Molecular Characterization of a Cluster of Imported Malaria Cases in Puerto Rico

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Abstract. The Caribbean island of Hispaniola is targeted for malaria elimination. Currently, this is the only island with ongoing transmission of malaria in the Caribbean. In 2015, six patients from Puerto Rico and one from Massachusetts, who traveled to Punta Cana, Dominican Republic, were confirmed to be infected with *Plasmodium falciparum*. Additional molecular analysis was performed at the Centers for Disease Control and Prevention to characterize the drug-resistant alleles and *Plasmodium* population genetic markers. All specimens carried wildtype genotypes for chloroquine, sulfadoxine-pyrimethamine, and artemisinin resistance genetic markers. A mutation in codon 184 (Y/F) of *Pfmdr-1* gene was observed in all samples and they shared an identical genetic lineage as determined by microsatellite analysis. This genetic profile was similar to one previously reported from Hispaniola suggesting that a clonal *P. falciparum* residual parasite population present in Punta Cana is the source population for these imported malaria cases.

In the Caribbean, Hispaniola (shared by the Dominican Republic and Haiti), is the only island where malaria remains endemic. Although Puerto Rico eliminated malaria in the mid-1950s, reintroduction remains a risk given the presence of competent vectors, such as *Anopheles albimanus*, as well as travel links to endemic areas, including Hispaniola.¹ For travelers this island, there are several chemoprophylaxis options including chloroquine (CQ), hydroxychloroquine, atovaquone/proguanil, doxycycline, and mefloquine; however, not all travelers take prophylaxis as a precaution.

Between 2000 and 2014, Puerto Rico reported 35 imported cases of malaria, three of which were imported from Hispaniola.² In July and August 2015, a cluster of 27 suspected imported cases of malaria was identified by the Puerto Rico Department of Health among travelers returning from Punta Cana, Dominican Republic. In September, two additional patients who traveled to Punta Cana were reported in Puerto Rico and Massachusetts. The patients in all 29 suspected cases did not use malaria chemoprophylaxis despite long-standing Centers for Disease Control and Prevention (CDC) recommendations. Samples from all patients were sent to the CDC for analysis.

Real-time polymerase chain reaction (PCR) confirmed a total of seven *Plasmodium falciparum* cases among the 29 specimens tested.² Further laboratory analysis was undertaken by CDC to characterize the genetic signatures of the *Plasmodium* sp. parasites present in these samples based on the drug-resistant profile and neutral microsatellite markers. Samples were genotyped by Sanger sequencing for *Pfcr*t (codons 72–76), *Pfdhfr* (codons 50, 51, 59, 108, and 164), *Pfdhps* (codons 436, 437, 540, 581, and 613), *Pfmdr-1* (86, 184, 1034, 1042, and 1246), and *PfK13* (propeller domain) using an Applied Biosystems 3130 capillary sequencer.^{3–5} *Pfmdr-1* copy number was determined by TaqMan real-time PCR (Stratagene MX3005P; Agilent Technologies, La Jolla, CA) using a previously described protocol.⁶ Additionally, seven

neutral microsatellites [MSTA1 (chromosome 6); MSPoly- α (chromosome 4); MSPfPK2 (chromosome 12); MSTA109 (chromosome 6); MS2490 (chromosome 10); MSC2M34 (chromosome 2); and MSC3M69 (chromosome 3)]^{7,8} were amplified by PCR and analyzed using the GeneMarker software v1.95. These molecular markers have been previously used to characterize clonal lineages in *P. falciparum* populations from South America, Central America, and Hispaniola.⁹

All samples exhibited an identical genetic profile for all drug-resistant markers tested (Table 1). These samples showed wildtype CQ-sensitive genotype CVMNK in codons 72–76. Similarly, all the isolates carried the sulfadoxine-pyrimethamine (SP)-sensitive ancestral wildtype alleles in *Pfdhfr* and *Pfdhps*, as well as the wildtype allele for the *PfK13* propeller domain, the latter indicating susceptibility to artemisinin (ART). In the *Pfmdr-1* gene, a mutation was identified in codon 184 (Y/F), and all samples had a single copy of this gene. The microsatellite data showed that all specimens demonstrated an identical microsatellite profile (A1) with the following alleles: MSTA1: 175, MSPoly: 151, MSPfPK2: 160, MSTA109: 176, MS2490: 80, MSC2M34: 217, and MSC3M69: 124. The investigation identified a *P. falciparum* strain with genetic signatures similar to those previously reported from samples originating from Hispaniola as shown by the Bayesian clustering algorithm used in Structure v2.3.4 and depicted in the phylogenetic tree constructed by Populations 1.2.31 and Mega6 (Figure 1). The A1 haplotype was similar to other samples present in 2010⁹; moreover, it belonged to the same cluster with an historic laboratory strain from the late 1970s. This observation suggests the strain found in all seven samples is genetically related to a parasite population that continues to circulate within Hispaniola.⁹ Importantly, all parasite isolates exhibited ART, CQ, and SP sensitive wild type genotypes consistent with historic data and indicating that the malaria parasites that continue to be transmitted throughout Hispaniola are sensitive to CQ. Additionally, the microsatellite profile found in these samples was different to the ones previously reported in countries from South America such as Peru,¹⁰ Colombia¹¹ and Guyana,⁵ suggesting a local and independent *P. falciparum* clonal population in

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TABLE 1
Drug-resistant genetic profile of the samples

Sample ID	PICRT					PFDHFR					PFDHPS					PIMDR1				
	C72S	V73	M74I	N75E	K76T	C50R	N51I	C59R	S108N	I164L	S436A	A437G	K540E	A581G	A613S/T	N86Y	Y184F	S1034C	N1042D	D1246Y
15-0116	C	V	M	N	K	C	N	C	S	I	S	A	K	A	A	N	F	S	N	D
15-0117	C	V	M	N	K	C	N	C	S	I	S	A	K	A	A	N	F	S	N	D
15-0160	C	V	M	N	K	C	N	C	S	I	S	A	K	A	A	N	F	S	N	D
15-0161	C	V	M	N	K	NA	NA	NA	NA	NA	S	A	K	A	A	N	F	S	N	D
15-0162	C	V	M	N	K	C	N	C	S	I	S	A	K	A	A	N	F	S	N	D
15-0223	C	V	M	N	K	C	N	C	S	I	S	A	K	A	A	N	F	S	N	D
15-113	C	V	M	N	K	C	N	C	S	I	S	A	K	A	A	N	F	S	N	D

NA = no amplification. Mutant alleles are indicated by bold letters.

Hispaniola. Recently, a binational agreement has been adopted between the Dominican Republic and Haiti to eliminate malaria by 2020.¹² The Dominican Republic has successfully reduced the number of malaria cases; nevertheless, the ongoing *Plasmodium* transmission in the island continues to represent a risk for travelers, a majority of which spend their vacations in Punta Cana. Although the efforts to eliminate malaria on the island of Hispaniola will be helpful in minimizing the risk of malaria transmission to travelers, it is important for public health authorities in the Dominican Republic to focus on eliminating ongoing residual malaria transmission in Punta Cana to prevent further transmission of malaria from this region. Finally, it is important for travelers to take steps to prevent the disease when traveling to malaria endemic destinations including use of mosquito avoidance measures and malaria chemoprophylaxis.

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FIGURE 1. Sample clustering using a neighbor-joining tree constructed with the neutral microsatellites of samples from Hispaniola using the Nei et al.'s (1983) DA distance. A1 (in red) represents the haplotype of the seven *Plasmodium falciparum* domestic cases reported in 2015. The clusters defined by structure are represented by the different colors.

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