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Letters to the Editor

First Case of Emergence of Atovaquone-Proguanil Resistance in *Plasmodium falciparum* during Treatment in a Traveler in Comoros▽

Atovaquone-proguanil (Malarone; GlaxoSmithKline) is now commonly used for the treatment and prophylaxis of falciparum malaria in France. We report here a treatment failure of atovaquone-proguanil in a patient who was infected during a 33-day visit without antimalarial prophylaxis in Comoros.

The patient presented with fever 10 days after the end of his trip, and a diagnosis of falciparum malaria was made. Treatment with atovaquone-proguanil was well tolerated. Isolated fever in association with falciparum parasites appeared 23 days after therapy. The patient was successfully treated with quinine.

In vitro susceptibility tests performed on blood samples from day 0 and day 23 showed a 50% inhibitory concentration (IC_{50}) value for atovaquone that was more than 100-fold greater on day 23 than on day 0 (Table 1). In addition, the IC_{50} for cycloguanil was increased by 18-fold.

Sequencing of the *cyt b* gene, encoding the atovaquone target (12), showed a wild-type *P. falciparum* strain on day 0 and a Y268S mutation on day 23.

Genotyping of the *dhfr* gene, encoding the proguanil target (5), showed a double mutation, C59R and S108N, on day 0, while a triple mutation, N51I, C59R, and S108N, was observed on day 23. However, proguanil likely does not act by itself in atovaquone-proguanil treatment but only facilitates the atovaquone activity (11).

Genotyping of the Pf*crt* gene (wild type, K76), encoding a transport protein involved in chloroquine resistance, and of the *dhps* gene (wild type, S436, A437, K540, A581, and A613), encoding the sulfadoxine target (5), showed wild-type, identical alleles.

The genotyping of the two isolates, using three of six microsatellite loci (7A11, Pf2802, C4M79, Pf2689, TRAP, and C4M69) (1), *msp1*, and *msp2* (5), showed differences between days 0 and 23 (Table 1).

The day 23 parasites presented a high IC_{50} for atovaquone associated with a Y268S mutation in *Cyt b*. Since 2002, fewer than 20 cases of genetically confirmed clinical resistance to atovaquone-proguanil had been reported (2–4, 6, 7, 9, 13, 14). Clinical failures were associated with in vitro-increased IC_{50}s for atovaquone between day 0 and the failure day only in five isolates (4, 7, 9). In some cases, the increased IC_{50} was moderate (7, 8). An in vitro atovaquone threshold of 1,900 nM was recommended to discriminate resistant isolates (10). Considering our results, this cutoff must be adjusted to >350 nM.

We were unable to detect *Cyt b* mutations on codon 268 and high IC_{50} to atovaquone in the pretreatment isolate. Reinfection was excluded because the patient was treated after returning to France. The atovaquone-resistant strain was probably present in the initial isolate but in the minority, making it undetectable by classical genotyping methods and in vitro testing. The isolate was polyclonal on day 0 and monoclonal on day 23.

This is the first observation of the clinical failure of atovaquone-proguanil treatment of *P. falciparum* infection in a traveler in Comoros, an area where the in vitro prevalences of isolates with reduced susceptibilities to classical antimalarial drugs were <7% (12).

Although clinical failures of atovaquone-proguanil therapy remain rare in travelers, an increased vigilance is required during their treatment followup, and surveillance of the parasite population should be reinforced as well.

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TABLE 1. In vitro drug susceptibility profiles and changes in genotyping profiles of the day 0 and day 23 *P. falciparum* parasites[a]

Sample	Parasitemia (%)	IC_{50} (nM) for:								*P. falciparum* genotyping result for gene or microsatellite locus				
										Allele group(s) of gene		Length (bp) of locus		
		CQ	QN	MQ	MDAQ	LMF	CYC	PYR	ATV	*msp1*	*msp2*	7A11	C4M79	C4M69
Day 0	0.5	32	206	25.3	37.8	14.6	14	241	2.9	K1, Mad 20	3D7, FC27	124	203	393
Day 23	1.3	62	652	34.1	30.5	15.2	250	2,512	390.0	K1	3D7	94	196	336

[a] Drug assays were performed at 1.5% hematocrit over a 60-h culture period using a [^{3}H]hypoxanthine incorporation microtest (12). Each isolate was tested once in triplicate against serial dilutions of antimalarial drugs over the following concentration ranges: 5 to 3,200 nM for chloroquine disphosphate (CQ; Sigma, Saint Louis, MO) and quinine hydrochloride (QN; Sigma), 3.2 to 400 nM for mefloquine (MQ; Hoffman-LaRoche, Bale, Switzerland), 1.56 to 1,000 nM for monodesethylamodiaquine (MDAQ; World Health Organization, Geneva, Switzerland), 0.5 to 310 nM for lumefantrine (LMF; World Health Organization), 10 to 20,000 nM for cycloguanil (CYC; Zeneca Pharma, Reims, France), 50 to 40,000 for pyrimethamine (PYR; Sigma), and 0.3 to 12,480 for atovaquone (ATV; GlaxoSmithKline).

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