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Performance, evaluation and integration of tNGS in diagnostic workflow for Tuberculosis

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BACKGROUND:

In vitro identification of Mycobacterium tuberculosis complex (MTBC) and its antibiotic resistance can be performed by genome sequencing.

Deeplex® Myc-Tb assay is a targeted NGS kit, which provide results to 15 anti-TB drugs (included Bedaquiline and Clofazimine) from culture-free specimens or strains. The purpose of this work was to evaluate the consistency of the tNGS kit for the integration in routine diagnostic MTBC workflow.

MATERIALS:

Strains selected on the basis of resistance profiles and biological samples from randomly selected patients with tuberculosis were analysed by Deeplex® Myc-Tb assay (GenoScreen). We tested 86 samples: 47 from solid or liquid medium culture (extraction with DNeasy Ultraclean, Qiagen) and 39 from biological samples (extraction QIAmp DNA Mini kit, Qiagen). Drug resistance profiles obtained by phenotypic antibiogram were statistically compared with the genotypic resistance profiles.

RESULTS:

39 biological samples were tested but 10 samples failed. Despite the minimun DNA concentration for testing was reached in all of them, the paucibacillary samples with very low and low bacterial load were not amplified. Whereas only 4 strains hadn't a sufficient quality at the end of the analysis.

The comparison between phenotypic and genotypic resistance was showed in figure 1. For rifampicin and isoniazid the differences were 1.47% and 1.49% respectively, whereas for pyrazinamide and ethambutol were 4.68% and 6.25%. We observed 100% concordance for linezolid, fluoroquinolones and aminoglycosides. Bedaquiline and Clofazimine were sensitive in all MDR infections.

Figure 1 - Comparison between phenotypic and genotypic resistance

DRUG	COMPARABLE ANALYSIS	NO INTERPRETATION ERROR	MAJOR ERRORS	VERY MAJOR ERRORS
RIFAMPICIN	68	66	1	1
ISONIAZID	67	66	1	0
PYRAZINAMIDE	64	61	2	1
ETHAMBUTOL	64	60	0	4
STREPTOMYCIN	21	18	1	2
FLUOROQUINOLONES*	21	21	0	0
KANAMYCIN	29	29	0	0
AMIKACIN	31	31	0	0
CAPREOMYCIN	26	26	0	0
ETHIONAMIDE	25	21	1	3
LINEZOLID	20	20	0	0

* They include levofloxacin, ofloxacin, moxifloxacin and ciprofloxacin.

CONCLUSION:

tNGS is faster than phenotypic antibiogram and allow more complete genotypic analysis of multidrug-resistant MTBC strains. In particular, this method is useful for testing susceptibility to the new generation drugs Bedaquiline and Clofazimine, for which a standardized phenotypic antibiogram is not yet available. For patients with intermediate or high bacterial load can be performed directly from specimens, whereas for ones with low bacterial load should be performed from positive liquid medium. The main strength of this technique is the improvement of personalized treatment outcomes to prevent the onset of new drug resistance and transmission.