SHORT REPORT: HIGH PREVALENCE OF MULTIDRUG-RESISTANT PLASMODIUM FALCIPARUM MALARIA IN THE FRENCH TERRITORY OF MAYOTTE

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Abstract. A drug-resistance survey was conducted in the French territory of Mayotte in the Comorian Islands in the Indian Ocean where malaria is endemic. A high prevalence of resistant Plasmodium falciparum parasites was observed, not only to chloroquine (88%) and pyrimethamine (99%), but more surprisingly to quinine (17%), mefloquine (9%), and amodiaquine (24%). This leaves few treatment alternatives other than artemisinine-mefloquine combinations. However, despite notification to French Health authorities three years ago, inadequate treatment (chloroquine plus sulfadoxine-pyrimethamine) is still used in this locality. Thus, people still die of malaria in this remote territory of France.

The island of Mayotte in the Comorian Islands in the Indian Ocean is in an unusual situation because from an epidemiologic and sociologic point of view, it is an African-like setting, yet it benefits from the French health system with diagnosis and treatment facilities that are of much higher standards than those in Africa. However, the population of Mayotte is of east African origin and lives in modest housing, including huts along rare roads in a forested landscape. In contrast, since 1975 when Mayotte decided to remain a French territory, a sophisticated health system has been implemented. This includes a modern, well-equipped central hospital, and numerous dispensaries run by doctors who deliver free medical care. This, along with other factors, has contributed to massive immigration from other Comorian islands.

Malaria has always been prevalent in this region and has not been decreased by vector control. Until 2001, treatment relied on classic schemes, i.e., chloroquine for acute uncomplicated cases and parenteral quinine together with adequate nursing for complicated cases. The increasing number of malaria fatalities, particularly among cases properly handled in the central hospital, led us to initiate a drug sensitivity survey to determine why many could still die of malaria in France in 2001, even though this is a remote territory of France. The results showed an unexpectedly high prevalence of resistance to many available antimalarial drugs.

The study was conducted from May 2000 to February 2001 among the patients from the different health centers of the island and those admitted to Mamoudzou Hospital. It was reviewed and approved by the health regional authority, and samples were obtained from patients after informed consent was provided. All patients had a Plasmodium falciparum–positive histidine-rich protein 2 (Cape Biotech Malaria Rapid Test; Abbott Diagnostic, Rungis, France) or parasite lactate dehydrogenase (LDH) test (Optimal; Diamed, Paris, France) result, which was verified by microscopic examination of thin and thick blood smears. A venous blood sample was taken either at the hospital or in dispensaries and kept at 4°C for a maximum of four hours before performing an in vitro culture. Drug sensitivity assays were determined for chloroquine sulfate, quinine hydrochloride, amodiaquine hydrochloride, mefloquine hydrochloride, pyrimethamine, and artemisinin. The conditions for in vitro culture of isolates in 96-well plates are the same as those previously described.1 Antimalarial drugs solutions were prepared for each assay. Drug sensitivity was determined using the colorimetric double-site enzyme-linked LDH immunodetection (DELI) test.2 The correspondence between results obtained with the DELI assay and the classic isotopic microtest has been previously established using laboratory-adapted strains2 and field isolates in Senegal,3 Burkina Faso,4 and Asia (Brasseur P, Druihle P, unpublished data). Two strains of P. falciparum (3D7 and Palo Alto) were used as internal controls.

The in vitro drug sensitivities of 132 isolates that were successfully cultured are shown in Figure 1. We observed an extremely high prevalence (88%) and an extremely high degree of resistance to chloroquine, which is consistent with the clinical failures reported by rural dispensaries and the increase in the number of cases referred to the hospital. The results also showed a substantial number (17%) of isolates resistant to quinine with high IC50 values (> 500 nM), which may explain the fatality rate recently observed in the hospital despite rapid infusions of quinine and adequate intensive care. Most likely as a consequence of quinine resistance, 9% of the isolates were found resistant to mefloquine, despite the rare use of this drug. This finding is consistent with previous observations of cross-resistance between quinine and mefloquine.

Despite the absence of use of sulfadoxine-pyrimethamine, all but one of the strains tested were resistant to pyrimethamine (IC50 > 2,000 nM). This is most likely due to numerous treatments with other antifolates for microbial infections (e.g., cotrimoxazole). In contrast with many chloroquine-resistant areas where amodiaquine is a suitable alternative to chloroquine as a first-line treatment,6 the prevalence of resistance to this drug is 24%. Therefore, amodiaquine cannot be recommended as an alternative to chloroquine for treatment of a disease that can be fatal within 24–48 hours.

Finally, as in many other areas where artemisinin derivatives have not yet been used, a number of isolates showed IC50 values greater than the threshold of 10 nM and 20% showed values of 5–10 nM. Although resistance to artemisinin derivatives remains debatable, it is known that even seven-day treatments have failure rates of 5–7%. This is consistent with in vitro findings. No significant difference in susceptibility was found between patients from rural areas, who constituted the majority (82%) of the study population, and those from urban areas. In addition, resistance to pyrimethamine was investigated in 40 additional parasite isolates with a nested polymerase chain reaction and restriction fragment length polymorphism as reported by Eldin de Pécoulas and...
FIGURE 1. Distribution of the 50% inhibitory concentrations (IC$_{50}$s) of the antimalarial drugs studied with 132 isolates of *Plasmodium falciparum* from Myotte in the Comorian Islands. Results for pyrimethamine are not shown because all isolates were resistant and only one showed borderline resistance. CQ = chloroquine; QN = quinine; AQ = amodiaquine; MF = mefloquine; AT = artemisinin. The shaded areas correspond to the threshold values between susceptibility and resistance. The values at the bottom of the figure are the geometric (Geo) mean ICSO concentration for each drug and the 95% confidence interval (CI).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Geo Mean</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>CQ</td>
<td>282.6</td>
<td>281-283</td>
</tr>
<tr>
<td>QN</td>
<td>229</td>
<td>227-230</td>
</tr>
<tr>
<td>AQ</td>
<td>36.3</td>
<td>35-37</td>
</tr>
<tr>
<td>MF</td>
<td>3.9</td>
<td>2.7-5.2</td>
</tr>
<tr>
<td>AT</td>
<td>1.9</td>
<td>0.6-3.2</td>
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others. This showed a point mutation in codon 108 (Ser (AGC) to Asn (AAC)) in the dihydrofolate reductase gene in 62% of the isolates (other mutations were not investigated).

Our results are puzzling and in marked contrast with those obtained in neighboring areas such as Madagascar, where results over the past 10 years have showed a moderate but stable prevalence of resistance to chloroquine, together with a low prevalence of resistance to other drugs. Less numerous data are available from the neighboring islands of Anjouan and other Comorian islands. The last survey conducted in 1992 showed a chloroquine resistance level of 22.2% in seven-day in vivo assays. This situation suggests that the improved availability in Mayotte, compared with Africa, of antimalarials and other drugs through a free and well-organized health system, has contributed to the emergence of multidrug-resistant *P. falciparum* malaria.

Given this state of affairs, it is somewhat puzzling that it took so many years of treatment failures to initiate a preliminary evaluation of the actual status of susceptibility. Our results should have at least triggered further in vitro and in vivo investigations, which are known to be complementary. It is shocking that given the degree of health care in Mayotte new and more adequate regimens have not yet been implemented. Indeed, the current recommendation for treatment is a combination of chloroquine with sulfadoxine-pyrimethamine, which in view of our observations, would constitute the worst possible option. In addition, this combination therapy has not been the object of rigorous in vivo investigations to determine its efficacy. It has unfortunately remained mandatory despite reports of treatment failures and communication of the present data in January 2001 to the health authorities in Mayotte, as well as to the Direction Generale de la Sante in Paris, and repeated recommendations to use artesiminin-based combinations.

In view of the data in this report, therapeutic options are scarce and few alternatives are currently available, other than drug combinations such as quinine-tetracycline or artesunate-mefloquine. However, the former combination has shown insufficient compliance during ambulatory treatment in dispensaries. In our opinion, this suggests that the three-day artesunate-mefloquine combination promoted by investigators in Thailand is mandatory.

One could argue that eradication through vector control might be achievable in Mayotte, as on other small islands such as la Reunion, Martinique, and Guadeloupe. However, several years of vector control in Mayotte has failed to prevent the present increase in endemicity and many breeding sites are hardly accessible in the relatively large, wild, forested areas of Mayotte. In this regard, it is worth mentioning that the increased use of artesiminin derivatives in Southeast Asia indicates that they also exert a gametocytocidal effect in contrast to sulfadoxine-pyrimethamine, which tend to increase transmission. Therefore, in the case of Mayotte, artesiminin may not only be the most efficient therapeutic option, but would moreover constitute an outstanding opportunity to investigate its ability to control transmission and potentially eradicate malaria in a small, well-delineated territory, i.e., without borders with other endemic countries.

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