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Antimalarial Drug Resistance of *Plasmodium falciparum* in northwest Madagascar

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Research Goal: To implement a sustainable system of testing for the emergence of drug-resistant *P. falciparum* parasites in northwest Madagascar.

BACKGROUND

Malaria parasites, notably *Plasmodium falciparum,* are among the top five reported causes of mortality in Madagascar[1]. Following widespread parasite resistance to chloroquine in the 1970s, artemisinin-based combination therapies were adopted as first-line treatment worldwide[2]. However artemisinin efficacy is now threatened by emerging resistance, and monitoring is crucial to a unified global effort to monitor resistance patterns and inform drug policy.

DESCRIPTION OF ORGANIZATION

This collaboration with the Institut Pasteur de Madagascar (IPM) was initiated to test for the emergence of drug-resistant *P. falciparum* parasites in northwest Madagascar. The Parasitology Unit at IPM is a key leader in national malaria surveillance and directly informs the Ministry of Public Health in Madagascar.

Capacity strengthening of the Parasitology Unit was a major focus, with the aim of creating a sustainable system of collecting *Plasmodium* isolates, culture-adapting parasite lines, and amplifying the genes of *pfmdr1, pfk13,* and most importantly *pfcrt* for which resistance to piperaquine is mediated via point mutations.

TABLES

	chloroquine resistance transporter (*pfcrt*)					multidrug resistance protein (*pfmdr1*)	
	p3805/p3806	p7950/p7949				p7820/p7821	
Sample number	Q271E	N326S/D	M343L	C350R-G353V-I356T/L	R371I	N86Y	Y184F
C008_J3	WT	X	WT	WT	WT	WT	Y184F
C009_J4	WT	X	WT	WT	WT	WT	Y184F
C010_J4	WT	X	WT	WT	WT	WT	Y184F
C011_J4	WT	X	WT	WT	WT	WT	Y184F
C012_J3	WT	X	WT	WT	WT	WT	X
C013_J3	WT	X	WT	WT	WT	WT	Y184F
C014_J2	WT	X	WT	WT	WT	N86Y	Y184F
C016_J8	WT	X	WT	WT	WT	X	X
C018_J4	WT	X	WT	WT	WT	X	X
C019_J2	WT	X	WT	WT	WT	X	X
C020_J1	WT	X	WT	WT	WT	X	X

Table 1. Sequencing results at *pfcrt* codons 271, 326, 343, 350/353/356, 371, and *pfmdr1* codons 86 and 184. “X” indicates unreadable or unavailable data.

Parasitology Unit, Institut Pasteur de Madagascar

METHODS

Plasmodium sp isolates were collected at a primary health center in Antanimbary, Madagascar (284 km northwest of Antananarivo). Venous blood samples arrived in Antananarivo within 24 hours and the species of *Plasmodium* infection was determined by microscopy. Samples presenting singularly with *P. falciparum* and with a parasitemia > 0.2% were placed into culture. Following RNA extraction and cDNA synthesis, reverse-transcriptase PCR was performed to amplify the drug resistance-associated genes of *pfcrt, pfmdr1, and pfk13*. Samples were sent to GenoScreen in France for sequencing and analyzed using DNASTAR.

Data show point mutations in *pfmdr1* codon 86 (asparagine to tyrosine) and codon 184 (tyrosine to phenylalanine). No mutations were found in *pfcrt* isolates. *Kelch-13* sequences did not capture the codons of interest (539 and 580).

DISCUSSION

Results show a high prevalence of *pfmdr1* mutant *P. falciparum* (55% of isolates) in northwest Madagascar. Technical progress must be made to monitor parasite susceptibility to the first-line combinations of artemisinin + amodiaquine, and importantly artemether + lumefantrine, to which resistance is mediated via point mutations in *pfmdr1*. The absence of *pfcrt* mutant strains warrants further investigation, as it contrasts with reported chloroquine treatment failures in Madagascar for which *pfcrt* mutations are considered the key determinant globally.[3-4]

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