Phillips and Lampe [1]. Given the rapid changes in HAART over the past decade—including marked improvements in potency, tolerability, and dosing frequency, it is doubtful that we will ever have an observational cohort in which patients receive the same therapy for 10 years. New therapies are certain to emerge that will be adopted, and the details of the research questions will change over time.

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Multiple Cytochrome b Mutations May Cause Atovaquone Resistance

To The Editor—Wichmann et al. [1] recently demonstrated, in a cross-sectional study of 504 cases of malaria imported to western and central Europe, that single-nucleotide polymorphisms in the cytochrome b codon 268 were not common and did not predict atovaquone resistance in Plasmodium falciparum. Although that study clearly demonstrated that codon 268, by itself, does not account for all atovaquone resistance, we would like to caution readers to not interpret the results to suggest that cytochrome b mutations are not useful molecular markers.

The poor correlation between the codon 268 mutation and atovaquone resistance is not surprising; in a 1996 review [2], mutations in almost 40 different cytochrome b loci were associated with resistance to inhibitors. Numerous different mutations have also been found in Pneumocystis jirovecii cytochrome b, probably because atovaquone is also used clinically as prophylaxis against P. jirovecii pneumonia (PCP). In 2 published studies of patients with PCP who had been exposed to atovaquone, 7 different nonsynonymous mutations in the Qo site were found, only 2 of which were seen in >1 patient [3, 4]. Cytochrome b is encoded in the mitochondrial genome, where replication has a 10-fold higher error rate [5]. The resulting high rate of mutation might explain not only the multiplicity of mutations but also why atovaquone resistance occurs readily when it is administered as a single agent [6].

Thus, specific point mutations in cytochrome b are unlikely to be useful in surveillance for atovaquone resistance. Methods that can pick up multiple mutations—such as high-throughput sequencing, single-strand conformation polymorphisms, and heteroduplex tracking assays—may be more appropriate.

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References


Reply to Meshnick and Trumpower

To the Editor—We thank Meshnick and Trumpower [1] for their comments regarding the usefulness of cytochrome b mutations as molecular markers for the resistance of Plasmodium falciparum to atovaquone. We support their point that further testing and sequencing of the cytochrome b gene can provide results with high sensitivity. This is exactly what was done in our study [2], as is shown in the Results section and later in the article. Isolates from patients who had therapeutic failure with atovaquone/proguanil treatment were sequenced at the cytochrome b gene, to detect mutations other than the one used for screening purposes. A 716-bp fragment of the cytochrome b gene that contains the encoding region of the putative atovaquone-binding domain was amplified [3]. Sequencing revealed the absence of all mutations previously described as being involved in atovaquone resistance in vivo and in vitro [4], except the mutation at codon 268 in 1 of the samples [2].

Still, only mutations at codon 268 have been associated with drug resistance in P. falciparum field samples [2]. This is why we chose the detection of this mutation as a screening method for all samples. However, as was indicated by our results, the usefulness of codon 268 as the only...
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 quantify 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 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 reflect 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,