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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

Claudio U. Köser^{1*†}, Josephine M. Bryant^{2†}, Iñaki Comas^{3,4†}, Silke Feuerriegel^{5,6}, Stefan Niemann^{5,6}, Sebastien Gagneux^{7,8}, Julian Parkhill² and Sharon J. Peacock^{1,2,9,10}

¹Department of Medicine, University of Cambridge, Cambridge, UK;

²Wellcome Trust Sanger Institute, Hinxton, UK; ³Genomics and Health Unit, FISABIO, Valencia, Spain; ⁴CIBER (Centros de

Investigación Biomédica en Red) in Epidemiology and Public Health, Barcelona, Spain; ⁵Molecular Mycobacteriology, Borstel, Germany; ⁶German Centre for Infection Research, Research Centre Borstel, Borstel, Germany; ⁷Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland; ⁸University of Basel, Basel, Switzerland; ⁹Clinical Microbiology and Public Health Laboratory, Public Health England, Cambridge, UK; ¹⁰Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

*Corresponding author. Tel: +44-1223-331664; Fax: +44-1223-336846; E-mail: cuk21@cam.ac.uk

†Contributed equally.

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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

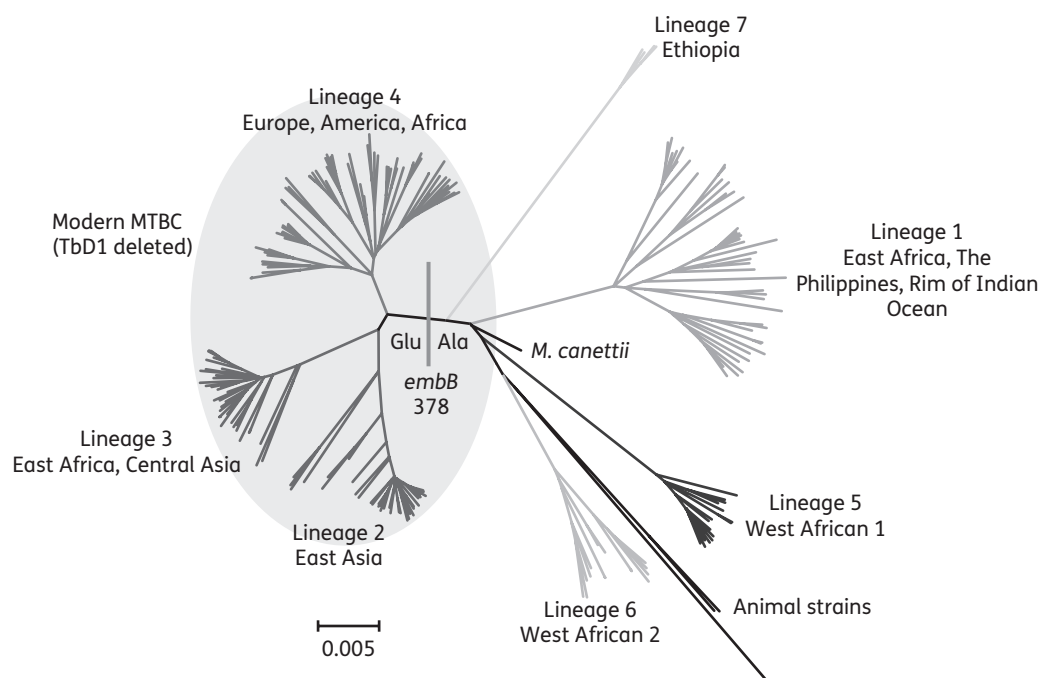


Figure 1. Whole-genome phylogeny of 219 isolates representative of all major MTBC lineages.⁹ Glu at codon 378 is a marker for modern MTBC, which all share the TbD1 deletion and include the lineage 4 *M. tuberculosis* H37Rv laboratory strain that is used as the reference/wild-type sequence for sequence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

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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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Transparency declarations

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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

Raquel Moure¹, Montserrat Español², Griselda Tudó³, Eva Vicente⁴, Pere Coll², Julian Gonzalez-Martin³, Virginie Mick⁵, Margarita Salvadó⁴ and Fernando Alcaide^{1*}

¹ Servei de Microbiologia, Hospital Universitari de Bellvitge-IDIBELL, Universitat de Barcelona, Barcelona, Spain; ² Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau de Barcelona, Universitat Autònoma de Barcelona, Barcelona, Spain; ³ Servei de Microbiologia, CDB, Hospital Clínic de Barcelona-Barcelona Centre for International Health Research (CRESIB), Universitat de Barcelona, Barcelona, Spain; ⁴ Laboratori de Referència de Catalunya, Barcelona, Spain; ⁵ Paris-Est University-ANSES, Animal Health Laboratory, Bacterial Zoonoses Unit, Maisons-Alfort, France

*Corresponding author. Tel: +34-932607930; Fax: +34-932607547; E-mail: falcaide@bellvitgehospital.cat

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