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).5–9 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 homoplasic 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.2,10

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References

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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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Keywords: M. tuberculosis, antituberculous resistance, molecular diagnosis, microarray
Sir,

We read with great interest the letter by Köser et al., responding to our article on the characterization of the embB gene in the area of Barcelona. The point that Köser et al. raise is very interesting and deserves further comment. Codon 378 has indeed been described by several authors as a phylogenetic polymorphism not related to resistance. However, in our opinion the role of this codon remains unclear, since some studies describe the existence of ethambutol-resistant isolates with a mutation only in this codon. Even one work cited by Köser et al. includes two isolates with a single Glu378Ala substitution and a decreased susceptibility to ethambutol.

The design of our microarray was based on the existing literature, taking into account all the possible embB codons that have been implicated in ethambutol resistance. We finally included the ones that were also found in our setting. The microarray system was not designed to determine the association of each mutation with the phenotypic resistance (allelic exchange experiments would be required for this purpose), but to reflect the variety of embB substitutions prevalent in our area. Moreover, the frequency of mutations in embB378 is low (<2% in our study), so we consider that the probability of misassigning a result of embB378 mutation to phenotypic resistance rather than to an epidemiological cause is negligible and does not compromise the effectiveness of the microarray.

Regarding the isolates, we included a collection of Mycobacterium tuberculosis complex (MTBC) clinical isolates (not identified to the species level). Isolate 233R has now been analysed and identified as M. tuberculosis/Mycobacterium canettii. It contains the embB378Glu variant; therefore, a homoplastic event may be present (experimental error was ruled out).

Likewise, the MIRU-VNTR genotyping was performed not for epidemiological purposes (i.e. for the identification of lineages), but to establish the real frequency of embB mutations among circulating MTBC isolates in our geographical area.

Finally, we stress that the main objective of this study was to highlight the relevance of mutations in embB codons apart from embB306, focusing on codon 406, which represents 20% of the embB mutations in our area. Our results show that this target (embB406) should be included in any genotypic method for rapid ethambutol resistance detection.

Transparency declarations
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Comment on: S-nitrosogluthathione (GSNO) is cytotoxic to intracellular amastigotes and promotes healing of topically treated Leishmania major or Leishmania braziliensis skin lesions

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Keywords: nitrosogluthathione, parasite, transcriptome, chemokines

Sir,
A previous study by Costa et al. published in this journal reported the action of S-nitrosogluthathione (GSNO) (CAS number: 57564-91-7) on skin disease induced by Leishmania parasites. The major findings of this study were: (i) GSNO following topical application reduced ulcerated skin lesions of infected mice; and (ii) the number of intracellular Leishmania major amastigotes is reduced in THP-1 macrophages exposed to 300 μM GSNO. The authors assume that the nitrosation by GSNO of host cell and parasite proteins could explain the in vitro leishmanicidal activity of GSNO in THP-1 cell culture.

We suggest a complementary mechanism of GSNO action. We have recently analysed the transcriptome of THP-1 cells exposed to 50 μM GSNO for 4 h. Some of our results might be of high

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