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

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References

1. Meshnick SR, Trumpower B. Multiple cytochrome b mutations may cause atovaquone resistance [letter]. J Infect Dis **2005**; 191:822 (in this issue).
2. Wichmann O, Muhlberger N, Jelinek T, et al. Screening for mutations related to atovaquone/proguanil resistance in treatment failures and other imported isolates of *Plasmodium falciparum* in Europe. J Infect Dis **2004**; 190:1541–6.
3. Gil JP, Nogueira F, Stromberg-Norklit J, et al. Detection of atovaquone and malarone resistance conferring mutations in *Plasmodium falciparum* cytochrome b gene *(cytb)*. Mol Cell Probes **2003**; 17:85–9.
4. Korsinczky M, Chen N, Kotecka B, Saul A, Rieckmann K, Cheng Q. Mutations in *Plasmodium falciparum* cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob Agents Chemother **2000**; 44:2100–8.

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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 quantifie 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 combination 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 finding might indicate that the parameters of the study and the methods of analyses were not very robust. It is particularly diffi cult 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 fitnes (i.e., reduced transmission between hosts and reduced persistence and growth within hosts) imposed by antimicrobial resistance could influ ence 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 fitnes costs by studying, at a phenotypic level, the resistance observed in primary drug-resistant strains versus those with acquired drug resistance (primary resistance is define as infection with a resistant strain; acquired resistance is define as drug resistance that emerges during chemotherapy). In contrast to acquired resistance, primary resistance reflect additional parameters, such as fitnes 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 define fit ness 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 fitnes 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 fitnes 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 fitnes 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 significan fitnes 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 find ings of Canetti [5] and the results reported by Burgos et al. [1], it was found that isoniazid-resistant strains in general were significantl 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 fitnes 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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References

1. Burgos M, DeRiemer K, Small PM, Hopewell PC, Daley CL. Effect of drug resistance on the generation of secondary cases of tuberculosis. J Infect Dis **2003**; 188:1878–85.
2. Cohen T, Sommers B, Murray M. The effect of drug resistance on the fitnes of *Mycobacterium tuberculosis.* Lancet Infect Dis **2003**; 3: 13–21.
3. Andersson DI, Levin BR. The biological cost of antibiotic resistance. Curr Opin Microbiol **1999**; 2:289–93.
4. Levin BR. Models for the spread of resistant pathogens. Neth J Med **2002**; 60(Suppl 7): S58–66.
5. Canetti G. Present aspects of bacterial resistance in tuberculosis. Am Rev Respir Dis **1965**; 92:687–701.
6. Cohn M, Kovitz C, Oda U. Studies on isoniazid and tubercle bacilli: the growth requirements, catalase activities, and pathogenic properties of isoniazid resistant mutants. Am Rev Tuberc **1954**; 70:641–64.
7. Böttger EC, Springer B, Pletschette M, Sander P. Fitness of antibiotic-resistant microorganisms and compensatory mutations. Nat Med **1998**; 4:1343–4.
8. Pym AS, Saint-Joanis B, Cole ST. Effect of katG mutations on the virulence of *Mycobacterium tuberculosis* and the implication for transmission in humans. Infect Immun **2002**; 70:4955–60.
9. Sander P, Springer B, Prammananan T, et al. Fitness cost of chromosomal drug resistance–conferring mutations. Antimicrob Agents Chemother **2002**; 46:1204–11.
10. Mariam DH, Mengistu Y, Hoffner SE, Andersson DI. Effect of rpoB mutations conferring rifampicin resistance on fitnes of *Mycobacterium tuberculosis.* Antimicrob Agents Chemother **2004**; 48:1289–94.
11. van Soolingen D, Borgdorff MW, de Haas PE, et al. Molecular epidemiology of tuberculosis in the Netherlands: a nationwide study from 1993 through 1997. J Infect Dis **1999**; 180:726–36.
12. van Soolingen D, de Haas PE, van Doorn HR, Kuijper E, Rinder H, Borgdorff MW. Mutations at amino acid position 315 of the *katG* gene are associated with high-level resistance to isoniazid, other drug resistance, and successful transmission of *Mycobacterium tuberculosis* in The Netherlands. J Infect Dis **2000**; 182:1788–90.

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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 difficul 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 refine understanding of the biology of drug-resistant tuberculosis in its natural population.

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References

1. Böttger EC, Pletschette M, Anderson D. Drug resistance and fitnes in *Mycobacterium tuberculosis* [letter]. J Infect Dis **2005**; 191:823–4 (in this issue).
2. Burgos M, DeRiemer K, Small PM, Hopewell PC, Daley CL. Effect of drug resistance on the generation of secondary cases of tuberculosis. J Infect Dis **2003**; 188:1878–85.

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