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Title: Assessment of molecular assays for detection of MDR Mycobacterium tuberculosis resistant to second-line injectable drugs

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Body: With the emergence of MDR-TB and XDR-TB rapid and accurate second-line drug-susceptibility testing became essential. The [GenoType® MTBDRsl] (Hain Lifescience, Germany) gives the possibility to identify mutations in the rrs, gyrA and embB genes. The aim of the present study was to assess the sensitivity of this molecular assay for the identification of M. tuberculosis resistant to second-line injectable drugs (SLIDs), as, to our knowledge, detection of rrs mutations is not sufficient for this purpose. 40 MDR M. tuberculosis strains, also resistant to SLIDs, were chosen from our culture collection. 20 SLIDs-susceptible strains were adopted as control. Three DNA targets were investigated: the rrs gene, the eis promoter region and the tlyA gene . The [GenoType®] was used to detect rrs mutations and SSCP and sequencing were performed for all three genes. All kanamycin and capreomycin-resistant cultures carried an A1401G substitution in the rrs gene and were detected with all the molecular assays, but one of the strains also had an eis mutation. 8 kanamycin-resistant strains carried various eis mutations, the most common being the C14T substitution, and thus could not be identified with the [GenoType®]. No mutations were found in the tlyA gene gene. One of the SLIDs-susceptible strains carried a C12T mutation. Therefore 20% of M. tuberculosis strains resistant to kanamycin could not be detected with the [GenoType®]. Mutations in the eis promoter region are found in kanamycin-resistant M. tuberculosis strains at high rates and should be included in molecular assays for detection of SLIDs resistance.