the studies could have been the individual variations in sample classification. This could be resolved by introducing SCAS into the quality control for bacilloscopy currently implemented by the NTCP.

In summary, cytological classification of sputum samples was feasible and beneficial for informing laboratories about the quality of sputum specimens. Misclassification of samples may lead to unwarranted discarding of sputum samples that could be used for the diagnosis of TB. This study highlights the need to improve sample collection to reduce the number of saliva samples submitted and the importance of prompt delivery of samples when a preservative is not used.

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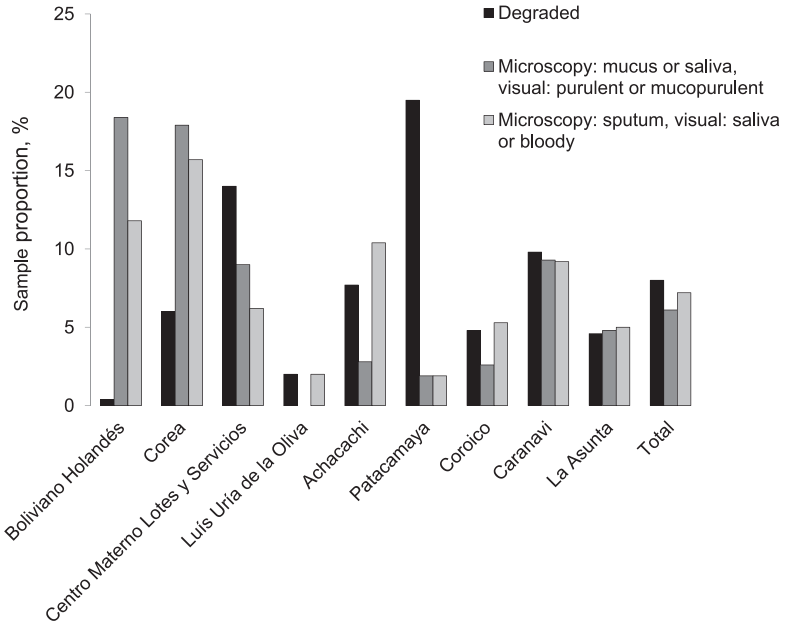

Figure Proportion of samples found to be degraded or misclassified by the laboratory.

involved in this study: D Magne, C Jemio, Y Cutili, J Flores, K Calle, E Pomacagua, B Mamani, V Feraudy and R Bustillos, R Troche, J Melgarejo, B Huallpo, S Sacaca, G Cardoso, J Apaza, D Rodríguez and N Nina; C R Aguilar and M P Lunasco for data collection; and the London School of Hygiene & Tropical Medicine (London, UK) and the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (Madrid, Spain) for their financial support.

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References

1 World Health Organization. Global tuberculosis report 2013. WHO/HTM/TB/2013.11. Geneva, Switzerland: WHO, 2013.
2 Ministerio de Salud y Deportes de Bolivia. Plan Estratégico para el control de la tuberculosis en Bolivia 2008–2015. Documentos técnico-normativos. La Paz, Bolivia: Ministerio de Salud y Deportes, 2008. [Spanish]
3 Steingart K R, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; 6: 664–674
4 Hepple P, Nguele P, Greig J, Bonnet M, Sizaire V. Direct microscopy versus sputum cytology analysis and bleach sedimentation for diagnosis of tuberculosis: a prospective diagnostic study. BMC Infect Dis 2010; 10: 276.
5 Ministerio de Salud y Deportes de Bolivia, Programa Nacional de Control de la Tuberculosis. Manual técnico de la red de laboratorios: baciloscopía. Documentos Técnico Normativos. La Paz, Brazil: Ministerio de Salud y Deportes, 2011. [Spanish]
6 Klarkowski D B. The use of sputum cytology on Ziehl-Neelsen stained smears to improve pulmonary TB diagnosis. 37th Union World Conference on Lung Health, Paris, France, 31 October–4 November 2006. [Poster discussion] Int J Tuberc Lung Dis 2006: 10 (11 Suppl 1): S154. [PC-61857-03].
7 Alisjahbana B, van Crevel R, Danusantoso H, et al. Better patient instruction for sputum sampling can improve microscopic tuberculosis diagnosis. Int J Tuberc Lung Dis 2005; 9: 814–817.
8 Khan M S, Dar O, Sismanidis C, Shah K, Godfrey-Faussett P. Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomised controlled trial. Lancet 2007; 369: 1955–1960.

RESUME

Un système de cytologie par microscope à faible puissance pour évaluer la qualité des crachats expectorés pour le diagnostic de la tuberculose (TB) a été piloté en Bolivie. Un total de 3688 échantillons a été soumis à un examen visuel et cytologique dans neuf laboratoires ; 591 (16%) échantillons ont été mal classées par examen visuel et 294 (8%) ont été trouvés dégradés. Le degré de discordance a varié d'un endroit à l'autre, et les laboratories recevant des échantillons de centres de santé isolés ont observé un plus grand nombre de spécimens dégradés. L'évaluation cytologique des crachats a été trouvée faisable et a identifié des domaines à améliorer dans le système de diagnostic de la TB en Bolivie.

RESUMEN

Un sistema citológico basado en microscopía de baja potencia para evaluar la calidad del esputo expectorado proporcionado para el diagnóstico de tuberculosis (TB) fue pilotado en Bolivia. Un total de 3688 muestras se sometieron a inspección visual y citológica en nueve laboratorios. Del total de las muestras, 591 (16%) fueron clasificadas erróneamente y 294 (8%) se encontraron degradadas. El grado de discordancia varió entre localizaciones y laboratorios, siendo en los establecimientos de salud más aislados en los que se observó un mayor número de especímenes degradados. La evaluación citológica del esputo mostró ser factible y ayudó a identificar áreas del sistema de diagnóstico de TB que precisan ser mejoradas.