Reply to Zimenkov, “Mutation in *luxR* Family Transcriptional Regulator Rv0890c Is Not a Marker of Linezolid Resistance”

Shashikant Srivastava,^a Tawanda Gumbo^a

^aCenter for Infectious Diseases Research and Experimental Therapeutics, Baylor Research Institute, Dallas, Texas, USA

We thank Dr. Zimenkov (1) for his interest in our work and the valuable comments on our paper (2) pointing out the possible cause of the discrepancies between the single-nucleotide variant (SNV) calls in our data and suggesting a reanalysis of our results. As Dr. Zimenkov noted, a possible cause of the differences noted in the SNV calls of the whole-genome sequence analysis might be the reference genome utilized. Indeed, we had used the reference genome NC_000962.2 (released on 21 December 2012), which was what was available when we did our original sequencing and SNV calls. After Dr. Zimenkov's letter, we realigned the sequences using the latest version of the sequence (NC_000962.3, released on 2 August 2016), and some of the previously reported SNVs in the *Mycobacterium tuberculosis* genome disappeared or had differences in nucleotides that would ultimately result in a coding region change different from what was seen with the older reference version. However, the mutations in question, such as Rv0890c (transcriptional regulator) and Rv0930, were the same in our reanalysis as in our wild-type laboratory strain at the start of the experiment. The average for the coverage was 338×, with a total read count of 337, a forward read count of 162, a reverse read count of 176, a frequency of 99.8, and a quality score of 38.4, and the probability was 1 for the reported nucleotide change at the particular position that Dr. Zimenkov commented on. Dr. Zimenkov's analysis found that the mutations that we noted are present in >99% of the 6,419 isolates screened. That is interesting and surprising and should open up further discussion. It should be noted that our RNA sequencing (which we reported in detail) identified an ensemble of genes that were upregulated, with the most prominent changes occurring with the ABC multidrug efflux pumps *rrrA*, *rrrB*, and *rrrC*. Upon whole-genome sequencing, SNVs were identified in a different ensemble of genes, including Rv0890c, which was also a surprise. We agree that ascribing “causation” for acquired linezolid resistance to this one gene would be controversial and is not a question that our experiments were designed to identify. All we can say is that the mutation occurred in drug resistance isolates, but whether this is what caused the phenotypic linezolid resistance, either alone or in the company of other SNVs, requires a different set of experiments. Dr. Zimenkov's analysis of clinical isolates clearly raises the question of whether the particular gene has anything to do with resistance at all, and the field is open for further studies, perhaps via site-directed mutagenesis and complementation experiments and examination of the strain's susceptibility to linezolid. We will report on those findings in due course.

Citation Srivastava S, Gumbo T. 2018. Reply to Zimenkov, “Mutation in *luxR* family transcriptional regulator Rv0890c is not a marker of linezolid resistance.” *Antimicrob Agents Chemother* 62:e02296-17. <https://doi.org/10.1128/AAC.02296-17>.

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to Tawanda Gumbo, tawanda.gumbo@bswhealth.org.

This is a response to a letter by Zimenkov (<https://doi.org/10.1128/AAC.02087-17>).

REFERENCES

1. Zimenkov D. 2018. Mutation in *luxR* family transcriptional regulator Rv0890c is not a marker of linezolid resistance. *Antimicrob Agents Chemother* 62:e02087-17. <https://doi.org/10.1128/AAC.02087-17>.
2. Srivastava S, Magombedze G, Koeuth T, Sherman C, Pasipanodya JG, Raj

P, Wakeland E, Deshpande D, Gumbo T. 2017. Linezolid dose that maximizes sterilizing effect while minimizing toxicity and resistance emergence for tuberculosis. *Antimicrob Agents Chemother* 61:e00751-17. <https://doi.org/10.1128/AAC.00751-17>.