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K13-Propeller Polymorphisms in *Plasmodium falciparum* Isolates from Patients in Mayotte in 2013 and 2014

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Plasmodium falciparum **isolates were collected from 29 malaria patients treated with artemether-lumefantrine in Mayotte in 2013 and 2014. Twenty-four cases (83%) consisted of imported malaria. Seventeen percent of the isolates presented mutations in one of the six K13-propeller blades (N490H, F495L, N554H/K, and E596G). A total of 23.8% of the isolates from the Union of Comoros showed K13-propeller polymorphisms. Three of the 18 isolates (16.7%) from Grande Comore showed polymorphisms (N490H, N554K, and E596G).**

Mayotte is a French island located in the Indian Ocean in the Comoros archipelago. *Plasmodium falciparum* malaria was a major public health problem in Mayotte until 2010, with 400 to 800 cases reported each year from 2003 to 2010 for about 180,000 inhabitants (1, 2). The number of reported malaria cases decreased to 99 in 2011, 74 in 2012, 80 in 2013, and 15 in 2014 (3). Legal and illegal immigration are important, and 40% of inhabitants are of foreign origin. Although the number of imported cases also decreased from 2011 to 2014, the rates of imported cases increased and represented 52.5% in 2011, 63.5% in 2012, 87.5% in 2013, and 86.7% in 2014. From 2007 to 2014, most of the imported cases came from Comoros (94%). The main island of Grande Comore accounted for 74% of the overall cases, with an increase from 69% in 2007 to 84% in 2013, whereas 16% of the cases were imported from Anjouan and 2%, from Moheli (3). A minority of the cases was imported from Madagascar (4%), Africa (1%), or other countries (3). The reduction in the prevalence of malaria in Mayotte (including autochthonous transmission and imported cases) can be explained by the anti-malaria campaigns—both logistically (involving long-lasting insecticide-treated bednets, rapid detection tests, and artemisinin-based combination therapy use) and politically (involving interisland cooperation, involvement of a national program, and participation of the private sector—conducted in Mayotte and the Comoros archipelago in recent years. From 2007 to 2009, indoor residual spraying was systematically proposed in all of the houses throughout Mayotte, with at least one treatment per year. Since 2010, indoor residual spraying has been progressively substituted by the distribution of long-lasting insecticidal nets (LLINs). In 2011, a mass distribution of LLINs was implemented in Mayotte. Currently, 60,000 LLINs have been distributed, and only seven villages have not been equipped. The health insurance system has evolved since 2005, but dispensaries are still free of charge in Mayotte, so most of the population can consult a health practitioner without having to pay. The detection of malaria by rapid diagnostic tests is mandatory for any consultant with a fever over 38.5°C. A 3-day regimen of artemisinin-based combination therapy (ACT) is used as first-line treatment for mild or uncomplicated malaria. Severe cases and pregnant women are hospitalized and treated with quinine. All of these actions have resulted in the passage of Mayotte in the *P. falciparum* elimination phase.

In 2007, 95% of the foreigners living in Mayotte were Comorian people. The government of the Union of Comoros introduced artemether-lumefantrine as first-line therapy for uncomplicated *P. falciparum* malaria and officially withdrew the use of chloroquine and sulfadoxine-pyrimethamine in 2004 (4, 5). Unfortunately, the ACT regimen did not lead to a decrease of malaria incidence, with approximately 100,000 annual malaria cases recorded from 2006 to 2012. To eliminate malaria transmission in this area of endemicity, mass drug administration with a therapeutic dose of artemisinin-piperaquine and a low dose of primaquine was launched in 2007 on Moheli and in 2013 on the island of Grande Comore. In parallel, a mass distribution of LLINs was implemented on Grande Comore, Moheli, and Anjouan in 2012 (6). Consequently, the annual malaria cases dramatically decreased from 114,537 in 2007 to 2,142 in 2014.

Twenty-nine *P. falciparum* isolates were obtained from patients diagnosed with malaria in the Hospital Center of Mayotte in 2013 and 2014 (Table 1). Venous blood samples were collected in Vacutainer ACD tubes (Becton Dickinson, Rutherford, NJ, USA) prior to patient treatment. Blood samples

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TABLE 1 Polymorphisms observed in the *K13* gene in 29 *P. falciparum* isolates collected in Mayotte in 2013 and 2014[a]

Sex	Area of collection in Mayotte	Country of infection	Parasitemia		Polymorphism			
			At D0[b] (%)	Control (%)	N490H	F495L	N554H/K	E596G
F	Mamoudzou	Grande Comore	0.02[c]		H[d]	F	N	E
F	Mamoudzou	Grande Comore	0.02		N	F	K[d]	E
F	Mtsangamouji	Grande Comore	0.18		N	F	N	G[d]
M	Mtsangamboua	Grande Comore	0.05		N	F	N	E
M	Barakani	Grande Comore	3	0 (D5)	N	F	N	E
M	Mtzamboro	Grande Comore	0.7		N	F	N	E
M	Kaweni	Grande Comore	0.8		N	F	N	E
M	Pamandzi	Grande Comore	0.03	0 (D13)	N	F	N	E
F	Mtsapere	Grande Comore	0.3		N	F	N	E
F	Dzoumogne	Grande Comore	1		N	F	N	E
M	Mrouale	Grande Comore	0.5		N	F	N	E
M	Mrouale	Grande Comore	0.2		N	F	N	E
M	Kaweni	Grande Comore	0.01		N	F	N	E
M	Kaweni	Grande Comore	0.01		N	F	N	E
F	Kaweni	Grande Comore	10	0.001 (D2)	N	F	N	E
M	Mamoudzou	Grande Comore	0.68		N	F	N	E
M	Pamandzi	Grande Comore	0.09		N	F	N	E
M	Mamoudzou	Grande Comore	2.5	0.001 (D3)	N	F	N	E
F	Mtsapere	Anjouan	1		N	F	H[d]	E
M	Mtsapere	Anjouan	0.01[c]		N	F	N	E
M	Dzoumogne	Mohéli	0.33		N	F	N	E
F	Pamandzi	Madagascar	0.63		N	F	N	E
F	Sada	Madagascar	0.01		N	F	N	E
F	Tsingoni	Madagascar	0.01		N	F	N	E
M	Chirongui	Mayotte[e]	4.7		N	L[d]	N	E
M	Mamoudzou	Mayotte[e]	0.12		N	F	N	E
M	Dembeni	Mayotte[e]	0.13		N	F	N	E
F	Pamandzi	Mayotte[e]	0.04	0 (D4)	N	F	N	E
F	Mamoudzou	Mayotte[e]	0.09	0 (D3)	N	F	N	E

[a] All data are relative to the PF3D7_1343700 reference coding sequence.
[b] D, day.
[c] Mixed isolate (*P. falciparum* and *Plasmodium malariae*).
[d] Identified mutation.
[e] Location of origin questionable.

were absorbed onto a Whatman FTA elute absorbent filter (Whatman, Inc., Florham Park, NJ, USA). The filter papers were sent to the Institute of Biomedical Research of the French Army (IRBA) in Marseille. Informed consent was not required for this study, because the sampling procedures and testing are part of the French national recommendations for the care and surveillance of malaria. Twenty-four cases (83%) consisted of imported malaria; of these, 18 of the cases (62%) were imported from Grande Comore, 3 were from Madagascar, 2 were from Anjouan, and 1 was from Moheli. The remaining 5 cases were indeterminate (possible autochthonous cases).

In response to increasing chloroquine resistance, Mayotte switched in 2007 to artemether-lumefantrine as the first-line treatment for uncomplicated malaria. However, the emergence of *P. falciparum* resistance to artemisinin and its derivatives, as manifested in delayed parasite clearance following treatment with artesunate monotherapy or ACT, has developed in Southeast Asia (7–9). Recently, mutations in the propeller domain of the *Kelch 13* (*K13*) gene were associated with *in vitro* resistance to artemisinin and with delayed clearance after artemisinin treatment in Southeast Asia (9–12).

The aim of this study was to characterize the variability of the *K13* gene for the first time in Mayotte. K13-propeller gene amplification and sequencing were performed, as previously described (13). The *K13* mutations were confirmed three times.

The mutations associated with *in vitro* resistance in Southeast Asia, such as Y493H, R539T, I543T, and C580Y, were not observed (9–12), other than the M476I mutation obtained *in vitro* in a Tanzanian strain after artemisinin pressure (10). Seventeen percent of the isolates presented mutations in one of the six K13-propeller blades (N490H, F495L, N554H/K, and E596G) (Table 1). Three of these mutations lie adjacent to the mutation described in Southern Asia: N554H/K near the previously described P553L, which is associated with delayed parasite clearance following treatment with artesunate monotherapy in Asia (9), and, in one patient from Mali (14), N490H and F495L near the previously described Y493H, which is associated with delayed parasite clearance (9, 10, 12). A mutation in codon 554 has also been found in one isolate each from Kenya (15), Mali (16), and Senegal (unpublished data). The F495L mutation has been found in two isolates from the China-Myanmar border (17).

A total of 23.8% of the isolates from the Union of Comoros showed K13-propeller polymorphisms. Three of the 18 isolates (16.7%) from Grande Comore showed a polymorphism (N490H, N554K, and E596G). One of the two isolates from Anjouan was mutated (N554H), and the other of the two isolates was from the

five indeterminate cases (possible autochthonous cases). None of the three isolates from Madagascar was mutated for *K13*. No mutation was identified in 97 isolates from Antananarivo, Madagascar (18). Currently, there are no data on *K13* in Comoros. Most of the patients left the hospital or dispensary after the first dose of artemether-lumefantrine and did not return due to an unstable living situation and fear of police (i.e., illegal immigrants). Disease control was assessed between days 2 and 13 for six patients, and no polymorphism on the K13-propeller gene was observed. Early resistance to the artemether-lumefantrine combination therapy was not observed in these six samples, which presented no polymorphisms. We have no follow-up for the 18 patients with isolates that had wild-type *K13* or for the five patients with mutated *K13* after treatment by the artemether-lumefantrine combination. We can assume that these patients were successfully treated, but acquisition of black-market antimalarial drugs for further self-treatment, clinical failure after returning to their country of origin, or patient death may be outcomes of no follow-up as well.

Molecular surveys of the prevalence of *K13* mutant alleles in Africa suggest that the changes are at a very early stage there (19). *K13* was identified at low prevalence (<5%) in almost all African locations, with many having different *K13* mutant alleles from those described in Southeast Asia (19). The phenotypes of these four coding substitutions are unknown and will require further characterization to better describe the clinical impact of artemisinin resistance and to survey the emergence of artemisinin resistance in Mayotte and the three other islands of the Comoros archipelago. There is now an important need for local studies of clinical resistance to artemisinin and for *in vitro* and *ex vivo* ring stage assay data to clarify the significance of K13-propeller mutations as markers of artemisinin resistance in Mayotte, the Union of Comoros, and Madagascar.

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