Detecting Drug Resistant Malaria and Tuberculosis in Africa

Highlighting achievements of Regional Technical Cooperation Project RAF/6/025

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This booklet was prepared as part of IAEA regional technical cooperation project RAF/6/025. The project was conducted by partners from Burkina Faso, Cameroon, Ethiopia, Ghana, Kenya, Madagascar, Mali, Nigeria, South Africa, Sudan, United Republic of Tanzania, Uganda and Zambia with support from IAEA staff and experts from Africa and Europe.

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Foreword

One of the most serious challenges confronting health authorities in developing and developed countries today is the increasing occurrence of tuberculosis (TB). The World Health Organization (WHO) estimates that 1.6 million deaths resulted from TB in 2005, with the highest number of deaths and the highest mortality per capita in the Africa region. In 2005, there were nearly 350 cases of TB per 100 000 population in Africa.[1] Malaria, another major communicable disease, is also of considerable concern, as it is the largest cause of mortality in Africa, particularly in children. About 90% of the world's malaria cases occur in Africa.

Project RAF/6/025, Detection of Drug Resistant Malaria and Tuberculosis, built on IAEA experience since 1997 in developing new diagnostic capabilities in the use of molecular and radionuclide techniques for the diagnosis of drug resistance in malaria and TB in African Member States. The main objective of the project was to develop innovative, effective strategies for strengthening regional capacity in new diagnostic tools for national disease control and surveillance programmes. Thirteen African countries participated in the project: Burkina Faso, Cameroon, Ethiopia, Ghana, Kenya, Madagascar, Mali, Nigeria, South Africa, Sudan, United Republic of Tanzania, Uganda and Zambia. The project began in 2001 and was completed in 2006.

This brochure summarizes the main findings of the project and provides a pooled analysis of results for all participating countries for malaria and TB. This analysis highlights interesting results relevant to policy makers and programme managers in Africa.

[1] WHO Factsheet No. 104 (2007).

Contents

Introduction

One of the most serious challenges confronting health authorities in developing and developed countries today is the increasing occurrence of tuberculosis (TB). Malaria, another major communicable disease, is also of considerable concern, as it is the largest cause of mortality in Africa, particularly in children. About 90% of the world's malaria cases occur in Africa.

The burden of both malaria and TB has recently been complicated by the emergence of drug resistance. The most pathogenic causative agent of malaria has developed resistance to all drugs currently in use, with the exception of the newly introduced artimisanin derivatives. The prevalence and magnitude of resistance to different drugs vary from one country to another. For example, in Kenya, resistance to the widely used chloroquine (CQ, the safest and most affordable drug) has reached 80%.

Dramatic outbreaks of multidrug resistant tuberculosis (MDR-TB) among institutionalized HIV infected patients have focused international attention on the emergence of strains resistant to antimycobacterial drugs. More recently, it has been shown that transmission of MDR-TB is not limited to HIV patients in institutional settings, as micro-epidemics of MDR-TB have been identified within communities in Africa. MDR-TB has serious consequences in resource-poor countries, where the availability of second line drugs is limited.

The diagnosis of new TB patients is based on microscope examination of sputum smears for the presence of acid fast organisms. No drug susceptibility testing is done in this protocol. Cases of primary drug resistant TB are thus missed, resulting in prolonged infectivity and the further spread of drug resistant TB. Conventional procedures for detecting drug resistance require culture sampling, which can take four to six weeks, followed by the determination of drug susceptibility of an isolate, which needs an additional three weeks.

Recent developments in the study of the molecular genetics of malaria and TB have led to the identification of mutations in genes involved in resistance to the front line drugs. Their species and strains are identifiable by molecular typing methods. Perhaps the greatest strategic advantage of this approach is in surveillance studies. For malaria, these techniques also provide a precise and large scale means to diagnose whether parasites that follow drug treatment are due to re-infection with new parasites or to the persistence of the parasites responsible for the original infection.

Since 1997, the IAEA has been involved in building new diagnostic capabilities in the use of molecular and radionuclide techniques for the diagnosis of drug resistance in malaria and TB in a number of sub-Saharan countries. These activities were undertaken in collaboration with national control programmes. Collaboration with the World Health Organization (WHO) on programme efficacy began in 1998.

The main objective of project RAF/6/025, Detection of Drug Resistant Malaria and Tuberculosis, was to develop innovative and effective strategies for strengthening regional capacity in new diagnostic tools for national disease control and surveillance programmes in Member States in Africa. Thirteen African countries participated in the project: Burkina Faso, Cameroon, Ethiopia, Ghana, Kenya, Madagascar, Mali, Nigeria, South Africa, Sudan, United Republic of Tanzania, Uganda and Zambia. The project was completed in 2006.

The study criteria for drug resistant malaria and TB were clearly defined for patients enrolled in the study. Molecular techniques applying radioisotopic and non-radioisotopic methods were used. Standard WHO protocols for TB and malaria were used for in vitro drug susceptibility assays and in vivo therapeutic efficacy studies in all the study sites. Specific country ethical approvals were sought before the project began.

In order to harmonize the processes and procedures for implementation of the project in the 13 countries, four workshops were convened and experts in the field of drug resistant malaria and TB were recruited. A final coordination meeting was held in June 2006 to document the completion of each country report.

The final country reports submitted by each counterpart varied in the degree of detail and information. This brochure summarizes the main findings of the project in each of the 13 countries. Findings are presented in two sections: one on malaria and one on TB. A pooled analysis of results for all participating countries for each disease is documented at the end of the relevant section.

The analyses highlight interesting results relevant to policy makers and programme managers in Africa. The results from each country

participating in the project have been published in local and international peer reviewed journals. The results generated were, in some cases, able to influence policy formulation for malaria or TB treatment and are an important achievement of the regional project. The study also recognized the need to strengthen links between counter-parts (or researchers) and policy or programme managers in order to support evidence based policy recommendations.

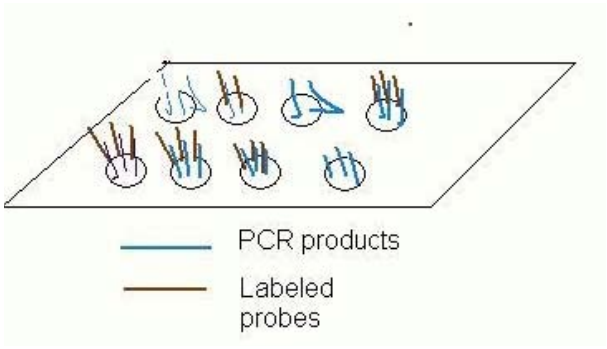
Monitoring the emergence of drug resistant TB strains is critical to controlling its spread. However, most countries with a high prevalence of TB continue to use slow, culture based methods to investigate suspected MDR-TB cases. These traditional phenotypic methods of detecting the drug resistant disease take a long time, due to the protracted growth rate of *M. tuberculosis*, and results often take weeks to obtain.

In the case of malaria, conventional assessment of resistance is also slow and requires field studies, where individuals need to be monitored for 14 to 28 days.

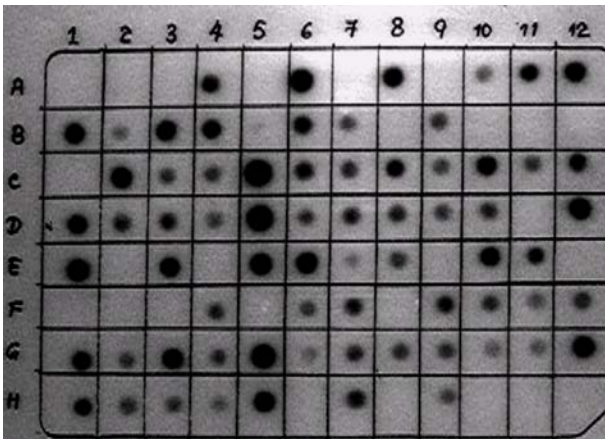
Genotypic prediction of drug resistance, using molecular techniques that apply radioisotopic methods, holds a significant advantage in that it is faster and many samples can be analysed together.

Radioisotopic methods are used in the research and diagnosis of many human and animal diseases. The sensitivity of radioisotopic probes has been demonstrated to be high, detecting as little as 0.1 pg of target DNA. In terms of reproducibility, the method has been shown to be quite robust. Dot blot assay combines polymerase chain reaction (PCR) amplification and hybridization of amplified products with radiolabelled allele specific probes. It has been extensively used for the detection of point mutations in a number of pathogens. The dot blot/probe hybridization technique can also be used for large scale epidemiological surveys of genes associated with drug resistance.

In summary, the dot blot assay involves the following steps. Dot blot PCR products known as amplicons are blotted onto a single solid support matrix — for example, a nylon membrane. The matrix can then be tested with a labelled probe, such as a radioactive phosphorus attached to ATP (γ-ATP-32) that emits gamma (γ) radiation. The probe is specific to a particular mutation or sequence of interest. The probe will form a complementary sequence present in the amplified PCR sequence. After the washing steps, autoradiography will reveal the specific PCR products for the probe used. The probe can be stripped off and the membrane can be rehybridized with other probes.



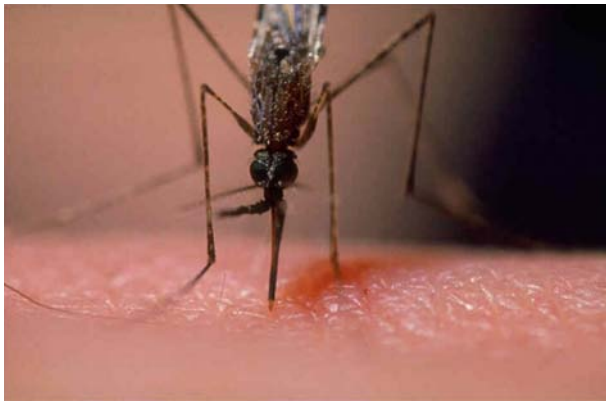
Oligonucleotides specifically hybridize to complementary strands of DNA (and RNA), tagged to radioisotopes used to probe for specific sequences of DNA (or RNA) of interest.



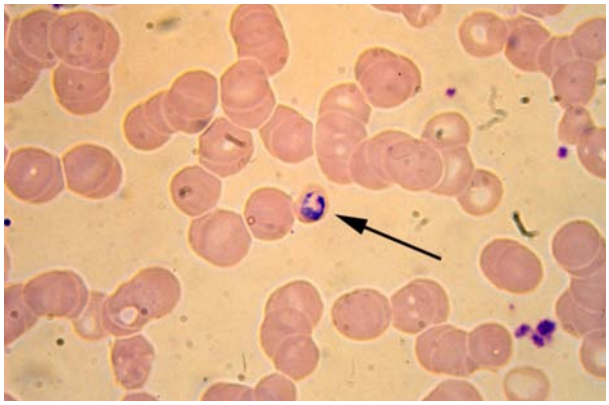
Dot blot autoradiography showing the use of radioisotopes to detect gene polymorphisms implicated in treatment failure.

Malaria

Malaria is the leading cause of mortality and morbidity in Africa, accounting for 90% of an estimated 300–500 million clinical cases worldwide every year and for one in five of all childhood deaths. Morbidity and mortality have increased, mainly due to the emergence of parasites that are resistant to the cheap and readily available antimalarials. *P. falciparum* is the most prevalent malaria causing parasite in sub-Saharan Africa, with reported resistance to the most commonly and easily available drugs, CQ and Fansidar. Levels of treatment failure vary from country to country, but are higher in east and southern African countries than in west Africa. CQ resistance was first noted in the 1980s in east Africa and by the 1990s a similar pattern of resistance was seen spreading throughout west Africa and the rest of Africa. Most countries in sub-Saharan Africa have now adopted either artesunate–amodiaquine or artemether–lumefantrine as their first line artemisinin combination therapy (ACT) for treatment of uncomplicated malaria.



Mosquito vector A. gambiae, the principal vector of malaria in Africa, is shown here taking a blood meal from a human host.



Slide taken from a malaria patient showing P. falciparum (a parasite causing malaria in humans) inside a red blood cell.

Burkina Faso

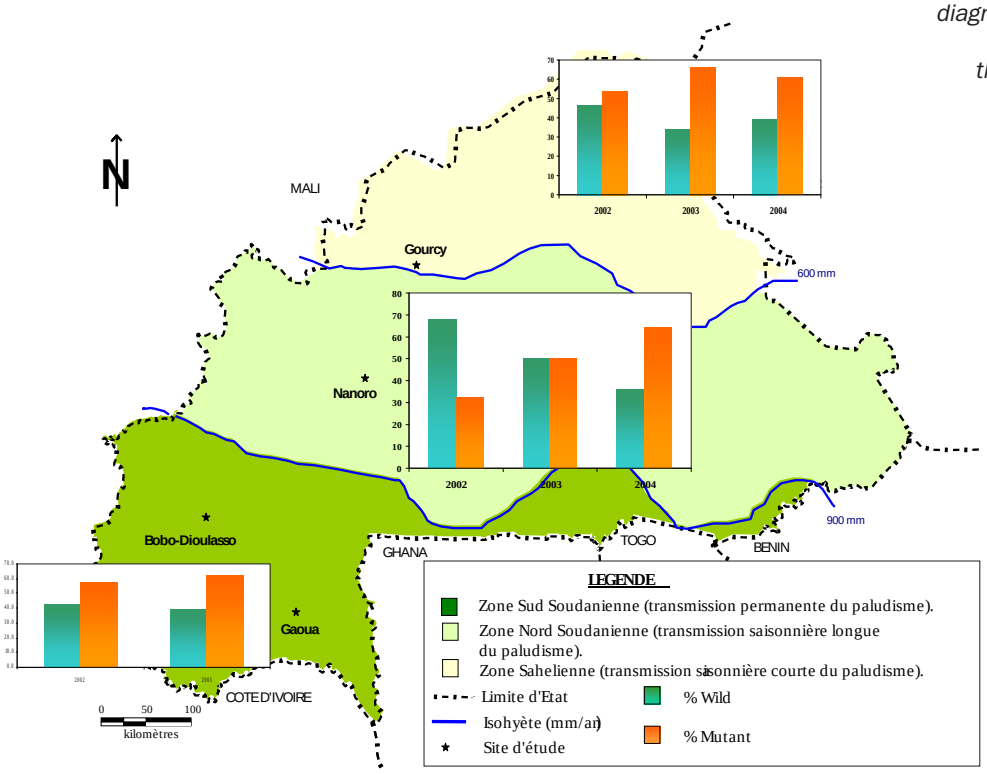
Malaria remains a major public health problem in Burkina Faso. It constitutes the first cause of consultations in health centres and accounts for more than 50% of hospitalizations. Infant mortality from malaria is relatively high, with around 50% of deaths occurring in children below the age of five.

The first case of CQ resistance was detected in 1988. The treatment failure rate remained below 15% in 2000, but by 2003 there were reports of widespread CQ treatment failure, as evidenced in the RAF/6/025 project. An important observation was the increasing prevalence of the CQ resistant genotypes reported between 2002 and 2003. These results highlighted the need to stop the use of CQ as an antimalarial drug for uncomplicated malaria and to adopt a more effective regimen. These findings contributed towards antimalarial drug policy change in Burkina Faso.

By 2004, more than 13% of patients with uncomplicated malaria did not respond to Fansidar. Under the project, antimalarial efficacy studies were carried out on amodiaquine (AQ) and Fansidar, as well as on the combination of these two drugs, for the treatment of uncomplicated malaria. This was followed by molecular genotyping of resistant markers to the antimalarials.

The important findings from the mutational analysis suggested that mutations *dhfr* 59R and *dhps* 437G in the genes dihydrofolate reductase and dihydropteroate synthetase, respectively, were linked more to Fansidar treatment failure.

Patient screening includes diagnosis for malaria parasites, temperature reading and the recording of patients for inclusion and treatment.



Pfcrt prevalence evolution from 2002 to 2006 in Burkina Faso.

The results highlighted the significance of molecular assays in determining resistance to different antimalarials, including AQ and Fansidar.

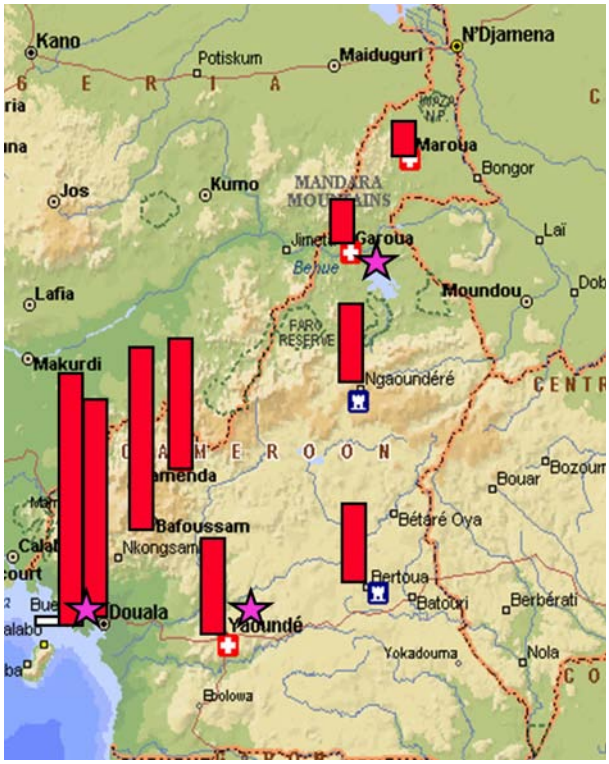
An important output of the project in Burkina Faso was the recommendation that the national malaria control programme (NMCP) adopt artemisinin based combination therapy as first line drugs for uncomplicated malaria. The change was suggested due to the treatment failure of Fansidar as evidenced through this project and other research projects. The NMCP recognized the counterparts as key partners in the monitoring of drug efficacy in the country.

Cameroon

In Cameroon, malaria accounts for 23% of hospitalizations and 35% of all deaths in children below the age of five. Resistance to CQ was first reported in Cameroon in 2001 and has since been reported in many parts of the country.

Within the project, the objective of the Laboratory for Public Health Biotechnology was the establishment of a molecular profile of markers causing resistance to antimalarials. The aim was to provide the NMCP of the Ministry of Health (MoH) with data to be used in the formulation of an antimalarial drug policy.

The therapeutic efficacy of AQ, Fansidar and AQ + Fansidar was also assessed. This was followed by correlation studies between in vivo treatment outcomes and known molecular markers pointing to resistance to the antimalarial. The study was conducted in different parts of Cameroon. The dot blot technique was found to be more sensitive and user friendly compared with the other methods of evaluating therapeutic response, including clinical and parasitological responses.

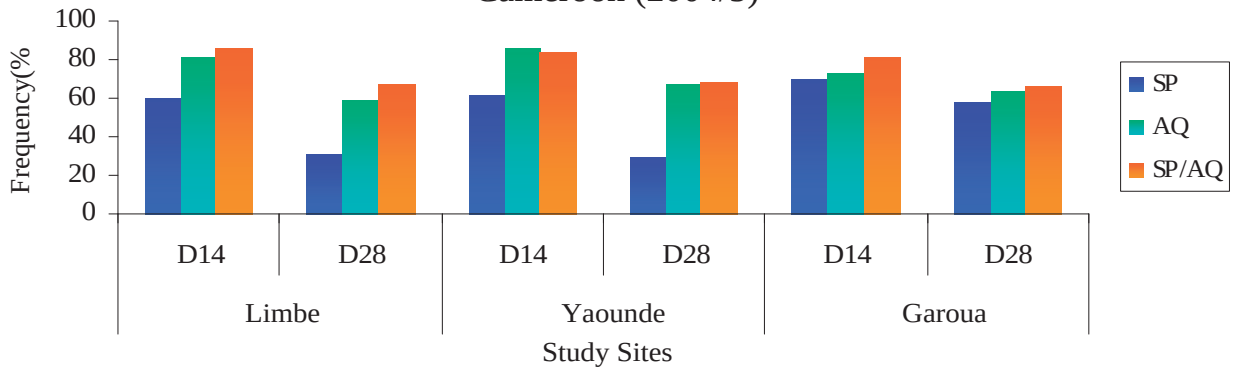


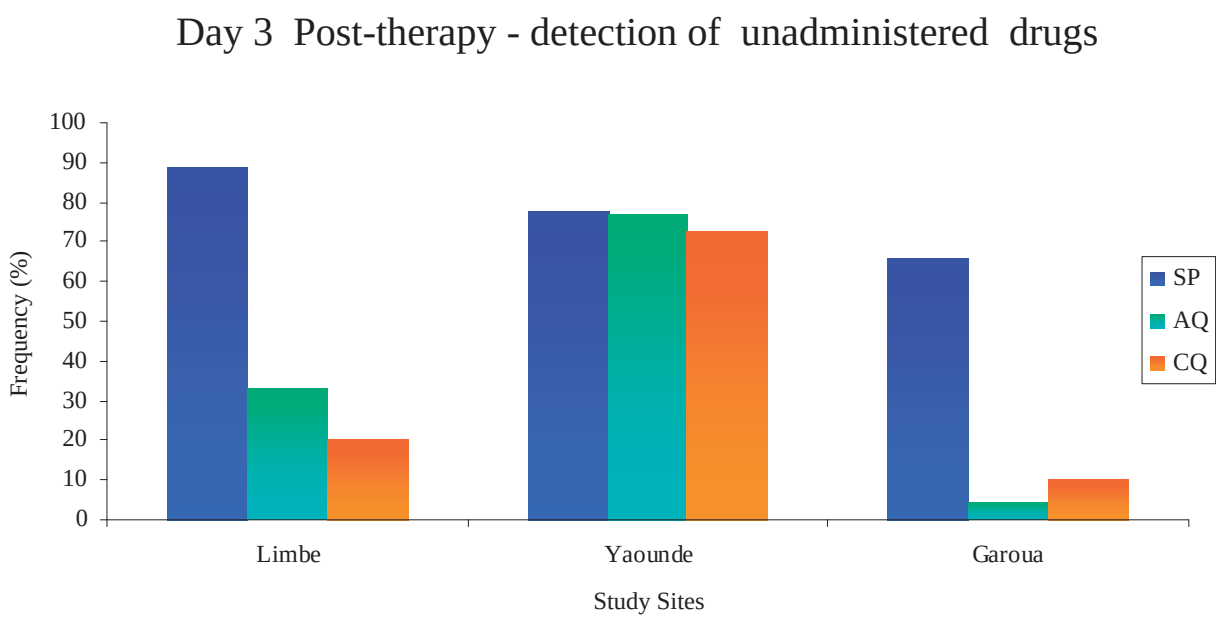
Over time, the geographic distribution of drug resistant malaria can be monitored more easily with molecular assays rather than with in vivo testing or in vitro drug sensitivity assays. The present data suggest the existence of a marked difference in antifolate resistance in different epidemiological regions of the country. The varying levels of resistant genotypes, as evidenced here, may suggest different drug usage patterns in the country.

With the participation of the NMCP, evidence on failing chemotherapy to CQ (46%), AQ (20%) and Fansidar (50%) was recognized within this project. The spread of antifolate resistant markers is one of the first studies conducted at a national level over an extensive geographic area, and constitutes the baseline data for mapping the distribution of *dhfr* alleles in Cameroon with the consequential change to artemisinin derivatives as the first line treatment of uncomplicated malaria.

RAF/6/025 results showed a difference in the regional spread of resistance, with greater resistance in Cameroon spreading from the south to the north and from the west to the east for the antimalarial CQ. This was similar for the AQ and Fansidar combination (see graphs below). Regional differences in efficacy may require regional policies within Cameroon.

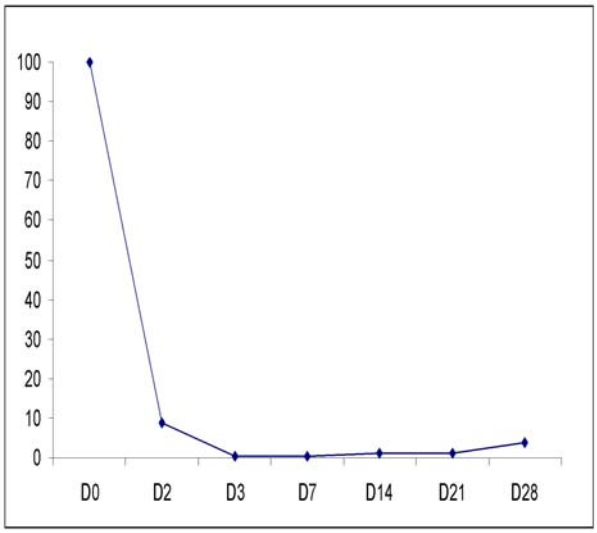
Adequate clinical and parasitological response SP, AQ, SP/AQ in Cameroon (2004/5)





The results suggest that drug pressure through continued use or misuse of antimalarial drugs can lead to an increased selection of mutant genotypes associated with treatment failure. The data presented here suggest that the misuse of antimalarial drugs is widespread even for patients who have received treatment.

Malaria remains a major public health problem in Ghana. It has been estimated that approximately 50% of all outpatient visits to clinics are attributable to malaria, and that about 25% of all childhood mortality is due to the disease. The current control strategy in Ghana has been through case management based on prompt recognition and adequate treatment.



Rate of malaria parasite clearance among study subjects treated on ART + AQ combination (x axis: follow-up days; y axis: percentage clearance).

The results from the project showed that by the year 2002, the antimalarial therapeutic data from CQ had a positive correlation between the prevalence of mutations in *pfcrt* (a gene associated with CQ resistance) and treatment failure in at least two study sites in Ghana. Similarly, almost 50% of *P. falciparum* infected patients had the *dhfr* and *dhps* antifolate resistant markers. This led to a rapid deterioration of Fansidar efficacy, resulting in a high therapeutic failure rate. At this time, Fansidar was used as the second line antimalarial drug in the country.

As a consequence of the results from RAF/6/025, Fansidar is no longer used as a second line drug. It is reserved for intermittent presumptive treatment among pregnant women in the country.

Within the same project, it was also possible to embark on a therapeutic efficacy study on an artemisinin based combination of artesunate + amodiaquine (ART + AQ), given to patients with uncomplicated malaria. This was to assist the NMCP maintain an up to date database on treatment responses to the new antimalaria drug. The Noguchi Memorial Institute for Medical Research (NMIMR) was mandated to conduct drug efficacy monitoring studies in sentinel sites across the country. These studies will also enable the NMCP to respond to the changing trends in the efficacy of the new treatment in a timely manner. In addition to project support, the NMCP is also providing additional financial support to NMIMR. The funds are from the Global Fund to Fight AIDS, Tuberculosis and Malaria programme. Such efforts help to ensure future sustainability of the project.

Kenya

P. falciparum malaria remains among the most prevalent parasitic diseases in Kenya. Over 70% of Kenya's population is at risk of the disease, with approximately 8.2 million clinical cases. It is the leading cause of under five year old mortality, causing the deaths of approximately 34 000 children in this age group annually.

A technician looking for malaria parasites from a blood smear.

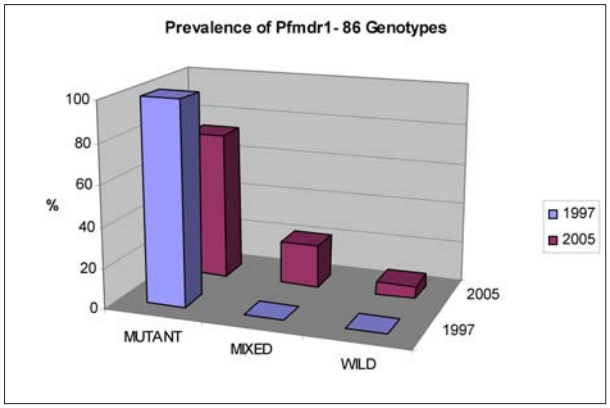
Malaria parasites can be identified by examining a drop of the patient's blood spread out as a 'blood smear' on a microscope slide. Prior to examination, the specimen is stained (most often with Giemsa stain) to give the parasites a distinctive appearance. This technique remains the 'gold standard' for laboratory confirmation of malaria

With the high treatment failure rates reported due to CQ resistance (66–87%), the Kenya Division of Malaria Control (DMC) replaced CQ with Fansidar as the first line drug for uncomplicated malaria in 1997.

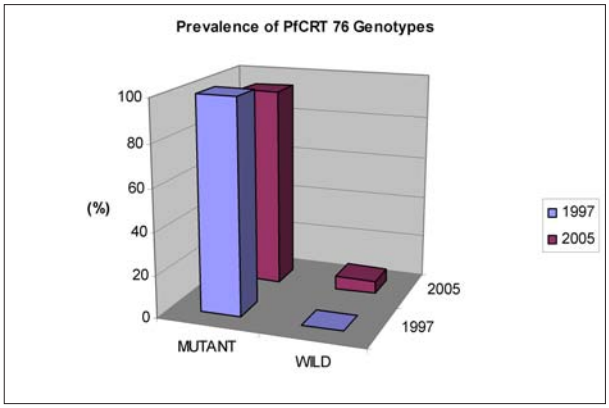
The project monitored the prevalence of gene polymorphisms on the *P. falciparum* multirug resistant gene 1 (*Pfmdr*1) and the *P. falciparum* CQ transporter gene (*Pfcrt*) associated with resistance to CQ in 2005, eight years after the cessation of the use of CQ. The results showed that 94% of field isolates from this site still contain the mutant type of CQ resistance gene, while 6% contained the wild type allele. The implication of this finding suggests that reversal to CQ sensitivity is slow and that CQ should not be reintroduced or used in combination with other antimalarials in Kenya.

Resistance to Fansidar in the malaria parasite is a well established phenomenon in Kenya. In 1997, the DMC changed the first line drug for uncomplicated malaria in Kenya from CQ to Fansidar, and in 2006 another antimalarial drug policy change was carried out, switching from Fansidar to artemether–lumefantrine (Co-artem). IAEA support was instrumental in obtaining the results, and recently the Malaria Unit at the Kenya Medical Research Institute submitted the results on antimalarial efficacy data (from 1997 to 2007) to the DMC, strengthening linkages that exist with the control programme. The establishment of the ten year database was only possible through the support of this project and has facilitated productive collaboration between policy makers and counterparts.

CHLOROQUINE THERAPEUTIC STUDY



Prevalence of Pfmdr1-86 genotypes.



Prevalence of Pfcrt-76 genotypes.

Madagascar

Prevalence of CQ susceptibility markers was analysed under project RAF/6/025. The population in Madagascar is estimated to be 19 million. An estimated 1 million people suffer from malaria annually — over 5% of the overall population. Recent statistics estimate that malaria or malaria related illness are responsible for 27% of deaths in children and 16% in adults. However, it is anticipated that malaria morbidity will reduce from 19% to 16% by 2008 (NMCP). This is the result of a concerted approach in the fight against malaria, made possible through the Global Fund to Fight AIDS, Tuberculosis and Malaria.

Patients, mostly children, waiting to be screened for malaria in a rural hospital in Madagascar.

Routine monitoring of the therapeutic efficacy of antimalarial drugs at peripheral health centres, together with malaria diagnosis, is part of the national malaria surveillance programme.

An overall level of CQ treatment failure of 15–40% as detected during the 14 day in vivo follow-up period has been recorded in the country. Project support coordinated this research outcome.

Clinics and dispensaries are quite rare in rural Madagascar and most are inadequately equipped. Patients with fever end up being treated with CQ. Home treatment (or self-medication) is quite common. The latter practice is worrying as it may promote the selection and spread of the mutant *pfcrt* 76T, already found under RAF/6/025. The *pfcrt* K76T was detected in six (3.3%) out of 183 *P. falciparum* isolates.

Policy recommendations proposed as a direct result of the project outcome, and also due to grants from the European Union, include:

- The adoption of ART + AQ combination as the first line treatment;
- Recognition of the usefulness of the genetic resistance markers for drug resistance surveillance;
- Demonstration that Fansidar and AQ are still highly efficacious in Madagascar.

The antimalarial drug efficacy assessment was conducted by the Institut Pasteur de Madagascar in collaboration with the MoH, showing a strong linkage between researchers and policy makers. Other research interests that exist between these two institutions include:

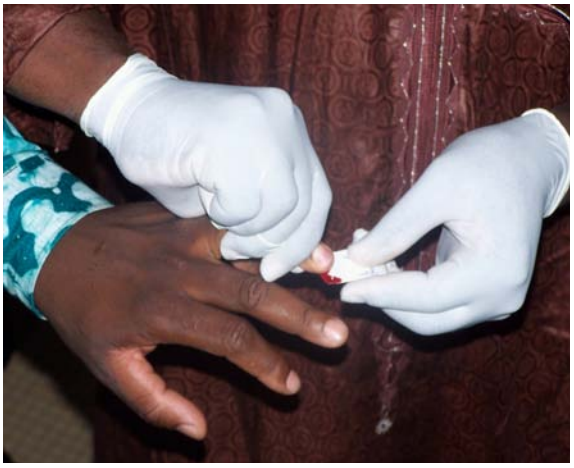
- An expansion in the use of insecticide treated nets and insecticide spraying;
- The use of Fansidar as a prophylactic tool in pregnant women as an intermittent preventive treatment in pregnancy;
- The evaluation of rapid diagnostic tests in epidemic-prone regions;
- ACT for case management;
- Home management of fever in children.

Demonstration of the impregnation of insecticide treated nets in a village in Madagascar as part of malaria control. Such strong partnerships exist between the Institut Pasteur de Madagascar and the MoH.

Mali

Mali has a population of 13.5 million. Malaria is responsible for 17% of childhood mortality. Approximately 15–20% of *P. falciparum* infections fail to respond to CQ treatment. The public health policy recommends treatment with Fansidar for cases that fail treatment with CQ.

The aim of the project was to correlate mutations in parasite genes with clinical and parasitological treatment failure following treatment with CQ and/or Fansidar in uncomplicated *falciparum* malaria.



A finger prick sample of blood being taken from a patient for diagnosis and molecular investigation.

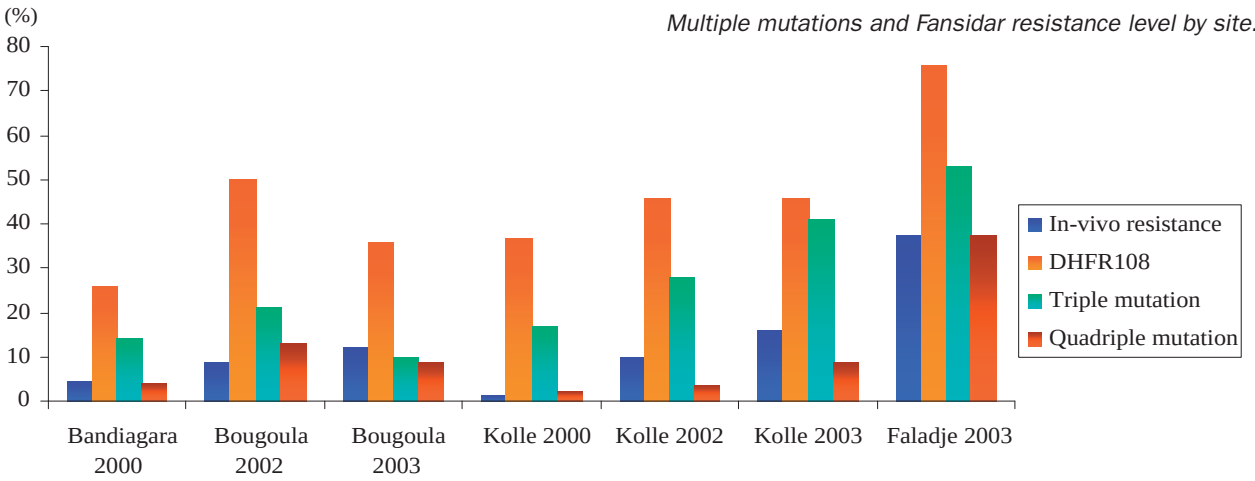
Molecular assays can be performed for a large number of finger prick capillary blood samples absorbed onto filter papers. The filter papers can be stored for long periods and can be transported with ease.

P. falciparum analysis indicated mutations on the *dhfr* gene (mutations 108N, 51I and 59R) and the *dhps* gene (mutations 437G and 540E), which were found to be strongly associated with Fansidar failure in Mali.

Interestingly, the results from Mali showed that the prevalence of these markers always exceeded the prevalence of treatment failure of the relevant drug, posing a challenge to the use of these markers as predictors of drug failure in regions with high malaria transmission. However, age (as a surrogate marker for immune protection) was strongly associated with the ability to clear CQ resistant parasites, and a model for using the ratio of molecular marker rates to parasitological resistance rate, adjusted for age, has recently been described. These ratios, termed the genotype resistance index (GRI), when adjusted for confounders (such as age, ethnicity, transmission), were very stable at four sites in Mali with different patterns of malaria transmission.

Molecular markers of, for example, CQ resistance have been shown to provide a precise measurement of the clearance of the drug resistant parasites, making it a plausible tool in relating phenotypic outcome to genetic outcome. In this regard an index encompassing both the phenotypic and genotypic outcome has been recognized within the project. The GRI is the ratio of the frequency of the resistant genotype to the frequency of the parasitological resistance to a particular drug in a given area. Such an index presents several unique opportunities for application in drug resistance studies.

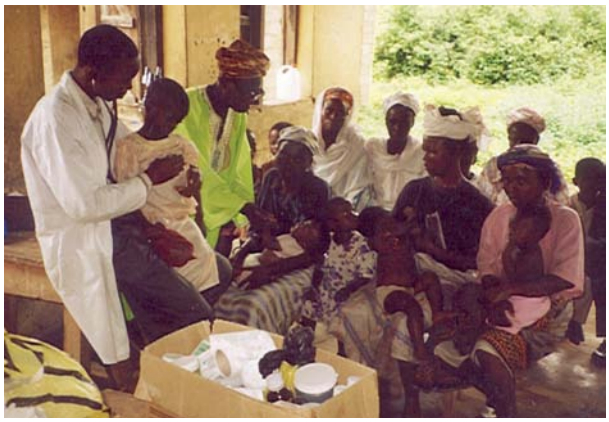
Multiple mutations and Fansidar resistance level by site.



- This approach permitted a comprehensive mapping of resistance levels across Mali without the need to carry out numerous repeated in vivo efficacy studies.
- In collaboration with the NMCP and the Association of Physicians from Community Health Centres, the GRI model was successfully used with local physicians working in rural areas to estimate rates of in vivo CQ resistance in their respective health districts.
- The GRI represents a practical tool for public health surveillance of antimalarial resistance, mainly in malaria endemic areas where microscopic diagnosis is not available.
- This approach may be applicable to other markers of drug resistance, such as molecular markers of ACT resistant malaria when available.

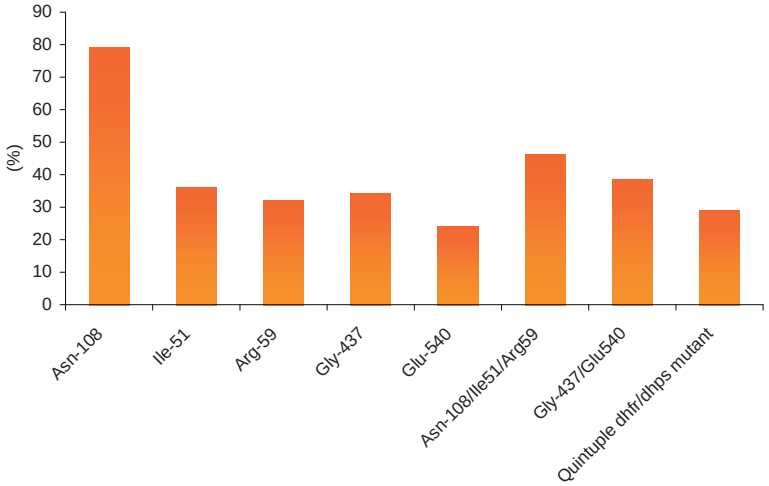
Nigeria

Nigeria has the largest population in Africa, with 120 million people (National Population Census, 1999). Malaria accounts for 63.4% of all reported diseases in Nigerian health facilities. A 2000 situation analysis in the country showed that malaria accounted for 25% of infant mortality, 30% of childhood mortality and 11% of maternal mortality. In the absence of an effective malaria vaccine, chemotherapy remains the main treatment and control of the disease.



Children with malaria being examined in a rural clinic at Olode Adetoun, Oyo State, south-west Nigeria.

Molecular methods that detect genetic markers of drug resistance in the malaria parasite have been shown as potential and powerful tools for tracking drug resistant infections. Indeed, the project in Nigeria showed that the presence of mutations in malaria parasites (*P. falciparum*) from patients prior to treatment could be used to accurately predict CQ and Fansidar resistance during a 28 day clinical efficacy trial.



Levels of mutations as observed in the malaria-causing parasite, before treatment with Fansidar.

It is anticipated that the dot blot assay under this project will be used for the development of a simple high throughput method for large scale monitoring/surveillance of drug resistant parasites to artemisinin and partner drugs used in combination therapy for malaria in Nigeria and other African countries.

A graduate student performing PCR for detecting drug resistant P. falciparum at the University of Ibadan.

The project provided a unique opportunity for investigators at the Malaria Research Laboratories, IMRAT, College of Medicine, to use the upgraded infrastructure for other research work. The latter included parasite population genetic studies and the identification of genetic markers resistant to AQ. In addition, investigators were able to identify specific mutations that may serve as simple and reliable markers to predict treatment failure in different age groups in Nigeria.

A long standing goal in malaria research has been to use molecular markers of drug resistance as a rapid means of surveillance to promote evidence based antimalarial drug treatment policy formulation. This was recognized within project RAF/6/025 and in collaboration with other research projects. The results generated were used as additional evidence to support the current change in antimalarial drug policy in Nigeria from Fansidar to artemisinin derivatives.

Sudan

Sudan is the largest country in Africa, covering over 8% of the entire continent. The total population is estimated to be 30.3 million inhabitants, of which 75% live in rural areas. There are 7.5 million malaria cases and 35 000 deaths every year due to malaria. The problem appears to have worsened in recent years, due to increasing levels of *P. falciparum* resistance against the two most commonly used antimalarials: CQ and Fansidar.

The overall aim of the project was to assess clinical response following treatment with CQ and Fansidar, and to determine the prevalence of *P. falciparum* gene polymorphisms known to be associated with drug resistance. Studies were conducted at two sites in central and eastern Sudan.

Whilst the whole population is at risk from the disease, there is a higher incidence among pregnant women and children under five years of age. This results in complicated pregnancies, low birth weight and infant mortality.

Results from in vivo studies showed failure rates of approximately 12% in Khartoum and the eastern part of the country and between 16% and 70% in other parts of the country.

One of the significant achievements of the project has been in capacity building. The young researcher here has been trained on radioisotopic techniques and is currently undertaking drug resistance work in the National Public Health Laboratories, Khartoum.

The results from this study also highlighted that other factors, such as acquired immunity, pharmacokinetic variations and previous drug use, may influence both the clinical and parasitological response to the treatment outcome. These studies are ongoing in different IAEA Member States. The major areas of investigations will include:

- Further statistical analysis of Fansidar data to adjust to major confounders such as age, ethnicity, residence, transmission, etc.
- Additional pharmacokinetic analysis of Fansidar.
- The investigation of human genetic factors involved in resistant parasite clearance.

Malaria: Pooled Analysis of Project Results for all Countries

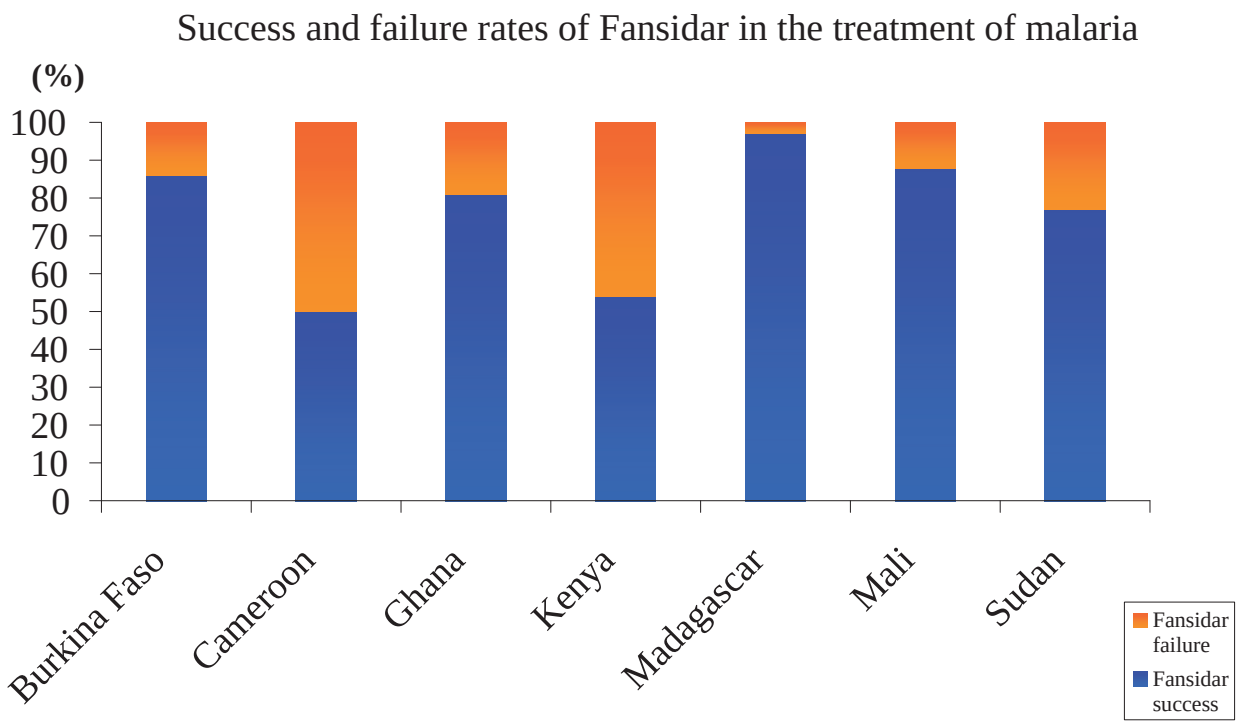
The regional technical cooperation project RAF/6/025, Detection of Drug Resistant Malaria and Tuberculosis, aimed to: (1) evaluate the efficacy of CQ and Fansidar antimalarial drugs in different sites across Africa; and (2) assess the frequency of markers for the development of resistance through molecular genetics. This provided evidence based results to support the work of policy and programme managers. Eight countries in Africa were involved.

Samples from patients with symptoms indicative of uncomplicated malaria were used in the RAF/6/025 study. The standard WHO protocol for therapeutic efficacy on uncomplicated malaria was used (MAL/2002 guidelines) for the determination of molecular markers to CQ and Fansidar. Association studies between the two methods (in vivo and dot blot molecular assays) were carried out. Recrudescence or re-infections were differentiated by analysis of the MSP_1, MSP_2 and GLURP genes (these are not highlighted in this brochure). The antimalarial drugs studied were mainly CQ and Fansidar, although AQ and ACT were included during the latter part of the study. The table below summarizes the project activities.

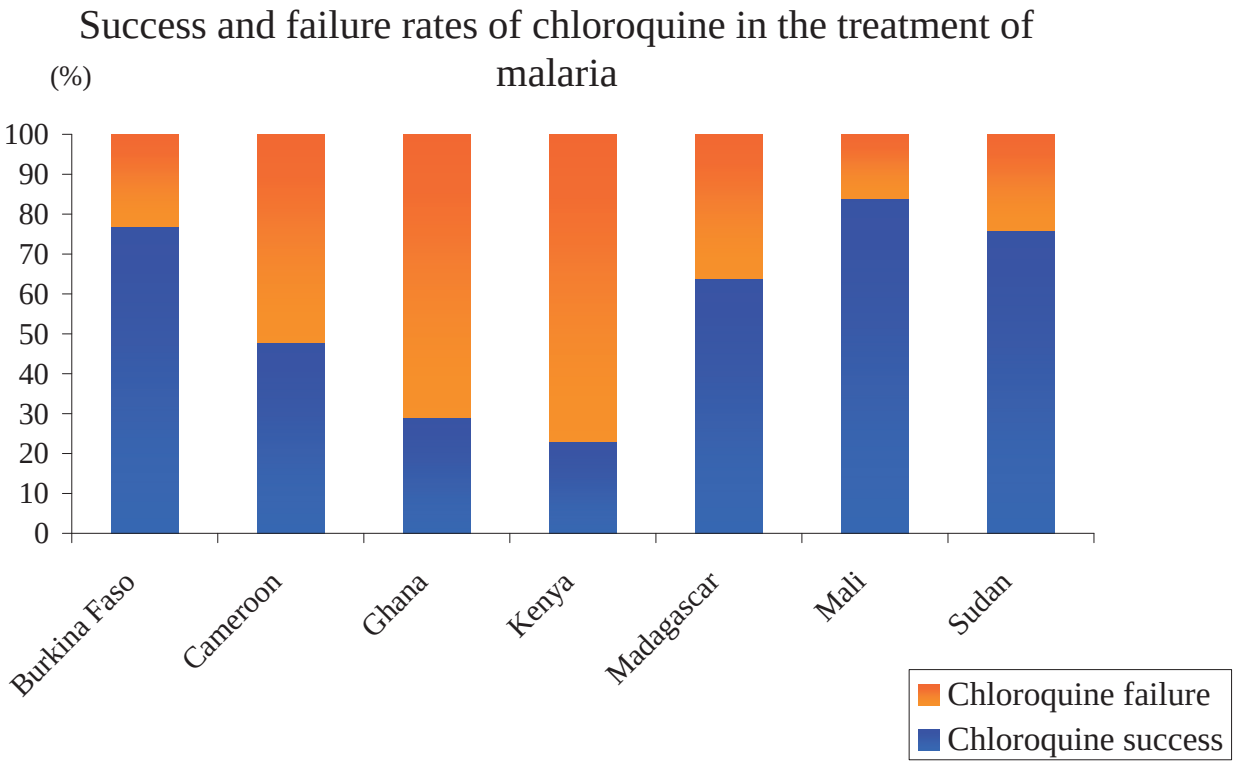
The results showed failure rates to CQ and Fansidar to be between 10% and 80% and 4% and 50%, respectively. Genotypic results on antifolate and CQ resistant markers from the dot blot technique were more predictive of the treatment outcome (see graphs on the next pages). A higher prevalence of mutations on the dihydrofolate reductase gene (*dhfr*) and on the *P. falciparum* transporter gene (*Pfcrt*) were observed from isolates from Kenya, Ghana, Cameroon and Sudan, compared with Burkina Faso and Madagascar. Interestingly, these translated to higher CQ and Fansidar failure rates, suggesting the reliability and usefulness of the mutational analysis using the dot blot technique.

Patients were enrolled in the study following the WHO standard protocol on malaria therapeutic response.

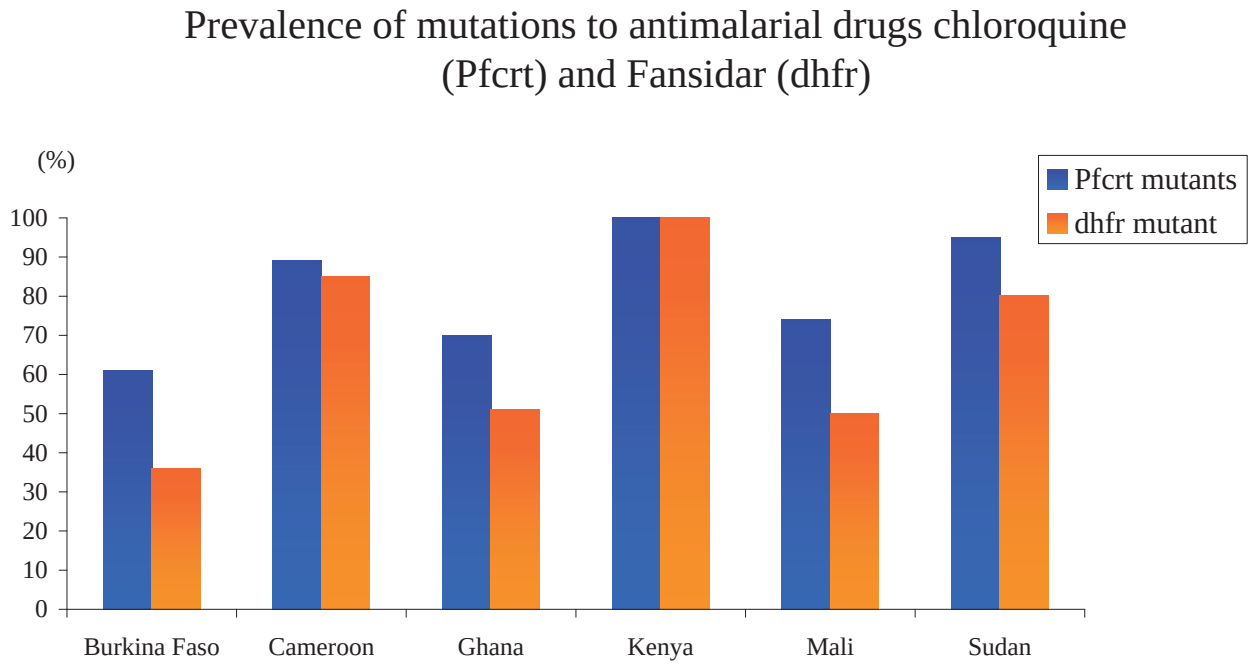
Assessment	**Follow-up days**							
	D0	**D1**	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**
Written informed consent	✓							
Inclusion/exclusion criteria	✓							
Clinical examination	✓	✓	✓	✓	✓	✓	✓	✓
Finger prick blood smears for microscopy	✓	✓		✓	✓	✓	✓	✓
Blood sample (filter paper for molecular investigation)	✓				✓	✓	✓	✓



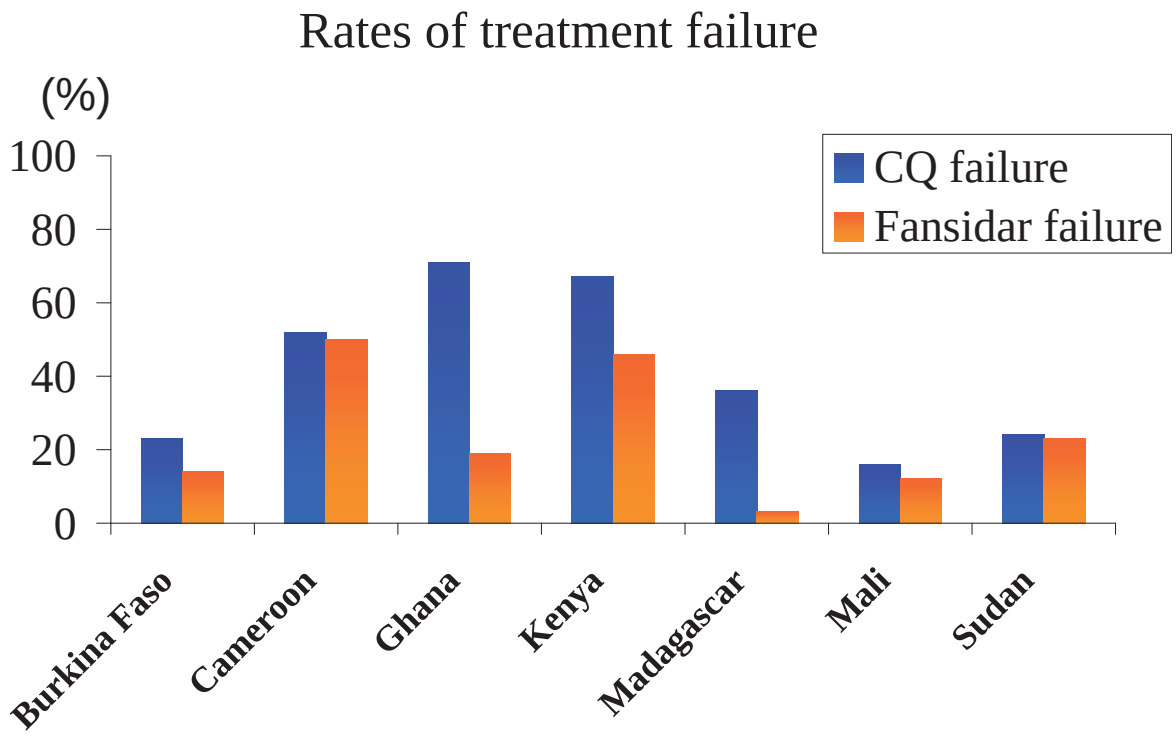
The failure rate of Fansidar is high in some countries (Ghana, Kenya) and low in others (Madagascar, Mali, Burkina Faso).



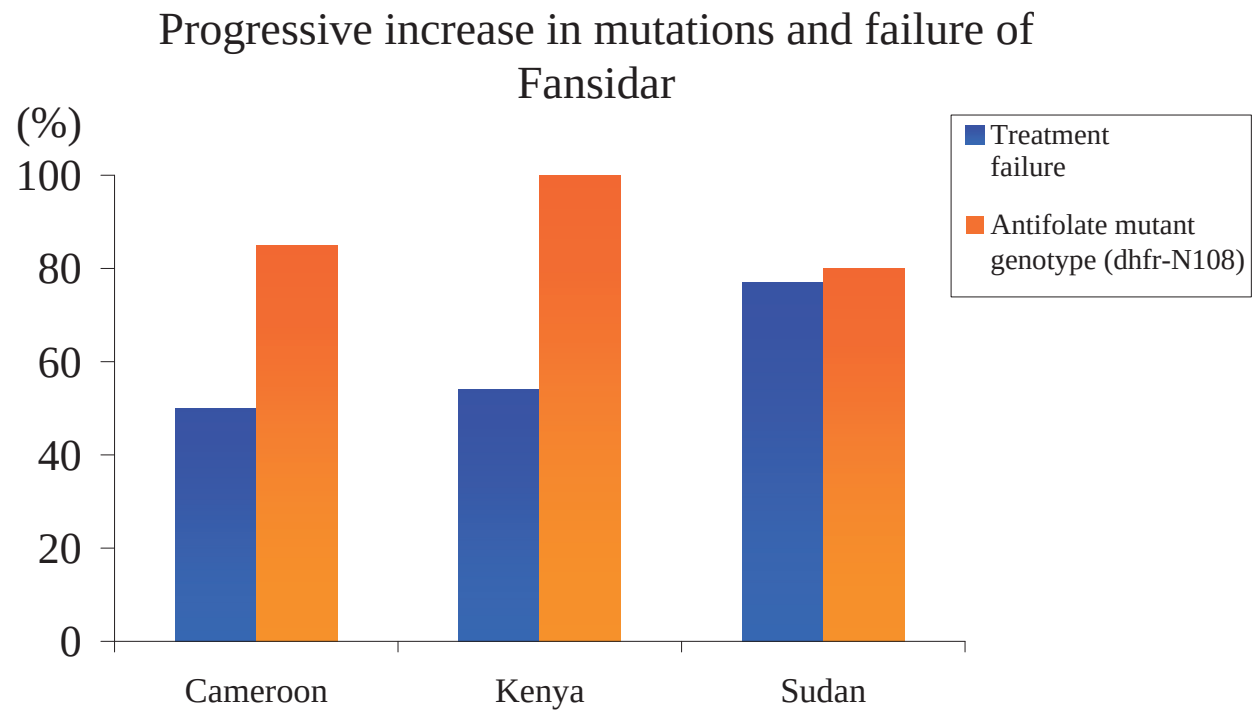
The failure rate of CQ in the treatment of malaria varied in different countries. The highest failure rates were observed in Kenya, Ghana and Cameroon.



The results show high prevalence of T76-Pfcrt and 108N-dhfr mutations. This may explain the failure of CQ and Fansidar therapy. Results are generated from the dot blot technique.



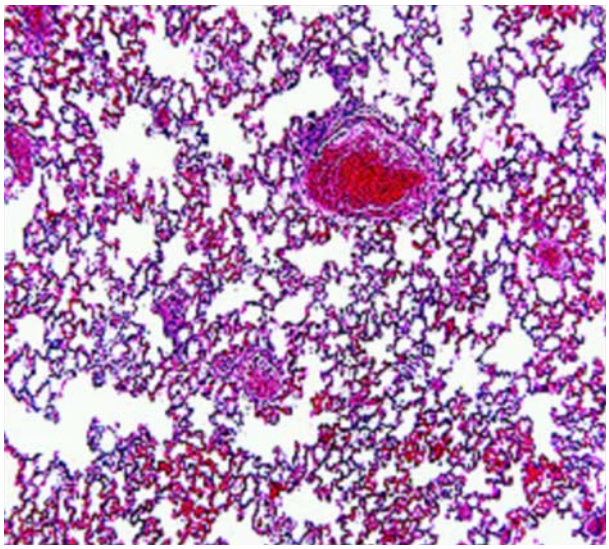
The high rate of CQ and Fansidar treatment failure suggests the adoption of alternative therapy for uncomplicated malaria.



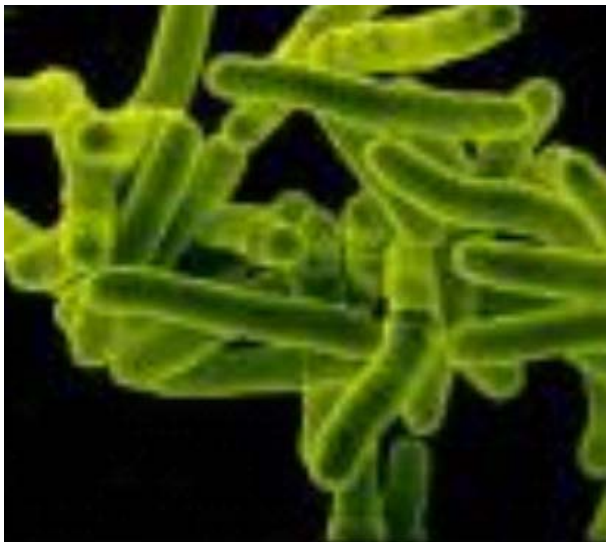
High prevalence of antifolate resistant markers may explain the reduced efficacy of Fansidar in the three countries.

Tuberculosis

The rising incidence of communicable diseases in sub-Saharan Africa has emerged as a major global health issue. The ease of transmission and the highly infectious nature of active pulmonary TB pose an increased burden in settings with high co-infection rates of HIV. In some parts of sub-Saharan Africa, incidences of TB exceed 300 cases per 100 000 inhabitants per annum. TB is primarily an illness of the respiratory system, and is spread by coughing and sneezing. The emergence of MDR-TB further complicates this picture. More recently, it has been shown that the transmission of MDR-TB is not limited to HIV patients in institutional settings and that micro-epidemics of MDR-TB are occurring globally. According to the WHO, drug resistant TB is widespread, and occurs as a result of treatment mismanagement.[2] Failure to achieve a definitive cure of the largely drug susceptible disease can lead to the development of drug resistant strains, and the difficulty of detecting and treating patients infected with these resistant strains allows continued transmission. The rise of MDR-TB in resource-poor countries, where the availability of second line drugs is limited, can have an immediate impact on TB morbidity and mortality. It has been estimated that as many as 200 000 MDR-TB cases may be found in sub-Saharan Africa. A joint WHO/International Union Against Tuberculosis and Lung Disease (IUATLD) project for surveillance of drug resistance in TB has been running since 1994, with the goal of ascertaining the global extent of the problem. The most recent report covers the 2002–2006 period. However, much of sub-Saharan Africa has not been included in the worldwide surveillance project so far, due to the poor infrastructure of mycobacteriology laboratories. Thus the true prevalence of drug resistant TB is unknown in this part of the world.



Electronic microscopy of a TB infected lung.



M. tuberculosis.

[2] WHO, Tuberculosis MDR-TB & XDR-TB, 2008 Report Factsheet.

Cameroon

In Cameroon, health centres are registering increasing numbers of TB cases in correlation with the spread of HIV/AIDS (30–40%, 2000). The incidence of the disease in 2000 was estimated at more than 150 cases per 100 000 inhabitants annually. The Yaoundé region includes almost one sixth of the total cases recorded in the country (National Tuberculosis Control Programme (NTCP), Cameroon), and despite the implementation of the direct observed treatment short course, the incidence is still increasing.

HIV also increases *M. tuberculosis* transmission rates at the community level, therefore threatening the health and survival of HIV seronegative individuals as well.

The results obtained in this study could have several implications, in particular for TB control programmes in Cameroon, namely:

- Mutational analysis by dot blot can indicate prominent outbreaks of drug resistant strains.
- Preliminary results from the project suggest that, if carried out on a larger scale, it could contribute to a better understanding of the transmission mode of MDR-TB within and between communities.
- There was observed resistance to rifampicin (RIF), and the mutations for isoniazid (INH) were also reported. Sensitivity was found to be 77% and specificity to be 90%. For INH sensitivity, this was 88% specificity, and 95% for RIF.
- Molecular studies can show the outbreak and transmission of MDR-TB strains. Results from the project were conveyed to the NTCP.

Table showing some of the results on sensitivity and specificity.

Number of samples	Drug	Sensitivity[a]	Specificity[b]
64	Isoniazid	77%	90%
	Rifampicin	88%	95%
	Streptomycin	—	—
	Ethambutol	50%	80%

[a] Number of resistant samples classified correctly.
[b] Number of susceptible samples classified correctly.

In Ethiopia, TB has seen an increase in reported cases in recent years of 5% to 10% per year because of the HIV/AIDS pandemic. TB/HIV co-infection has been recorded at approximately 50%. Over 100 000 new cases were reported between 2001 and 2002.

TB drug resistance in Ethiopia was reported in the first national surveillance data, supported by WHO/IUATLD during the 2003–2005 study period. 749 new patients were tested, of which 1.5% were reported with multidrug resistance (MDR).

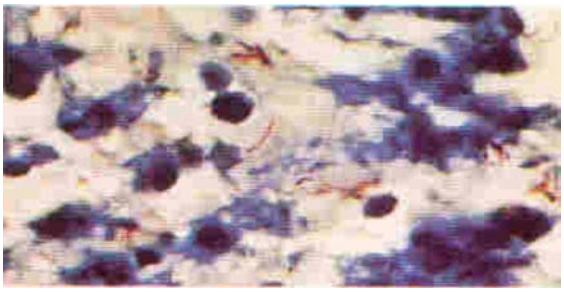
Rapid molecular methodologies have been developed that detect mutations that can predict resistance to, for example, RIF. The project examined the *rpo*B gene encoding the β subunit of bacterial DNA dependent RNA polymerase and has been demonstrated to have a high level of accuracy for detecting RIF resistant strains of *M. tuberculosis.*

Project results based on the dot blot and direct susceptibility test to INH indicated that the sensitivity was 46.3%, whereas the specificity was 92.3%. Similarly, with regard to the drug RIF, for the *rpo*B gene marker, sensitivity was found to be 65.6% and specificity was 97.5%.

The Director of the National TB and Leprosy Control Programme and the research institution, the Ethiopian Health and Nutrition Research Institute, have collaborated in the national drug resistance surveillance programme and have reported on the progress of the project on the molecular drug resistant detection of TB.

The results from the multicentre study showed that resistance to RIF was found to be highly predictive of MDR-TB, and that these rapid tests may be used to investigate suspected MDR-TB cases or to monitor high risk patients such as those failing standard treatment regimens.

Research results from the project were provided to local authorities and there is good collaboration with the National TB Control Programme.



Acid fast bacilli microscopy test.

Microscopy for mycobacteria has traditionally involved staining the specimen with carbol fuchsin dye, which is retained by the cell wall on washing with acid alcohol (for example, Ziehl-Neelsen). Laboratories must screen specimens for acid fast bacilli and culture and identify isolates as quickly as possible.

Kenya

The number of new cases of TB emerging annually in Kenya has increased fivefold in the past decade, with an estimated 155 000 new cases in 2001. Only 47% of these cases were detected and treated in 2001.

The project aimed to strengthen the capacities of the specialized TB laboratory and to incorporate the laboratory into the WHO surveillance programme.

The project's other objective was to screen suspected TB patients in Nairobi in order to identify the presence of MDR Beijing/W type and other genotypes of *M. tuberculosis* in the country. Thirty-three isolates resistant to one or more drugs (resistance ratio method), including 15 MDR isolates and 40 susceptible isolates selected at random, were analysed by dot blot hybridization for mutations associated with resistance to INH, RIF, streptomycin (SM) and ethambutol (EMB). All strains were genotypically classified using spoligotyping. The results showed that of the 33 drug resistant isolates, 21 (64%) were from males and 12 (36%) were from females. Mutations associated with resistance to INH (*kat*G315) and RIF (*rpo*B526, 531) were confirmed in 83.3% and 100% of the isolates, respectively, and in 87% of the MDR isolates. Mutations were detected in 25% and 71.5% of the isolates resistant to SM (*rps*L43) and EMB (*emb*B306), respectively. No mutations were detected in the drug susceptible isolates. Spoligotyping grouped the isolates into 25 groups. Ten of these groups corresponded to previously identified strain groups, including seven families in the international database. One of these families (CAS1) comprised six (40%) of the 15 MDR isolates. Another family (Beijing) had six (8.3%) isolates, of which two (33.3%) were MDR (Beijing/W).

Interestingly, mutations were detected which had not been picked up using the conventional techniques, thus suggesting a higher sensitivity of the molecular detection methods.

In summary, the project identified the Beijing/W and MDR-TB strains in the country, leading the MoH to establish a task force for further surveillance and implementation of a policy for management of these strains.

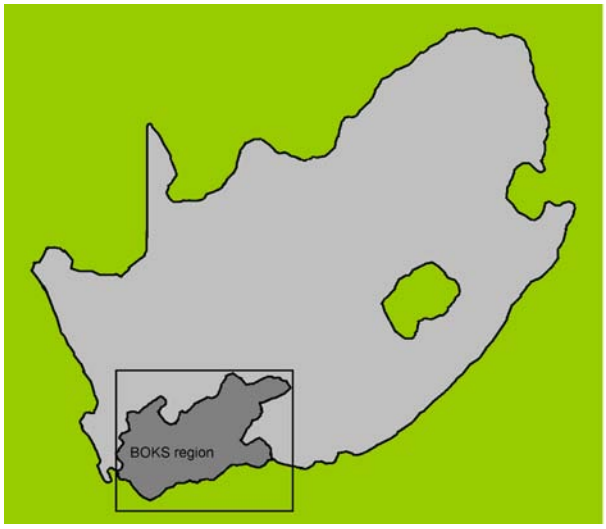
This study is the first in Kenya and the second in sub-Saharan Africa to report the presence of MDR Beijing/W type and other possible drug resistant outbreak strains. The application of molecular techniques and markers will allow the spread of existing drug resistant strains and the appearance of new ones to be monitored.

Furthermore, the outcome of the project recommended that future use of the dot blot technique be considered for inclusion in the national TB surveillance programme for monitoring MDR-TB.

The Kenya Medical Research Institute has established close linkage with the National Leprosy and TB Control Programme.

The capacities of a specialized laboratory were successfully strengthened.

In South Africa, for every 100 000 people, 558 are infected with TB. The incidence is higher in the Boland, Overberg, Karoo and Southern Cape region of South Africa (the BOKS region), where 1300 cases per 100 000 people have been reported. The high incidence of TB in the BOKS region, recorded in 72 clinics, motivated the RAF/6/025 study. Drug resistant TB in the region is managed by a team of physicians and primary health care workers devoted to the management of TB in the public sector. The geographical study area covers 93 000 km^2, with an estimated population of 1 348 405 in the year 2001.



BOKS study area, South Africa.

The RAF6/0/25 study method included the following: a collected aliquot of decontaminated sputum samples (*n* = 500) was submitted to the routine TB laboratory (BOKS region) for TB diagnosis on a weekly basis. The samples were then subjected to a short term mini-culture. The sensitivity and specificity for RIF resistance was 90% and 98%, respectively. Dot blot results were also compared with sequence results and 95% accuracy was obtained, showing reproducibility of the dot blot technique.

It is well known that RIF resistance can be used as a marker for MDR-TB. Therefore, after 5 days of incubation, PCR was performed on the *rpo*B gene followed by dot blot and sequencing. Dot blot results were compared with direct sputum test (DST) results.

By applying the dot blot technique directly on sputum samples for RIF resistance, molecular results was obtained within 10 days and, therefore, directly benefited the patient, the community and the TB Control Programme.

Waiting room, South Africa.

Results for dot blot analysis from the BOKS region were obtained directly from the microscopy positive sputum samples, and the results correlated well with DST results. In addition, DNA sequencing confirmed that the dot blot is reproducible.

A centre of excellence for TB research has been established at the University of Stellenbosch by the South African government in recognition of the excellent work achieved.

The RAF/6/025 study also screened 235 MDR isolates from the BOKS region for EMB resistance. Routine investigation (using the agar diffusion method) detected only 1.7% (EMB) resistant TB stains. Molecular analysis identified 20% of these isolates to be resistant to EMB. The molecular results were confirmed by sequencing. These mutations were confirmed by retesting samples in BACTEC (a proprietary testing kit), and again this confirmed the sensitivity of the dot blot assay. Other observations included:

- No direct comparisons between culture and dot blot methods were possible for EMB resistance, as the 'gold standard' is not accurate.
- RAF/6/025 findings, together with those of others (international), suggest that the culture based method and the critical drug concentration used for EMB testing need to be re-examined.

In terms of cost for comparative TB tests, it was found that the dot blot test would be cheaper than the BACTEC method for MDR-TB cases, as indicated below.

Test	Cost (US $)
1. BACTEC	10.00 per drug
2. PCR	3.00 per sample
3. Dot blot	3.00 per sample

The cost excludes labour, estimated to cost US $5.00 per assay

The research output of the project has identified that the dot blot technique can be applied to samples from known 'hot spot' regions identified through TB surveillance studies to determine epidemic outbreak strains of TB or MDR-TB, as well as to clinically difficult to treat TB strains in order to assist clinicians to deal with untreatable or difficult TB cases. An additional advantage of the molecular dot blot application is that all the above scenarios can be carried out directly from sputum, as demonstrated in the project.

United Republic of Tanzania

In 2005, TB cases in the United Republic of Tanzania stood at 342 per 100 000 people per year. TB prevalence registered at 496 per 100 000 people in the same year. MDR cases were 1.8% of TB cases in 2004.

Muhimbili University College of Health Sciences.

Several institutions participated in the project, including:

- Muhimbili University College of Health Sciences;
- NIMR (National Institute of Medical Research);
- MoH;
- NTLP (National Tuberculosis and Leprosy Control Programme).

The research findings of the project showed that strains of TB resistant to INH were 5.0%, to EMB, 12%, and to RIF and SM, 9.0%.

Further data analysis of the dot blot indicated that sensitivity was 91.7%, and the specificity was 99.2% to RIF. INH sensitivity was 93.4% and specificity was 88.9%; SM sensitivity was 100% and specificity was 99%; and EMB sensitivity was 85.7% and specificity was 97%. These results were in agreement with the mutational analysis on three codons (*rpo*B531, *rpo*B526 and *kat*G315). The study identified up to 90% of the MDR-TB cases.

The achievements of the project were as follows:

- Collaboration was established with the NIMR, which receives funding from the MoH under the NTLP.
- Dot blot results were obtained within 48 h of receiving a culture on a solid media, compared with 5–7 days and 6–8 weeks for the radiometric BACTEC 460TB and conventional Löwenstein–Jensen proportional methods, respectively.
- High sensitivity and specificity was acquired.

The dot blot technique can be adopted for screening MDR-TB in the National TB Reference Laboratory. This will allow alternative and effective treatment of TB patients and will curb the spread of MDR-TB.

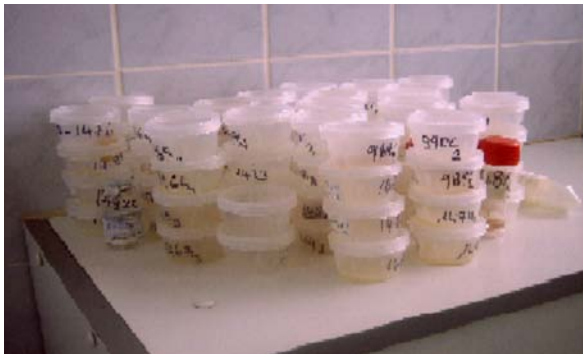
Uganda

In Uganda, the *M. tuberculosis* notification (incidence) rate, according to the WHO, currently stands at 138 cases per 100 000 people. The TB and HIV co-infection rate is 1532 cases per 100 000 people.

12% of all patients tested showed resistance to at least one first line drug (RIF 1.4%, INH 7.9%, SM 6.1%, ETH 0.9%), and resistance to more than one TB drug has been recorded to be 4.7%. Specificity to RIF was 98%; sensitivity to RIF was 32%. Similar observations were made for INH.

The Uganda National Tuberculosis and Leprosy Control Programme collaborated closely in the project, providing the TB samples (cryopreserved *M. tuberculosis*).

Most high prevalence countries continue to use slow culture based methods to investigate suspected MDR-TB cases. These traditional phenotypic methods of detecting the drug resistant disease are slow, due to the protracted growth rate of *M. tuberculosis*, with results often taking weeks to obtain.



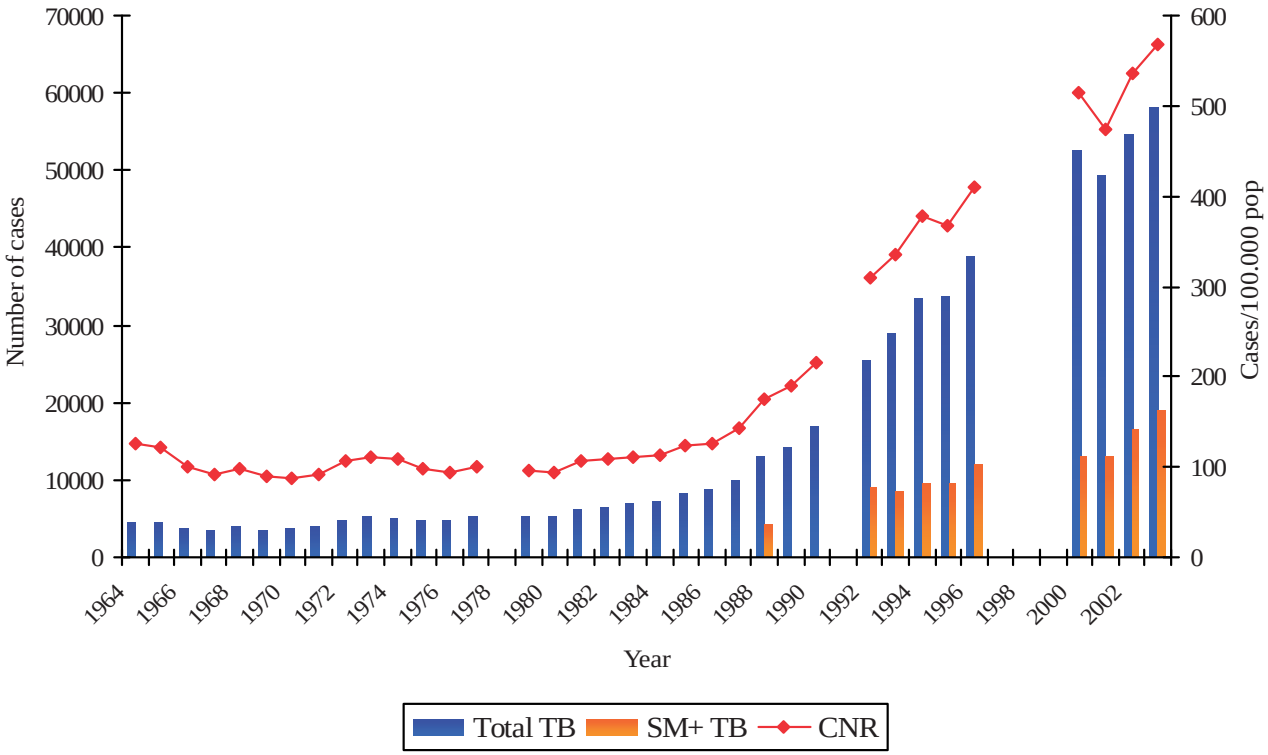
M. tuberculosis sputum samples.

Zambia

The population of Zambia is approximately 10 million. The country is divided into nine provinces and 72 districts. TB notification rates had risen to 580 per 100 000 by 2004. This is mainly due to the HIV/AIDS epidemic. Estimated HIV sero prevalence is between 70% to 80% among TB patients.

Collaboration with the Chest Diseases Laboratory was established at the beginning of the project. In addition, the Regional Tuberculosis Reference Laboratory was mandated by the MoH to provide quality assurance services. A molecular laboratory was established and staff were trained in basic molecular biology techniques. This allowed the Zambian Tropical Disease Research Centre (TDRC) to attract projects that require molecular biology analysis.

The TDRC is now called upon to assist with various forms of training, including of the District Health Management Team's laboratory personnel. It is also being asked to assist in the supervision of diagnostic laboratory services in the northern region of the country.



TB notifications in Zambia.

M. Tuberculosis: Pooled Analysis of Project Results for all Countries

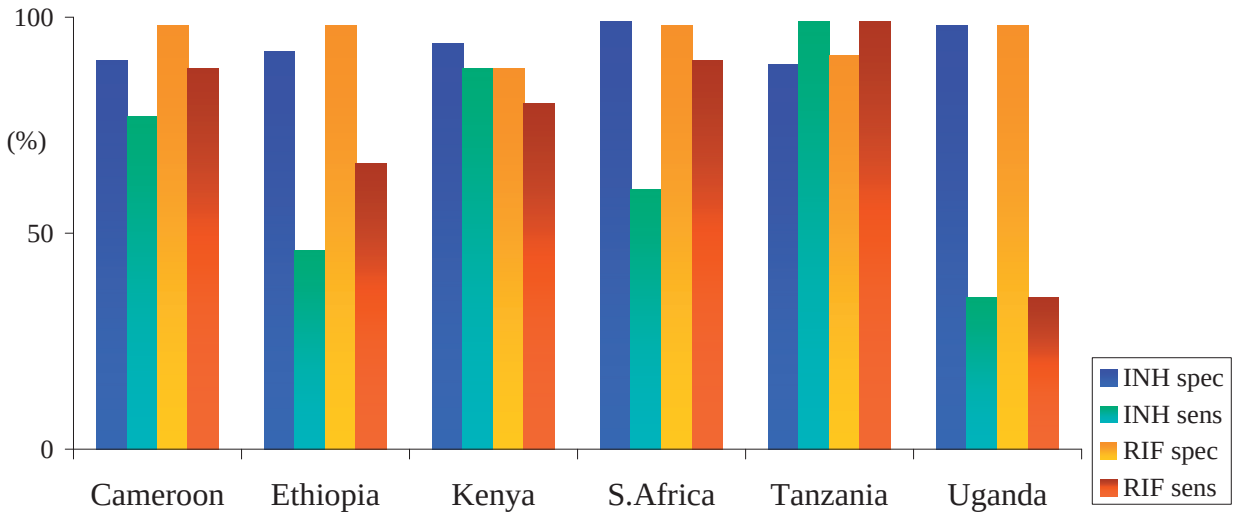
A multicentre study was conducted under project RAF/6/025 with the objectives of validating the dot blot technique for detecting *M. tuberculosis* drug resistance and comparing the technique with other current methods. The anti-TB resistant samples were obtained from local clinical isolates of *M. tuberculosis*. Anti-TB drugs included INH, RIF, SM and EMB. Seven countries with high burdens of MDR-TB were identified. The study population was individuals found to have smear- and culture-positive samples, identified mostly through national drug resistance surveillance studies. All samples resistant to at least one of the drugs were included in this study. In addition, a systematic sample of cultures susceptible to all four drugs was selected by a sampling strategy. *M. tuberculosis* reference strains H37Rv, together with well characterized clinical isolates of *M. tuberculosis*, were used as positive controls. Selected wild type and mutant specific oligonucleotide probes for each codon investigated were 5' end labelled by phosphorylation with [γ-32P]-ATP: *kat*G315, *rpo*B511, *rpo*B516, *rpo*B526, *rpo*B531 and *emb*B306. Mutation analysis by dot blot hybridization was performed by each laboratory on the local isolates. External quality control was performed by sending discordant results for sequencing to a TB reference laboratory. For each drug, samples found to contain drug resistant and drug susceptible *M. tuberculosis* using the conventional method were used to evaluate the performance of the dot blot method. The sensitivity and specificity of the dot blot method against the conventional method was calculated for each country and combined across all countries. These molecular techniques were central to the description and control of outbreaks of MDR-TB and the detection and monitoring of emerging MDR-TB strains.

External quality control for the conventional method was carried out for all countries represented in this report. For both conventional and molecular techniques, internal quality control was performed using standard protocols for the tests.

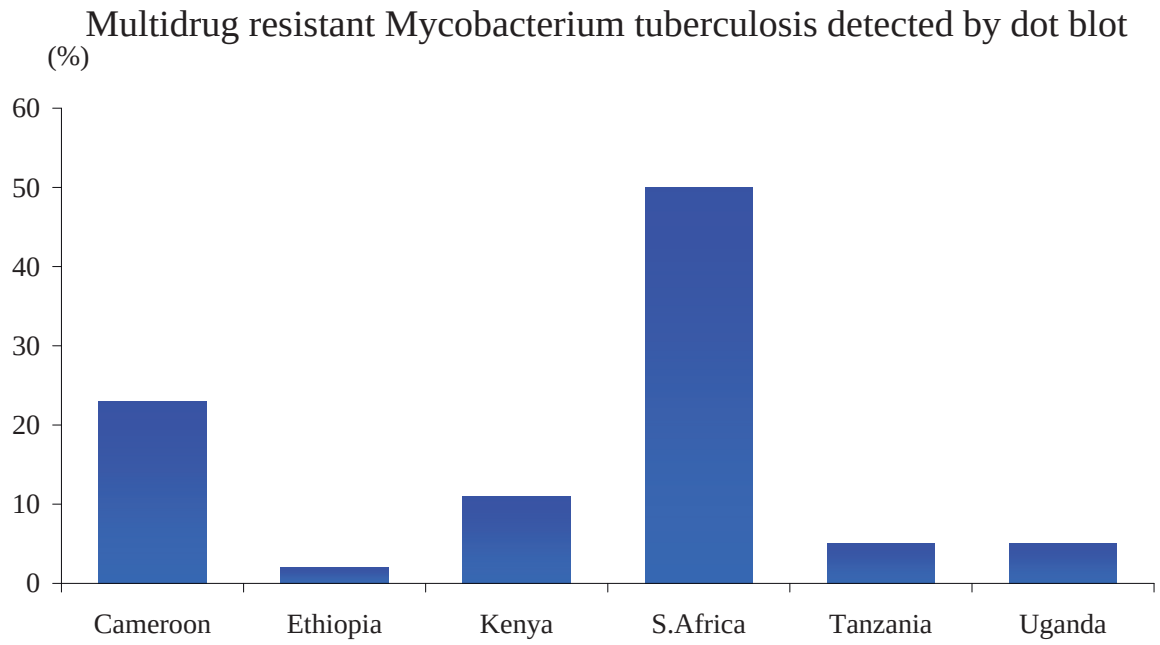
Over 7800 *M. tuberculosis* samples were subjected to in vitro culture assay and over 370 samples were analysed for genetic mutations associated with treatment failure of the anti-TB drugs. The study population was high risk patients that were found to have smear- and culture-positive samples from national drug resistance surveillance studies.

The sensitivity and specificity values varied for different countries and were: for INH 58–100% and 90–100%, respectively; and for RIF 32–90% and 95–100%, respectively. Countries such as Uganda and Ethiopia reported lower sensitivity values. The unusual discrepancies could be attributed either to a poor performance of the phenotypic tests or the presence of unknown targets for these drugs in the tubercle bacilli. The introduction of quality assurance systems, particularly for samples from the national TB control programmes, has been emphasized. Dot blot results were also compared with sequence results and 95% accuracy was obtained, demonstrating excellent reproducibility of the dot blot technique. Both external and internal quality control measures were included.

Dot blot versus conventional assay: sensitivity and specificity



In some settings, resistance to RIF has been shown to be highly predictive of MDR-TB (Kenya, South Africa). Therefore, this may be used to investigate suspected MDR-TB cases or to monitor high risk patients such as those failing standard treatment regimens. The dot blot system validated in the RAF/6/025 participating countries can be used for such activities in countries where resources are limited.



Summary of Achievements

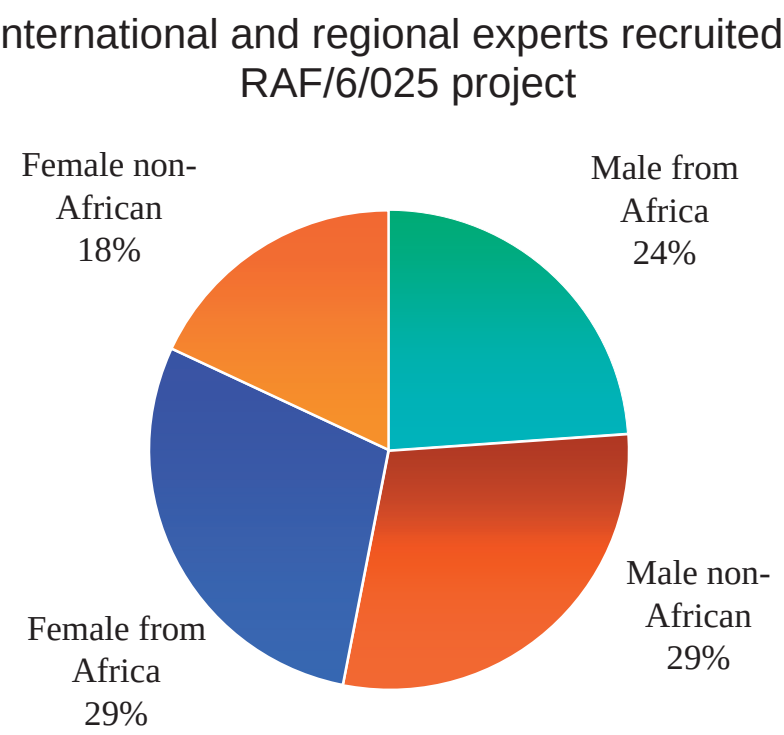
A total of 4310 malaria patients were enrolled into in vivo drug efficacy trials, and 7359 molecular tests, including radioisotopic assays (P-32), were performed. 3192 samples from TB patients were collected and were submitted to drug susceptibility testing for four anti-TB drugs by culture (*n* = 7839) and molecular analyses (*n* = 376), including radioisotopic methods (P-32).

The results showed the presence of drug resistant malaria and TB in all of the participating countries, and MDR-TB was detected in nearly all countries. For malaria, good correlations were obtained between molecular markers and treatment failure, and data were presented in terms of GRI values. Results from the studies were used directly to influence policy changes in first line antimalarial use in six countries. For TB, there was also a good correlation between phenotype and genetic markers. These molecular techniques were central to the description and control of outbreaks of MDR-TB, and the detection and monitoring of emerging MDR-TB strains.

The project resulted in 30 scientific publications in international journals, three publications in national journals and 38 scientific presentations at international and national conferences. The project resulted in the upgrading of laboratory facilities in eight countries, and the establishment or strengthening of molecular capacity in all 12 countries. The IAEA contributed approximately $2 million to capacity strengthening for this project.

Extensive training was carried out through four regional training courses, 22 fellowships and 22 expert visits. A total of 36 people in 11 African countries benefited. The project contributed towards postgraduate training for 33 MSc and PhD candidates. Counterparts also organized 20 local training courses, fellowships and postgraduate training. The IAEA contributed in some cases towards operational costs.

Over 70 collaborative networks were established: 17 locally or intra-institution, over 26 nationally and 27 internationally. Ten of the counterparts were successful in obtaining significant governmental or international funding to continue and develop the project. Awareness was raised not only through publications and collaborative networks, but also in the public health sector, at various levels, including seven reports, 26 local presentations, as well as 22 TV and radio interviews and seven articles in local newspapers. Through these methods, health care and disease control programme workers became aware of the emergence and spread of highly resistant strains of TB and malaria which are difficult to treat.



Gender differences and gender inequalities can give rise to dissimilar health status and access to health care. The Gender and Malaria Statement of November 2005, delivered at the Roll Back Malaria Partners' Forum V in Yaoundé, Cameroon, emphasizes that "women are recognized as equal partners and stakeholders in the fight against malaria". There is also an increasing international awareness of the need for countries to design and promote gender sensitive health policies and strategies. A major achievement of project RAF/6/025 was the empowering of women researchers in 13 countries in technical aspects of malaria and TB drug resistance control. More than ten women received fellowships to undertake masters or PhD programmes in universities away from their home institutions. In addition, three female experts from Africa were called upon to train others within the region in techniques of molecular applications incorporating the use of radionuclide based techniques in the detection of resistant malaria and TB infections. Furthermore, four regional training workshops were attended by 37 participants, which included seven young women trainees. These efforts are very relevant in supporting gender mainstreaming in the area of radionuclide technology to alleviate the malaria and TB disease burden in Africa and the world in general.

Finally, the participating laboratories made excellent progress in meeting the objectives of the project. The successful engagement, from the outset of the project, of national governments, disease control programme managers and clinicians has laid the foundations for sustainability and for moving the technology into a clinical setting. The project has also generated a critical mass of expertise within the African region, which could be expanded to include similar technologies for other high burden diseases.

Issues for Consideration

Research outcomes from the project identified key areas to be considered in future activities. These include:

Malaria:

- Use known markers such as *pfcrt* and *pfmdr*1 where ART + AQ is used.
- Use *pfmdr*1-86 and 1042 codons where Co-artem (lumefantrine + artemether) is used.
- Look for new genetic resistance markers for upcoming artemisinin derivatives.

M. tuberculosis:

- The dot blot technique can be applied in drug susceptibility testing for drugs for which conventional testing cannot be carried out easily, for example PZA and EMB.
- In surveillance studies of 'hot spot' (epidemic-prone) regions, the dot blot technique can be used in the drug resistance evaluation of *M. tuberculosis*.
- Screening for RIF mutation and the use of RIF as the marker for MDR-TB.
- The detection of drug resistant TB from sputum samples for countries with limited culture facilities.

With regard to gender in malaria research, there are a number of study areas to explore, including:

- Malaria and pregnancy: pregnant women with malaria are vulnerable to complications such as mortality, low birth weight for newborns, etc. Investigations into what determines the impact of malaria outcomes (whether mortality, low birth weight, etc.) in relation to gender at the household level will highlight gender related challenges.
- Home based treatment: women are the primary caregivers (or sufferers) at home; they have minimal or no control over resources. Studies on how such social factors impact on malaria control from the gender perspective can be examined.
- Access to health care: health services are not always suited to the needs of women. Research on how best to integrate gender perspectives in allowing access to health services is another area that can be explored.

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Abbreviations

ACT	artemisinin combination therapy
AQ	amodiaquine
BACTEC	a proprietary TB testing kit
BOKS	Boland, Overberg, Karoo, Southern Cape regions of South Africa
CQ	chloroquine
DMC	Division of Malaria Control (Kenya)
DNA	deoxyribonucleic acid
DST	direct sputum test
EMB	ethambutol
GRI	genotype resistance index
HIV/AIDS	human immunodeficiency virus/acquired immune deficiency syndrome
IMRAT	Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria
INH	isoniazid
IUATLD	International Union Against Tuberculosis and Lung Disease
MoH	Ministry of Health
MDR	multidrug resistance
MDR-TB	multidrug resistant tuberculosis
NIMR	National Institute of Medical Research (United Republic of Tanzania)
NTCP	National Tuberculosis Control Programme
NTLP	National Tuberculosis and Leprosy Control Programme (United Republic of Tanzania)
NMCP	national malaria control programme
NMIMR	Noguchi Memorial Institute for Medical Research (Ghana)
PCR	polymerase chain reaction
RNA	ribonucleic acid
PZA	pyrazinamide
RIF	rifampicin
SM	streptomycin
TB	tuberculosis
TDRC	Tropical Disease Research Centre (Zambia)
WHO	World Health Organization

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