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Myobacterium tuberculosis Beijing Type Mutation Frequency

To the Editor: A striking finding in the study by de Steenwinkel et al. (1) is the high frequency of mutation to rifampin resistance by 2 Mycobacterium tuberculosis Beijing strains, which might play a role in the association between the Beijing strains and multidrug-resistant tuberculosis. Earlier reported frequency of mutation to rifampin resistance by M. tuberculosis has been \(10^{-8}\) CFU (2,3), including the Beijing genotype (3,4). Of note, the Beijing 2002–1585 strain, for which frequency of mutation to rifampin resistance is \(10^{-3}\) CFU (1 mutant/1,000 CFU), showed a moderate frequency of \(10^{-6}\) CFU in another study (4). We think that a mutation frequency increase of 100,000 \(\times\) is remarkably high. In contrast, rifampin-resistant mutants of the Beijing 1585 strain did not emerge in low-density cultures (5 \(\times\) \(10^3\) CFU/mL) used for time-kill kinetics experiments, although frequency of mutation to rifampin resistance was determined to be \(10^{-3}\) CFU.

Mutation frequency is determined by fluctuation assays. To exclude preexisting mutations, which would bias the mutation frequency by so-called jackpots, a series of low-inoculum cultures is typically used (5). However, for unknown reasons, de Steenwinkel et al. used only 1 high-density culture of \(10^6\) CFU of each strain to determine mutation frequency. This strategy is not recommended because mutations can occur early or late, resulting in substantial mutation frequency fluctuation between test episodes. A strain with known mutation rates should preferably be included to rule out possible technical errors.

We propose the following explanations for the remarkable results: 1) the rifampin concentration for selecting mutants might have been too low, enabling growth of some colonies of drug-susceptible bacteria; 2) rifampin mutants arose early or preexisted in the cultivation of Beijing strains 1585 and 1607, producing jackpots; or 3) the 2 Beijing isolates might contain rifampin-resistant subpopulations (heteroresistance). The capacity of the Beijing strain to develop and, especially, transmit multidrug-resistant tuberculosis remains to be further analyzed.

Jim Werngren
Author affiliation: Swedish Institute for Communicable Disease Control, Solna, Sweden
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In Response: We explain the differing frequencies of mutation to rifampin resistance mentioned by Werngren (1). First, the strains of Mycobacterium tuberculosis that we tested differed from those previously tested (2). Second, we used different rifampin concentrations in subculture plates. For Beijing strain 2002–1585, Bergval et al. (3) found a mutation frequency of \(4–24 \times 10^{-3}\) at a subculture concentration of 8 mg/L, whereas we found a mutation frequency of \(3–4 \times 10^{-3}\) at a subculture concentration of 1 mg/L and a lower mutation frequency at 2 mg/L. Thus, the concentration of drugs in subculture plates is crucial to mutation frequency assays. Absent a subculture concentration standard, we applied rifampin at 1 mg/L (4) because bacteria growing at this concentration are considered resistant to rifampin. Our mutation frequency and time-kill kinetics assay results are not contradictory.
Because in the time-kill kinetics assays, the subculture rifampin concentration was 4 mg/L.

We performed no classical fluctuation assays. We compared the Beijing genotype with the East African/Indian genotype to learn how M. tuberculosis strains differed in their capacity to withstand antituberculosis drug treatment. For reference strain H37Rv, mutation frequency was $1.5 \times 10^{-6}$, higher than that found with higher subculture concentrations.

With regard to the 3 other issues, our drug-susceptibility testing of mutants showed a stable rifampin-resistant phenotype. We agree that these bacteria might represent preexisting mutants selected during drug exposure in a certain drug concentration window. By using different concentrations in subculture plates in our mutation frequency assay, we detected such preexisting mutants. Heteroresistance probably does not explain our observations because in our time-kill kinetics experiments, the whole mycobacterial population decreased over time in a drug concentration-dependent way, and regrowth of a drug-resistant subpopulation was not observed.

By not sticking to the fixed test conditions as used in the classical drug-susceptibility assays, research leads to highly interesting findings. One can conclude that serendipity flourishes with variation.

Jurriaan E.M. de Steenwinkel, Dick van Soolingen, and Irma A.J.M. Bakker-Woudenberg

Author affiliations: Erasmus University Medical Center, Rotterdam, the Netherlands (J.E.M. de Steenwinkel, I.A.J.M. Bakker-Woudenberg), National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands (D. van Soolingen); and Radboud University Medical Center, Nijmegen, the Netherlands (D. van Soolingen)

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Address for correspondence: J.E.M. de Steenwinkel, Erasmus MC, Room L-327, PO Box 2040, 3000 CA, Rotterdam, the Netherlands; email: j.desteenwinkel@erasmusmc.nl

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The name of author Arina Zanudana was misspelled in the article Vaccination of Health Care Workers to Protect Patients at Increased Risk for Acute Respiratory Disease. The article has been corrected online (http://wwwnc.cdc.gov/eid/article/18/8/11-1355_intro.htm).

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