Measuring resistance to malaria

The paper by Randrianarivelojosia and colleagues in this issue of the *Journal* (p. 47) describes the *in vitro* susceptibility of *Plasmodium falciparum* in Madagascar and the Comoros Union to three of the commonly used antimalarial drugs in the region — quinine, mefloquine and cycloguanil (the active metabolite of proguanil). Severe malaria in the Comoros Union and in Madagascar is invariably caused by *P. falciparum*, as it is in the rest of sub-Saharan Africa. All of 243 isolates assessed were sensitive to quinine, the drug recommended throughout the region for treatment of severe malaria. With regard to the two chemoprophylactic agents studied, all 67 isolates assessed were sensitive to cycloguanil and only 1 of 128 isolates was mefloquine-resistant. The mefloquine-resistant isolate was 1 of 110 evaluated from Madagascar; none of the 18 isolates from the Comoros Union was resistant. The authors argue that their findings confirm the sensitivity of the parasite to the 3 drugs most commonly used in their countries for both treatment and prophylaxis. They submit, on the basis of their findings, that current policy for treatment of severe malaria with a 7-day course of quinine, and prophylaxis with either mefloquine or cycloguanil-based regimens, is justified by the *in vitro* laboratory findings that they have shown.

These conclusions are interesting and important as malaria morbidity and mortality is rising. This is principally a result of increasing antimalarial resistance, and the results seem reassuring.[1,2] Undoubtedly there are public health benefits to knowing the drug sensitivity profile of *P. falciparum* in malaria endemic areas and to applying such knowledge to guiding drug policy for malaria case management and prophylaxis of the disease. When one considers how antimicrobial sensitivity testing *in vitro* has guided antibiotic selection and policies for many other infectious diseases, it is logical and makes good sense that a similar approach should be employed for malaria. The public health benefits of a comprehensive profile being determined across the African continent of *P. falciparum* drug sensitivity and changes in resistance over time by a collaborating network of competent laboratories would be considerable. This, in short, is the importance of the Antananarivo programme.

However, since Randrianarivelojosia *et al*. derive several clear-cut recommendations for the treatment and prevention of *P. falciparum* malaria from their findings it is necessary to look critically at the justification of their claim and the reliability of their findings.

As there is little evidence to support the efficacy of proguanil as a single agent, proguanil is generally only recommended in combination with chloroquine (in chloroquine-sensitive areas) or atovaquone (in the fixed-dose combination Malarone). To conclude that recommendations for prophylaxis are justified, it is important to consider the efficacy of these widely used partner drugs, as well as that of cycloguanil. Resistance is thought to arise from spontaneous chromosomal point mutations or gene duplications, which are independent of drug selection pressure. *De novo* resistance is determined by the intrinsic frequency with which these point mutations occur, and the degree of resistance conferred by the change.[3] High-level atovaquone resistance develops by the selection of a single mutant cytochrome b gene, explaining why resistance develops so rapidly when atovaquone is used as monotherapy.[4] These more resistant parasites have a survival advantage in the presence of antimalarial drugs. Several factors encourage the spread of resistance. They include the proportion of transmissible malaria infections exposed to sub-therapeutic drug concentrations, the drug concentration profile (a long elimination phase favours resistance), the pattern of drug use, and the level of immunity in the community. Thus, resistance frequently develops first to the antimalarials most widely used in the treatment of uncomplicated malaria, particularly chloroquine and sulfadoxine-pyrimethamine. However, the treatment policy for uncomplicated malaria in the area is not addressed by this study. The authors refer to the presence of chloroquine resistance in the study area, particularly in the Comoros Union, which is not surprising given its prevalence across almost all of sub-Saharan Africa. The efficacy of proguanil (cycloguanil)-based regimens cannot be concluded from this study alone.

It is also necessary to consider, in general, how feasible it is to extrapolate *in vitro* results to clinical outcome. As resistance means that there is a shift to the right in the dose-response (concentration-effect) relationship, it is to be expected that this might be reliably quantified *in vitro*. *In vitro* studies are not influenced by partial immunity acquired after repeated *P. falciparum* malaria infections (which do influence the *in vivo* response), and *in vitro* findings would therefore be as relevant to non-immune travellers, as to semi-immune locals. However, extrapolation of *in vitro* results to clinical outcome is not straightforward, for several reasons. In the first place, the authors have set drug concentration levels for each drug, above which the parasite is regarded as resistant and below which it is seen as being sensitive. But these levels are in the main arbitrary, and not universally agreed upon or in accordance with those set by others. Moreover, the results are critically dependent on precise details of the laboratory method; even minor changes in methodology might significantly influence the result. Unless the methods used for *in vitro* drug sensitivity testing are standardised between laboratories, subjected to robust quality assurance and monitored accordingly, comparison between laboratories cannot be made and general inferences that influence policy and clinical

decisions may not be derived. There are, in addition, inescapable limitations to extrapolating *in vitro* findings to the clinical situation. *In vitro* testing does not allow for drug behaviour in the body — the absorption, metabolism, distribution and elimination characteristics that significantly affect the antimalarial action of drugs. Nor can it take into account the complex immune response that takes place in conjunction with the drug action, including cytokine production, acute phase reactions, and the contribution of the spleen to the response in acute malarial infection. These are particularly important determinants of quinine effectiveness as quinine binds to acute phase reactants, resulting in a decrease in free quinine levels with increasing disease severity. This is one of the reasons for using a loading dose of quinine in severe malaria.[5] Treatment failure from under-dosing and poor adherence, both widespread reasons for lack of antimalarial effectiveness, are not captured in either *in vitro* or *in vivo* studies.

For the findings from Antananarivo and the conclusions that have been derived from them to be persuasive, and for them to influence policy in their own countries and beyond, more is required than is reported in their paper. The drug concentrations that are set for defining resistance of *P. falciparum* would need to be defended against clinical evidence, and laboratory methodology, quality control and monitoring, all of which should be robustly applied. The efficacy of partner drugs used in combination regimens would also have to be reported, and the *in vitro* sensitivity of those drugs at greatest risk of developing resistance as a result of intrinsic mutations (such as atovaquone) or increased drug pressure (generally those antimalarials recommended for the treatment of uncomplicated malaria) should also be assessed. Every detail of the laboratory method would have to be standardised, within and between laboratories. If policy decisions are to be made from such findings the most important element is to show that sensitivity profiles are changing over time. More general agreement is needed on how *in vitro* laboratory findings might be used in deciding on policy change, and in comparing the situation between countries and regions. The World Health Organisation has developed guidelines for the conduct of *in vitro* drug sensitivity testing, and the use of the methodology for deciding policy,[6] that need to be followed for the findings reported in this issue of the *Journal* to have general application. The laboratory approach must be affordable for it to take root in Africa. And finally, it should be remembered that drug resistance of the parasite is not the only explanation for a failed response in malaria.

A mosquito whirs on only two wings. By setting up harmonic vibrations in the air and in its own thorax, it gets more flaps out of its wings than its nerves or muscles could sustain alone. (From The Insects*, Life Nature Library, 1964).*

If all these requirements can be met, and only if they can be met, the work of the Madagascar and Comoros Union scientists will come to be seen as an important foundational step in determining malaria drug policy on the African continent.

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