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Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs

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The novel Deeplex Myc-TB molecular assay shows a high degree of accuracy for extensive prediction of susceptibility and resistance to 13 anti-tuberculous drugs, directly achievable without culture, which may enable fast, tailored tuberculosis treatment https://bit.ly/3bAvcAt

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ABSTRACT Conventional molecular tests for detecting *Mycobacterium tuberculosis* complex (MTBC) drug resistance on clinical samples cover a limited set of mutations. Whole-genome sequencing (WGS) typically requires culture.

Here, we evaluated the Deeplex Myc-TB targeted deep-sequencing assay for prediction of resistance to 13 anti-tuberculous drugs/drug classes, directly applicable on sputum.

With MTBC DNA tests, the limit of detection was 100–1000 genome copies for fixed resistance mutations. Deeplex Myc-TB captured *in silico* 97.1–99.3% of resistance phenotypes correctly predicted by WGS from 3651 MTBC genomes. On 429 isolates, the assay predicted 92.2% of 2369 first- and second-line phenotypes, with a sensitivity of 95.3% and a specificity of 97.4%. 56 out of 69 (81.2%) residual discrepancies with phenotypic results involved pyrazinamide, ethambutol and ethionamide, and low-level rifampicin or isoniazid resistance mutations, all notoriously prone to phenotypic testing variability. Only two out of 91 (2.2%) resistance phenotypes undetected by Deeplex Myc-TB had known resistance-associated mutations by WGS analysis outside Deeplex Myc-TB targets. Phenotype predictions from Deeplex Myc-TB analysis directly on 109 sputa from a Djibouti survey matched those of MTBSeq/PhyResSE/Mykrobe, fed with WGS data from subsequent cultures, with a sensitivity of 93.5/98.5/93.1%

and a specificity of 98.5/97.2/95.3%, respectively. Most residual discordances involved gene deletions/indels and 3–12% heteroresistant calls undetected by WGS analysis or natural pyrazinamide resistance of globally rare "*Mycobacterium canettii*" strains then unreported by Deeplex Myc-TB. On 1494 arduous sputa from a Democratic Republic of the Congo survey, 14 902 out of 19 422 (76.7%) possible susceptible or resistance phenotypes could be predicted culture-free.

Deeplex Myc-TB may enable fast, tailored tuberculosis treatment.