4. van Ingen J, Rahim Z, Mulder A, Boeree MJ, Simeone R, Brosch R, et al. Characterization of *Mycobacterium orygis* as *M. tuberculosis* complex subspecies. Emerg Infect Dis. 2012;18:653–5. http://dx.doi.org/10.3201/eid1804.110888
5. Gey van Pittius NC, Perrett KD, Michel AL, Keet DF, Hlokwe T, Streicher EM, et al. Infection of African buffalo (*Syncerus caffer*) by oryx bacillus, a rare member of the antelope clade of the *Mycobacterium tuberculosis* complex. J Wild Dis. 2012;48:849–57. doi: 10.7589/2010-07-178.

Address for correspondence: Jakko van Ingen, Medical Microbiology, Radboud University Nijmegen Medical Center, PO Box 9101, Nijmegen 6500 HB, the Netherlands; email: vaningen.jakko@gmail.com

Mycobacterium 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^{-8} CFU in another study (*4*). We think that a mutation frequency increase of 100,000× is remarkably high. In contrast, rifampin-resistant mutants of the Beijing 1585 strain did not emerge in low-density cultures (5×10^5 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 mutants, 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^{10} 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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References

1. de Steenwinkel JEM, ten Kate MT, de Knegt GJ, Kremer K, Aarnoutse RE, Boeree MJ, et al. Drug susceptibility of *Mycobacterium tuberculosis* Beijing genotype, association with MDR TB. Emerg Infect Dis. 2012;4:660–3.
2. David, HL. Probability distribution of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*. Appl Microbiol. 1970;20:810–4.
3. Werngren J, Hoffner SE. Drug-susceptible *Mycobacterium tuberculosis* Beijing genotype does not develop mutation-conferred resistance to rifampin at an elevated rate. J Clin Microbiol. 2003;41:1520–4. http://dx.doi.org/10.1128/JCM.41.4.1520-1524.2003
4. Bergval I, Kwok B, Schuitema K, Kremer K, van Soolingen D, Klatser P, Anthony R. Pre-existing isoniazid resistance, but not the genotype of *Mycobacterium tuberculosis* drives rifampicin resistance codon preference in vitro. PLoS ONE. 2012;7:e29108. http://dx.doi.org/10.1371/journal.po ne.0029108
5. Gillespie SH. Evolution of drug resistance in *Mycobacterium tuberculosis*: clinical and molecular perspective. Antimicrob Agents Chemother. 2002;46:267–74. http://dx.doi.org/10.1128/AAC.46.2.267-274.2002

Address for correspondence: Jim Werngren, Unit of Highly Pathogenic Microorganisms, Dept of Preparedness, Swedish Institute for Communicable Disease Control, Nobels väg 18 S-17182, Solna, Stockholm S 17182, Sweden; email: jim.werngren@smi.se

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 $4–24 \times 10^{-8}$ 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