

target for the surveillance of plasmodial atovaquone/proguanil resistance has to be questioned.

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Drug Resistance and Fitness in *Mycobacterium tuberculosis* Infection

To the Editor—In a recent article, Burgos et al. [1] investigated the effect of drug resistance on the generation of secondary cases of tuberculosis, because previous studies have yielded contradictory data on the pathogenicity and transmission of drug-resistant strains of *Mycobacterium tuberculosis* [2]. On the basis of epidemiological data, those authors quantified the number of secondary cases generated by drug-resistant versus drug-susceptible strains, to calculate the relative secondary case-rate ratio (SR). They concluded that, in the context of an effective tuberculosis control program, strains that were resistant to isoniazid either alone or in com-

bination with other drugs were less likely to result in secondary cases than were drug-susceptible strains. However, there were large differences in SRs for resistance to different drugs, such as a decrease in SR for isoniazid resistance (SR, 0.29), no effect on SR for streptomycin resistance (SR, 0.88), and an increase in SR for rifampicin resistance (SR, 2.33). Unless there are convincing reasons provided to explain these differences, these findings might indicate that the parameters of the study and the methods of analyses were not very robust. It is particularly difficult to explain why rifampicin resistance should increase the number of secondary cases. A priori, there is no reason why drug resistance should increase the SR, unless one assumes general factors, such as prolonged transmission due to ineffective drug treatment. This should, however, affect the drugs equally, if standard treatment procedures are used.

Experimental data and mathematical models have suggested that the reduction of bacterial fitness (i.e., reduced transmission between hosts and reduced persistence and growth within hosts) imposed by antimicrobial resistance could influence the frequency of drug-resistant microorganisms in a population [3, 4]. In this respect, we refer to a very eloquent article by G. Canetti [5]. Canetti addressed the question of resistance-related fitness costs by studying, at a phenotypic level, the resistance observed in primary drug-resistant strains versus those with acquired drug resistance (primary resistance is defined as infection with a resistant strain; acquired resistance is defined as drug resistance that emerges during chemotherapy). In contrast to acquired resistance, primary resistance reflects additional parameters, such as fitness and transmission. Canetti found that, for isoniazid but not for streptomycin, the proportion of high-level drug resistance was considerably lower for strains with primary resistance than for strains with acquired resistance. This seemed to indicate that, in contrast

to streptomycin, high-level isoniazid resistance is associated with a defined fitness cost.

The results of epidemiological investigations are complemented by studies that have examined the mechanisms of drug resistance at a molecular level, particularly those that have addressed the question of fitness cost related to the acquisition of a resistance determinant. Although corresponding studies in mycobacteria have been scarce [6–10], some of them have provided interesting insights, such as those that relate to resistance to streptomycin and to isoniazid.

The fitness cost of various chromosomal mutations was experimentally determined and was found to be dependent on the chromosomal alteration that mediates resistance to streptomycin [9]. Drug-resistant mutants obtained by in vitro selection in the laboratory are characterized by a variety of different possible resistance mutations. In contrast, streptomycin resistance in clinical *M. tuberculosis* isolates nearly invariably is associated with the lysine→arginine alteration at amino acid 42 of rpsL [7]. Interestingly, the lysine→arginine alteration at amino acid 42 of rpsL is the only streptomycin resistance determinant among the mutational alterations studied that was found to not carry a fitness cost, as determined experimentally in vitro [9]. These basic molecular studies provide a mechanistic explanation for the pioneering epidemiological observations by Canetti.

Most of the many chromosomal alterations that result in resistance to isoniazid are associated with a significant fitness cost [6, 8], although the serine→threonine resistance mutation at amino acid 315 of KatG was found to not affect in vivo growth in an experimental animal model [8]. In accordance with the early findings of Canetti [5] and the results reported by Burgos et al. [1], it was found that isoniazid-resistant strains in general were significantly less clustered than were isoniazid-susceptible strains [11]. However,

more-refine analysis of the epidemiological data revealed that aa-315 isoniazid-resistant mutants led to secondary cases of tuberculosis as often as drug-susceptible strains [12].

A picture emerges in which, among various resistance mutations that appear with similar rates, those associated with the least fitness cost are most likely to become selected in the population. To allow the implementation of rational strategies to combat the problem of drug-resistant tuberculosis, we need an integrated view that combines epidemiology, molecular mechanisms of resistance, and a relevant experimental determination of how drug resistance affects the entire life cycle of *M. tuberculosis* (the establishment of infection, progression to disease, and transmission).

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Reply to Böttger et al.

To the Editor—We appreciate the comments of Böttger et al. [1] concerning our recently published article [2]. In our analysis, we estimated the number of secondary cases that arose from all drug-susceptible and drug-resistant cases by assuming that strains were transmitted to other patients if the drug-susceptibility test results and genotype patterns were identical. We calculated the number of secondary cases for each drug-resistance pattern and determined the secondary-case rate ratio (SR). Böttger et al. questioned the large differences in SRs, especially the increased SR for rifampin-resistant cases. The most likely explanation for the high SR observed among rifampin-resistant cases was the increased incidence of HIV infection among patients with this resistance pattern. We found that the secondary cases caused by rifampin-resistant strains occurred mainly among patients with HIV infection; 77.8% (7/9) of the secondary cases with rifampin resistance occurred in HIV-positive individuals [1]. In addition, almost every case

of rifampin-resistant tuberculosis in San Francisco was in an HIV-infected individual. Therefore, it is difficult to draw conclusions about the effect of rifampin resistance on bacterial fitness. The heterogeneity of SR values found among persons with infections resistant to isoniazid and to streptomycin in our study are in agreement with the literature cited by Böttger et al., so it is not clear why the authors are puzzled by these findings. We concur with Böttger et al. that the application of epidemiological methodologies, in conjunction with molecular and genomic tools, is needed to achieve a more refined understanding of the biology of drug-resistant tuberculosis in its natural population.

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