Letter to the Editor (New Data)

Multiprimer PCR System Diagnosis of Pulmonary Tuberculosis in Cochabamba, Bolivia.

Bolivia has one of the highest incidence rates of tuberculosis (TB) in the Americas. An estimated 15,000 new cases are detected per year (1) which corresponds to an incidence rate of 112 cases per 100,000 population; 1,600 deaths are reported to occur annually due to TB (10). These figures are likely to be higher, because similar to many other developing countries, routine TB diagnosis in Bolivia is still primarily based on cultures or microscopic examination of specimens taken from individuals suspected of having TB. The drawback of this approach are that (i) neither culture or microscopy allow for differentiation of *Mycobacterium* species; (ii) a large number of *Mycobacterium* bacilli are required for positive identification (i.e. microscopy has poor sensitivity); (iii) though specific, cultures are time-consuming; and (iv) sensitivity and specificity of both diagnostic approaches are dependent on the expertise of the laboratory personnel (e.g. with regards to specimen collection, processing and evaluation). PCR has become a much used approach for the detection of mycobacteria in cultured strains and uncultured clinical samples (5,9), and a range of PCR protocols have been developed and tested to detect *Mycobacterium tuberculosis* (3,6,8). However, few protocols have been evaluated let alone operationalized in a developing country setting.
We used a published multiprimer system PCR (MS-PCR) protocol (4) to detect Mycobacterium spp. in sputum samples of suspected TB patients attending a regional TB laboratory in Cochabamba, Bolivia. Tested PCR protocol was compared to routine culture and microscopy according to the guidelines of the Bolivian TB Control Program (2), i.e. cultures being grown on Lowenstein-Jensen medium at 37°C for 60 days and slides being processed with Ziehl-Nielssen stain, respectively. For MS-PCR, samples were decontaminated using the Petroff method (7) prior to storage at -20°C. Unlike the protocol by Portillo et al. (4) we extracted DNA from samples using a commercial DNA extraction kit (DNAzol®, Invitrogen, Carlsbad, CA). All samples were amplified with the following primers according to previously published conditions (4): PT1 and PT2 (species-specific, targeting the MTP40 gene), MT1 and MT2 (genus-specific, targeting the gene coding for the 32kDa alpha antigen) and IS5 and IS6 (complex-specific, targeting the IS6110 insertion sequence). Each amplification cycle included negative (no DNA, DNA from an uninfected person and a dog) and positive (water-lysate mixtures of reference strain cultures) controls. Amplification products (10 ul) were visualized under UV light after electrophoresis on 1.5% agarose gels. To avoid cross-contamination, separate areas were used for DNA extraction, PCR sample preparation, and amplification; PCR-grade H2O was used throughout.

Between July and September 2004, of 284 patients attending the Regional TB Laboratory with suspected pulmonary TB, 63 patients satisfied the study inclusion criteria (i.e. non-salival sputum sample of patients >15 years of age, productive cough for three weeks prior to date of study inclusion). Because of logistical and technical constraints only 33 were randomly selected to participate in our study and had sputum samples taken for study purposes. Of tested study samples, 11/33 (33%), 7/33 (21%) and 11/33 (33%) tested positive
by MS-PCR, microscopy and culture, respectively. Assuming that culture is the diagnostic ‘gold standard’, both microscopy and MS-PCR were 100% specific. Sensitivity was 100% and 67% for MS-PCR and microscopy, respectively. Used MS-PCR protocol clearly identified all clinical samples due to *M. tuberculosis*, with the characteristic, species- and genus-specific bands readily observed in all cases.

For pulmonary TB, PCR has previously been shown to have a sensitivity between 77 and 95% and specificity between 95% and 100% (3,6,8), the fluctuation being due to whether samples tested where compared to samples from clinically suspected cases with or without positive microscopy or culture. Our study shows that a MS-PCR approach can readily be implemented in Bolivia. Based on our results, we intend to carry out further studies to develop a novel diagnostic algorithm including MS-PCR allowing for prompt and cost-effective diagnosis of TB patients in Cochabamba. Such algorithm could then be scaled up to national level in order to contribute to the improved patient management and better surveillance of the disease in the country.

REFERENCES


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