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A NEW COLORIMETRIC PLATE FOR THE RAPID DIAGNOSIS OF EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS DIRECTLY IN SPUTUM SAMPLES

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Objectives: The nitrate reductase assay (NRA) assay was recently approved by WHO for rapid rifampicin and isoniazid susceptibility testing of *Mycobacterium tuberculosis*. A modification of the technique is proposed, which consists in employing a multi-well plate format and enriched agar medium. The objective of this study is to evaluate the plate-NRA directly on sputum samples (D-NRA) for early detection of extensively drug-resistant (XDR-TB).

Methods: This preliminary study is currently performed in Buenos Aires, Argentina. Smear-positive sputum samples from 26 consecutive TB patients at risk of treatment failure were assayed. Sputa were decontaminated with NALC-NaOH. Rifampicin, isoniazid, ofloxacin, kanamycin, amikacin and capreomycin were tested at 1, 0.2, 2, 6, 2, and 5 µg/ml, respectively, in 24-well plates containing Middlebrook 7H11-KNO_3 agar. P-nitrobenzoic acid (PNB) was used for differentiating *M. tuberculosis* from other mycobacteria, and *PRA-hsp65* was used as species identification reference method. Accuracy, turnaround time and cost were evaluated using the MGIT 960 system as gold standard method.

Results: Out of 26 samples, 2 were both MGIT and NRA negative. The other 24 were identified as *M. tuberculosis* complex by both PNB test and PRA-*hsp65*. One NRA result for capreomycin was indeterminate and one isoniazid well was found contaminated. As for the remaining results, only one discordance was observed between both methods. The sensitivity and the specificity of the D-NRA was 100% for all drugs except for capreomycin with a sensitivity of 100% and a specificity of 94.7%. The detection rate on day 14 was 50% for D-NRA and 9.5% for MGIT. Mean *time* to results was 16.5 and 21.9 *days,* respectively *(P: <0.001)*. The local cost of supplies per sample was US$24.7 for D-NRA and US$50.5 for MGIT.

Conclusion: Advantages of this fast colorimetric plate version include simplification of media dispensing and/inoculation procedures, and reduction of storage/ incubation space. Plate-NRA is simple and appears to be accurate to detect XDR-TB. In comparison with the MGIT system, D-NRA was faster and cheaper.

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RPOB POLYMORPHISMS IN *MYCOBACTERIUM TUBERCULOSIS* COMPLEX FROM A POPULATION IN GUINEA-BISSAU

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The global tuberculosis (TB) threat is difficult to evaluate in countries with scarce laboratory facilities where little is known about the genetic basis of drug resistance, including the *rpoB* RRDR (rifampicin resistance determining region) of the M*ycobacterium tuberculosis* complex (MTC). The present study aimed at performing a preliminary evaluation of *rpoB* polymorphisms in a DNA set of MTC isolates from Guinea-Bissau patients.

Ninety-four sputum specimens (74 bleach processed and 20 unprocessed) were sent to Lisbon for molecular analysis (n=94) and drug susceptibility testing (n=20). A 369bp region of the *rpo*B gene (including the 81bp RRDR), was amplified. Sequencing was used as the gold standard for the identification of point mutations.

Two polymorphisms were identified: The alteration S531L, present in 3.2% of the specimens, and a new mutation, present in 28.7%, corresponding to nucleotide and amino acid polymorphism C224T and S469L, respectively, detected upstream from the RRDR, and not associated with RMP resistance. An NCBI BLAST revealed *M. tuberculosis* K85 (*M. africanum*) as the only strain presenting this polymorphism.

The circulation of S531L mutated stains, associated with RMP resistance, points to the urgent need to further investigate RMP resistance in this African region. Moreover, the strains presenting the S469L polymorphism showed no other mutations suggesting that its role on RMP susceptibility/ resistance or in