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Comment on: Characterization of the *embB* gene in *Mycobacterium tuberculosis* isolates from Barcelona and rapid detection of main mutations related to ethambutol resistance using a low-density DNA array

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Sir,

We agree with Moure *et al.*[1] that fast genotypic methods will play an increasingly prominent role in drug susceptibility testing for

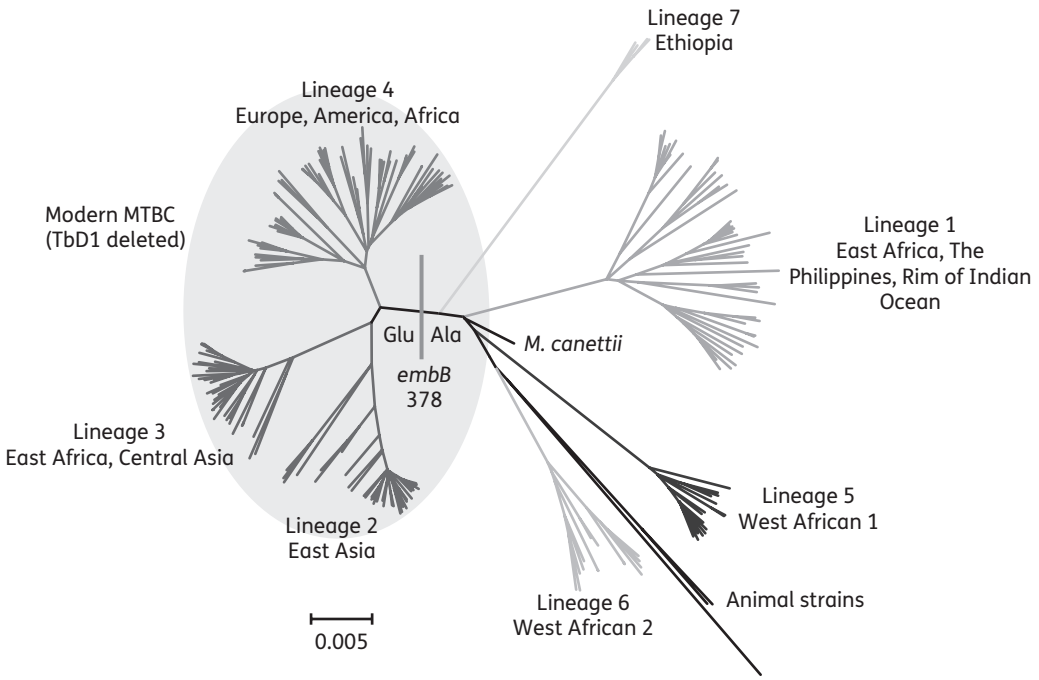

Figure 1. Whole-genome phylogeny of 219 isolates representative of all major MTBC lineages.[9] Glu at codon 378 is a marker for modern MTBC, which all share the TbD1 deletion and include the lineage 4 *M. tuberculosis* H37Rv laboratory strain that is used as the reference/wild-type sequence for sequence analyses.[10]

the *Mycobacterium tuberculosis* complex (MTBC).[2,3] We would, however, like to point out that the *embB* (*Rv3795*) Glu378Ala polymorphism, which is detected by probe 3 of their newly developed low-density DNA array, is not a marker for ethambutol resistance.[4–7] Instead, Ala represents the ancestral amino acid at this codon (Figure 1), whereas Glu is present in all modern MTBC (lineages 2, 3 and 4).[6–9] The MIRU–VNTR data of the 51 ethambutol-resistant isolates from the study by Moure *et al*.[1] are largely congruent with this finding. All 49 phylogenetically modern MTBC isolates had the *embB* 378 Glu variant. Isolate 5765 was a representative of *Mycobacterium bovis*, which is consistent with the fact that it harboured the Ala variant and was pyrazinamide resistant. By contrast, it was unclear why isolate 233R, which appeared to be *M. bovis* based on its MIRU–VNTR signature, had the Glu variant (experimental error or a homoplastic event might account for this discrepancy).

In light of these data, the results of probe 3 would be predicted to lead to systematic false-positive reports, which calls into question the validity of this probe. This underlines that the entire MTBC diversity has to be considered when designing and validating genotypic drug susceptibility testing assays.[7,10]

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Disclaimer

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Characterization of the *embB* gene in *Mycobacterium tuberculosis* isolates from Barcelona and rapid detection of main mutations related to ethambutol resistance using a low-density DNA array—authors' response

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