REPLY TO SHAW:

Challenges for enrichment analysis of AMR gene-bearing plasmids

You Che[a], Yu Yang[a], Xiaoqing Xu[a], Karel Břinda[b,c], Martin F. Polz[d,e], William P. Hanage[c], and Tong Zhang[a,1]

We thank Liam Shaw for his letter (1), "Null models for gene enrichment in plasmids," and appreciate the detailed simulation regarding the enrichment analysis of AMR genes on different types of plasmids, which confirms our original conclusions that conjugative plasmids are enriched in these genes (2). We agree that considering the plasmid size is a good point when using a null model for the enrichment analysis as it tests for a completely neutral accumulation of genes. However, using a completely neutral model for all genes on plasmids does not capture the reality of gene content constraints. Conjugative plasmids contain a much larger portion of genes that are involved in transfer and maintenance than other plasmids. Hence, the proportion of genes that can be readily exchanged is much smaller than suggested by the entire genome size. Additionally, we note that different plasmid types have very different biology, and hence the plasmid type is an important category to test for.

In a broad sense, the successful establishment of AMR genes on different plasmids will be shaped by factors related to plasmid-host evolutionary adaptations, plasmid mobility, and extrinsic selection pressures that may impact the fate and maintenance of the resistance. For example, previous research showed that transfer of AMR gene-bearing conjugative plasmids, even when costly, is sufficiently fast to exhibit maintenance in the absence of positive selection (3). On the other hand, compensatory adaptation and positive selection of plasmid-borne resistance are important to maintain the nonmobilizable plasmids (4). The extent to which interaction between the above factors and the potentially paradoxical role of these process, together with the phylogenetic barriers for plasmid transfer (e.g., most nonmobilizable plasmids circumscribed to a single bacterial species, while conjugative plasmids spread across bacterial families) (5), contribute to the distribution of AMR genes in different plasmid categories is an intriguing question that currently remains unresolved.

Regarding the description of the "shared" AMR genes on different plasmids, we use the term "transferred" (2), which may need further clarifications. Our initial attempt was to obtain an overall distribution of AMR genes in different plasmid categories based on network analysis. We reconstructed the network with plasmids and AMR genes as the network nodes; when an AMR gene was "shared" between two plasmids, both plasmids were linked to the AMR gene by edges. We don't introduce a distinction between "transferred" and "shared" terms simply because the network analysis is an extended analysis of the results presented in ref. 2, SI Appendix, figure S3 that indicates extensive horizontal transfer of AMR genes across plasmid categories, and, importantly, the significant role of conjugative plasmids in mediating the transfer of AMR genes within the plasmid community is inferred based on the network topology, which is fully supported by previous studies (5, 6). We are, nevertheless, grateful for the opportunity to clarify this point.

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[a]Environmental Microbiome Engineering and Biotechnology Laboratory, Department of Civil Engineering, The University of Hong Kong, Hong Kong, China; [b]Department of Biomedical Informatics, Harvard Medical School, Boston, MA 02115; [c]Center for Communicable Disease Dynamics, Department of Epidemiology, Harvard T. H. Chan School of Public Health, Harvard University, Boston, MA 02115; [d]Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; and [e]Department of Microbial Ecology, Centre for Microbiology and Environmental Systems Science, University of Vienna, 1090 Vienna, Austria

Author contributions: Y.C. and T.Z. designed research; Y.C. performed research; Y.C., Y.Y., X.X., and T.Z. analyzed data; K.B., M.F.P., and W.P.H. provided valuable advice on data analysis; and Y.C. and T.Z. wrote the paper with advice from M.F.P.

The authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: zhangt@hku.hk.

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