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Supplementary appendix

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Appendix

Depending on the nature of the resistance mechanism(s) to a novel antibiotic, not all technologies may be appropriate to detect them. Rapid molecular assays, such as the Cepheid GeneXpert system, can be used directly on clinical samples but only interrogate short stretches of bacterial DNA.[1] In the case of PA-824 (pretomanid) resistance can arise through mutations in five non-essential genes (*fgd1*, *fbiC*, *fbiA*, *fbiB* and *ddn*) with a total length of 6.4 kilobase pairs.[2-4] Therefore phenotypic drug-susceptibility testing or rapid whole-genome sequencing might be the only diagnostic option to detect resistance to this agent,[5, 6] unless one particular set(s) of mutations are found to be dominant in clinical isolates, as is the case with *katG* mutations and high-level isoniazid resistance.[7, 8] Irrespective of the technology used, a comprehensive understanding of the natural diversity in the resistance gene(s) for each antibiotic is crucial to avoid systematic false-positive results due to polymorphisms that do not cause resistance.[9-12]

The study of large collections of *in vitro* and *in vivo* mutants to correlate different mutations with minimum inhibitory concentrations (MICs) might inform the design of pre-clinical and clinical trials and, subsequently, improved diagnostic and screening tests.[12] If certain mechanisms or mutations led to only marginally elevated MICs (as is the case with *inhA* mutations and isoniazid[9] or with certain *rpoB* mutations and rifampicin[13]), higher doses or more frequent dosing of the novel therapeutic agent to overcome this low level of resistance could be evaluated.[14] This would benefit patients, clinicians and pharmaceutical companies by increasing the number of TB cases that could be treated with that particular drug. The resulting knowledge could also be used to determine appropriate breakpoints for phenotypic assays – which is not straightforward for MTBC[15, 16] – and to validate the reliability of these assays.[17] Finally, individual mutants could be used to quantify the risk of developing further resistance, as certain low-level resistance mechanisms have been found to increase the chance of developing high-level resistance.[11, 18]

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