IMPACTFUL MAPS AND ASSOCIATED VISUALISATIONS ON ANTIMALARIAL DRUG RESISTANCE FOR MALARIA PROGRAMMES AND POLICYMAKERS.

Dr Frank M. Kagoro, PgDip, MSc

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Medicine

University Of Cape Town

October 2024

Supervisors: Prof Karen I. Barnes and Prof Richard J. Maude

Abstract

The spread of antimalarial drug resistance threatens global, regional and national malaria elimination efforts. Despite being preventable and treatable, malaria still claims over half a million lives globally, with over 95% of malaria cases and deaths occurring in Africa. Antimalarial drug resistance threatens progress in malaria control and elimination, especially in less-resourced health systems in developing countries. Malaria control and elimination efforts are facing stagnation of funding and competing resources with other potential pandemic pathogens like COVID-19, HIV, tuberculosis and non-communicable diseases. Regrettably, the malaria programmes and policymakers who are at the forefront of confronting antimalarial drug resistance often lack timely monitoring tools for evidence-based decision-making.

This thesis used an iterative, sequential, explanatory, mixed-methods design to strengthen evidence on *Plasmodium falciparum* antimalarial drug resistance in Asia and South Africa. Through four thesis chapters of peer-reviewed manuscripts, the thesis presents innovative approaches to developing impactful policymaker-friendly tools for detection, reporting and responding to antimalarial drug resistance. Through co-design techniques, the thesis addresses major data challenges and developed guidelines and tools to support near-real-time antimalarial resistance monitoring. The thesis also highlights important processes and pinch points in rolling out an early warning system for antimalarial drug resistance in a pre-elimination setting in South Africa.

This research contributes to best practices in summarising evidence for antimalarial drug resistance for policymakers and decision-makers. Furthermore, this thesis provides insights on the process of establishing an early warning system in a pre-elimination malaria setting, a context reflective of where several Southern African countries and Island states are heading in this decade.

Inclusion of Publications Declaration

I, Frank M. Kagoro, confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s).

Signed by candidate

Signature:…… Date: 25 October 2024

List of Publications

1. Kagoro, F. M., Barnes, K. I., Marsh, K., Ekapirat, N., Mercado, C. E. G., Sinha, I., Humphreys, G., Dhorda, M., Guerin, P. J., & Maude, R. J. (2022). Mapping genetic markers of artemisinin resistance in *Plasmodium falciparum* malaria in Asia: a systematic review and spatiotemporal analysis. The Lancet Microbe. 3 (3), e184-e192.
2. Raman, J. and Kagoro, F. M., Mabuza, A., Malatje, G., Reid, A., Frean, J., & Barnes, K. I. (2019). Absence of kelch13 artemisinin resistance markers but strong selection for lumefantrine-tolerance molecular markers following 18 years of artemisinin-based combination therapy use in Mpumalanga Province, South Africa (2001-2018). Malaria Journal, 18(1).
3. Kagoro, F. M., Allen, E., Mabuza, A., Workman, L., Magagula, R., Kok, G., Davies, C., Malatje, G., Guérin, P. J., Dhorda, M., Maude, R. J., Raman, J., & Barnes, K. I. (2022). Making data map-worthy—enhancing routine malaria data to support surveillance and mapping of *Plasmodium falciparum* antimalarial resistance in a pre-elimination sub-Saharan African setting: a molecular and spatiotemporal epidemiology study. Malaria Journal, 21(1), 1–19.
4. Kagoro, F. M., Allen, E., Raman, J., Mabuza, A., Magagula, R., Kok, G., Malatje, G., Guérin, P. J., Dhorda, M., Maude, R. J., & Barned K. I (2024). Factors affecting the integration of an early warning system for antimalarial drug resistance with a routine surveillance system in a pre-elimination sub-Saharan African setting. Plos One (Under review)

Plagiarism Declaration

I, Frank M. Kagoro, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature:… Signed by candidate Date: 25 October 2024

Acknowledgements

I extend my heartfelt appreciation to God for granting me the remarkable opportunity to contribute to the realm of knowledge and for bestowing upon me good health throughout this significant endeavour.

I would like to express my eternal gratitude to my esteemed supervisors, Prof Karen I Barnes from the University of Cape Town and Professor Richard Maude from Mahidol Oxford Tropical Medicine Research Unit (MORU). Their guidance, expertise and unconditional support have been invaluable my growth as a person and that of my research.

I am immensely thankful to my co-supervision team, which comprises Dr Jaishree Raman from the National Institute for Communicable Diseases, Elizabeth Allen from the University of Cape Town and the University of Oxford, Mehul Dhorda from MORU, and Philippe Guerin from the University of Oxford. Their collective efforts and support have greatly enriched my academic journey.

My special thanks to the UCT Clinical Pharmacology Division and MORU Epidemiology Team for their unwavering and instrumental support. I would like to thank the generous assistance and collaboration of the Infectious Diseases Data Observatory (IDDO) and the Mpumalanga Malaria Elimination Programme.

Lastly, I would like to express my profound gratitude to my wife – Dr Zyleen Kassamali; I cannot thank you enough for your priceless love and support in sharing every part of this journey with me – family and friends for their trust in me and understanding throughout this challenging endeavour. Your presence has been a source of immense strength and inspiration, enabling me to reach the culmination of this daring journey.

I learnt about system thinking in my Diploma at Swiss TPH, and I became curious about how to apply it to drive science to less-resourced areas. So, when I was introduced to mapping in my MSc in Oxford in 2017, I started digging into how people understand and use maps for infectious disease interventions. At first, I wanted to work in antibiotic resistance but data was the main challenge. Luckily enough, Prof Richard Maude was working on antimalarial drug resistance, and he gave me an opportunity to advance my curiosity, taking it as my thesis topic for my MSc & internship. This led to me wondering what was happening back home in Africa. I was introduced to Prof Karen Barnes at the end of my internship in April 2018, resulting in working with her on piloting surveillance of molecular markers of antimalarial resistance, and this journey of wonder and a lot of learning led to this PhD.

Table of contents

Summary of the Chapters

In **Chapter One**, this thesis addresses the lack of up-to-date and policymaker-friendly antimalarial drug resistance maps in Asia. Thus, through an iterative, sequential, mixed-methods design, literature was systematically reviewed, and evidence from published articles and genomic surveillance data on artemisinin resistance in Asia were collated. Applying a descriptive spatiotemporal design, the study summarised trends and geographic distributions of artemisinin resistance markers and conducted usability assessments to achieve optimal visualisations of antimalarial drug resistance for malaria programmes and policymakers. The study found that there was a steady increase in molecular markers of artemisinin resistance (over time and space). The study identified a lack of consistent data collection over extended periods in the same areas and delays in sharing of data to better inform policy decisions; the median time observed to evidence sharing on artemisinin resistance was 3.6 years. Moreover, data was reported in a heterogeneous way, leading to difficulties in pooling, comparisons and interpretation. Thus, guidelines were developed for minimal standards to be used by genomic surveillance teams to enhance the quality, consistency and completeness of data shared for tracking *P. falciparum* artemisinin resistance.

In **Chapter Two**, to address the gap of the unknown prevalence of *P. falciparum* artemisinin and partner drugs resistance in in Mpumalanga Province, a pre-elimination malaria-endemic area of South Africa, we used routine surveillance data and applied the knowledge learnt from Asia for spatiotemporal mapping of the prevalence of molecular markers of antimalarial drug resistance (MMR) to support Mpumalanga's Malaria Elimination Programme. This study found no evidence of artemisinin MMR but showed increasing parasite tolerance to the artemisinin partner drug, lumefantrine. This could lead to increased drug pressure on the artemisinins, increasing the threat of artemisinin resistance. Hence, there was a need for an early warning system to track antimalarial drug resistance in near real-time.

In **Chapter Three**, the thesis builds on the lessons learnt from Asia and Mpumalanga, highlighting the need to develop an early warning system on MMR to support prompt, effective malaria treatment policy decision-making. This study aimed to look at the feasibility of integrating MMR monitoring into the routine malaria surveillance system in the Nkomazi sub-district, Mpumalanga, South Africa. Through a sequential, explanatory, iterative, mixed-methods approach, we developed a workflow of surveillance and molecular resistance monitoring in near real-time. The study showed that it is feasible to roll out an antimalarial drug resistance monitoring system as an early warning system where molecular surveillance using samples collected during malaria diagnostic testing could be integrated within routine malaria notification information systems in a pre-elimination, sub-Saharan African context. Near-real-

time mapping of MMR on a fine spatial scale provided a rapid and efficient early warning system for emerging resistance. However, data quality issues, a need for regular training, close supervision, and strong programmatic support were identified as key to the sustainability of such a strategy. This identified a need to understand the bottlenecks of rolling out such an early warning system.

In **Chapter Four**, to address the question of barriers to and enablers of integrating an early warning system for tracking antimalarial drug resistance within routine malaria surveillance described in Chapter Three, an explanatory, iterative, mixed-methods study was conducted to explore factors affecting the roll-out of such an early warning system. Using a process-oriented logic model, this study investigated these factors as part of a complex health intervention. Malaria case detection and notification, dried blood spot sample collection, case investigation, analysis and reporting were identified as the five tangible processes required for successful implementation. While healthcare facilities and district and provincial-level healthcare staff were identified as the key stakeholders, and the malaria programme as the main beneficiary, workload, training, ease of use, supervision, leadership, and resources were recognised as cross-cutting influencers affecting the programme's performance.

In **Chapter 5**, the discussion pulls together the new knowledge generated through the thesis and reflects on its strengths and limitations, as well as future research priorities. The thesis set out to identify key gaps and share knowledge gained through impactful visualisations and maps of changes in antimalarial resistance over space and time for malaria policymakers. It summarised an iterative framework that focuses on quality evidence, audience, co-design methodologies and iterative evaluation to achieve final products with a significant impact on the malaria elimination programmes. The thesis generated new evidence on the spatiotemporal distribution of antimalarial drug resistance while sharing knowledge on developing an up-to-date, early warning system for tracking antimalarial drug resistance in an elimination region (Asia) and pre-elimination sub-Saharan African setting (Nkomazi sub-district, Mpumalanga), respectively. Additionally, the thesis developed and shared tools for best practices, such as guidelines to assess the essential information needed for optimal surveillance of artemisinin resistance markers, as well as training materials to ensure accurate capturing of Global Positioning System (GPS) coordinates for malaria case investigation and reporting. As a complex intervention, this thesis proposes using a process-oriented logic model as the best approach to monitor the integration of early antimalarial resistance warning interventions into existing surveillance systems. The thesis also emphasises the importance of data accuracy, investment in data curation, and piloting early warning systems. However, the gains of such an integration are highly dependent on the strength of the existing system.

Therefore, we recommend rigorous, supportive supervision and feedback loops whereby the existing system is further strengthened.

List of Tools

List of Figures

Supplementary Figures

List of Tables

Introduction

The emergence and spread of antimalarial drug resistance have been shown to pose a threat to global, regional and national efforts to eliminate malaria [1]. Despite being preventable and treatable, malaria still causes over half a million deaths globally annually [2]. Following a 30% decline in annual malaria case incidence rates between 2000 and 2019, 2020 showed a 3% increase with no further change for the past three years [2]. The emergence and spread of antimalarial drug resistance endangers further progress, especially in less-resourced health systems of developing countries. Progress towards elimination has been particularly strong in Southeast Asia [3, 4], but the threat of antimalarial resistance posed a major threat to these efforts, despite substantial investment to combat it [5]. However, in Africa, the threat of artemisinin resistance has already been realised in East Africa and the Horn of Africa and is compounded by the stagnation of funding and competing resources with pandemic potential pathogens like COVID-19, HIV, tuberculosis and non-communicable diseases [6]. Unfortunately, the malaria programs and policymakers in Africa and Asia, who are at the forefront of addressing antimalarial drug resistance, often lack the necessary, timely monitoring tools for making evidence-based decisions.

Malaria is an infectious disease caused by a protozoal parasite of the genus *Plasmodium (P.)*. Among the five species of malaria affecting humans (*P. falciparum*, *ovale*, *vivax*, *malariae* and *knowlesi*), *P. falciparum* is responsible for most cases and deaths [6]. Considering the significant morbidity and mortality associated with malaria worldwide, there have been several global mobilisation efforts undertaken to eliminate the disease. Historical examples include mobilisation campaigns in the late 19th century and mid-20th centuries, as well as the ongoing elimination involving at least 35 countries aiming to achieve elimination by 2030 [7, 8]. The key to making progress towards malaria elimination lies in the effectiveness of the malaria interventions, including insecticide-treated bed nets, indoor residual spraying, rapid diagnostic tests and – the focus of this thesis - antimalarial drugs. Better use of these resources, supported by their evidence-based targeting, is crucial to advance the current World Health Organization phased strategy for elimination by 2030. However, the emergence and spread of resistance to key insecticides, rapid diagnostic tests and antimalarial drugs, threatens these efforts.

Artemisinin and its derivatives are the cornerstones for the treatment of both uncomplicated and severe *P. falciparum* malaria. Artemisinin derivatives include dihydroartemisinin, artesunate, and artemether [9]. Artemisinin-based compounds are combined with a longer-acting partner drug from a different class (with a different mechanism of action) to create artemisinin combination therapy (ACT). The World Health Organization (WHO) changed the

policy for the first-line treatment regimen in 2004 after the extensive spread of resistance to Chloroquine and Sulfadoxine-Pyrimethamine (SP) treatment, as well as the concern of resistance against artemisinin monotherapies [10]. The WHO-recommended partner drugs currently include amodiaquine, lumefantrine, mefloquine, sulfadoxine/pyrimethamine, piperaquine, and pyronaridine [11]. Antimalarial drug resistance can be assessed 1) in the laboratory through *in vitro* studies, 2) by identifying the genetic (molecular) markers associated with reduced drug susceptibility and 3) by assessing clinical and parasitological treatment response in drug efficacy studies [11].

The Global Technical Strategy for Malaria 2016 – 2030 sets goals and builds on the past malaria efforts. It aims to guide country and regional plans towards malaria elimination, with the third pillar being transforming malaria surveillance into a core intervention [8]. This includes advancing monitoring antimalarial drug efficacy and drug resistance using appropriate methods according to the malaria burden in different areas. Malaria burden can be classified by transmission intensity, using the annual parasite incidence per 1000 population (API) and malaria parasite prevalence. WHO classifies transmission as high when ≥ 450 API or *P. falciparum* malaria prevalence of ≥ 35%; moderate, 250–450 API or a *P. falciparum* / *P*. *vivax* prevalence of 10-35%; low, 100-250 API and *P. falciparum / P. vivax* prevalence of 1-10 % or very low < 100 API and *P. falciparum* / *P*. *vivax* prevalence of 0-1%. WHO recommends therapeutic efficacy studies (TES) as the gold standard for areas assessing drug efficacy. Due to their resource intensiveness, TES may not be feasible for areas of low and very low malaria transmission where any given health facility is only able to recruit a small sample size, hence the recommendation of integrated drug efficacy surveillance (iDES) in both low and very low transmission areas. Surveillance of MMR is also recommended for low and very-low-transmission areas[11].

The 2014 discovery of *P. falciparum* mutations in the propeller region on the *Kelch13* (*K13*) gene associated with artemisinin resistance [12] characterised by the delayed parasite clearance phenotype has led to their widespread use in molecular surveillance and studies on antimalarial resistance [13, 14]. According to the WHO, artemisinin resistance is suspected in a population if more than 10% of patients are still carrying parasites three days after the start of ACT treatment and confirmed when there is also a validated K13 mutation present [11]. WHO maintains an established list of validated and candidate K13 mutations, which is regularly updated depending on the evidence available. According to WHO, a mutation is classified as a validated K13 mutation when it has demonstrated a significant link with over 5 hours of half-life clearance or day three parasitemia across at least 20 clinical cases [11]. Additionally, the mutation must showcase parasite survival of over one percent using the ring stage assays in at least five separate isolates. If only one of these criteria is met, the mutation

is considered a candidate marker [11]. K13 mutations have become a useful tool for detecting and monitoring artemisinin antimalarial resistance and supporting policymakers in prioritising resources. However, molecular markers such as K13 required further research to understand their role and feasibility as part of routine surveillance, particularly in pre-elimination settings.

Since the discovery of *k13* gene mutations, there have been country, regional and global initiatives focused on mapping the geographical distribution and changing pattern of artemisinin (and partner) drug resistance over time [15, 16]. These, however, seldom incorporate the perspectives of policymakers as the users who are on the frontline of responding to antimalarial drug resistance. It remains unclear whether the preference for using thematic maps is due to their simplicity or if this stems from limited resources and personnel available to develop more precise or tailored maps that can support malaria program decision-making more effectively. It is also unclear whether such preferences would pertain equally to malaria burden and antimalarial resistance.

Thematic, spatial, temporal or modelled maps are frequently used to support the planning, implementing and monitoring of malaria interventions [17, 18]. Maps are all highly dependent on the data from which they are generated. Although seldom thoroughly appraised, the success of these analytical visualisations is deeply embedded in the data from which they are generated [19–22]. Previous work on evaluating how people interact with and interpret maps has shown that the audiences' interpretation can be affected by the design of the maps, the message being presented, and user or context factors [23]. For instance, graphical data interpretation varies with a user's experience, role (e.g., policymaker or scientist), and the data's time frame. Language and colour vision also influence users' interpretation [24, 25]. Therefore, the universality of maps can be questioned, and there is a need to explore which mapping techniques and formats are most suitable for different audiences in different geographical contexts, especially in the understanding of malaria transmission and the emergence and spread of antimalarial drug resistance.

Malaria cases in the Greater Mekong Sub-region (GMS) have declined sharply, with a 77% reduction from 2012 to 2022, accounting for 94% and 48% reduction in *P. falciparum* and *P. vivax* respectively, with the latter overtaking and becoming the predominant parasite in the GMS[5]. By 2022, the WHO Southeast Asia Region contributed 2% of the malaria burden globally, with China and Sri Lanka certified malaria-free. India carries the majority of the burden, with over 65.7% of all malaria in the region, with Myanmar noting a resurgence of malaria cases[2]. However, the progress made remains threatened by antimalarial drug resistance. Artemisinin resistance was first reported in 2008 in Cambodia, only a few years after ACTs became the drug of choice for the treatment of *P. falciparum* malaria in 2004 [26].

By the end of 2016, artemisinin resistance had been documented in Myanmar, Thailand, China (Yunnan Province), Cambodia, Vietnam and Lao Peoples' Democratic Republic (Lao PDR), with reports of its spread to parts of Eastern India [27–29], followed by reports of concomitant resistance to the ACT partner drugs in many of these areas.

Recognising the critical importance of controlling antimalarial drug resistance, the WHO called for coordinated efforts to combat artemisinin resistance in Southeast Asia. The Emergency Response to Artemisinin Resistance (ERAR) was launched in the GMS in 2013 [30]. This initiative emphasised the need for additional tools and resources to effectively address antimalarial resistance and stop its further spread throughout Asia, to Africa and beyond.

As of 2018, reports of genetic markers of artemisinin resistance in Africa were sporadic [28]. Since then, there has been a growing list of African countries that have identified K13 mutations associated with delayed parasite clearance, which, from genetic analysis, appear to have arisen independently [31–34]. This poses a significant threat to Africa, where the malaria burden is highest. The historical spread of chloroquine resistance from Asia to Africa and South America in the 1990s highlights the need for vigilant monitoring and a prompt and effective response to this threat [29]. This may be particularly crucial in; 1) low transmission areas where communities are non-immune, drug pressure is high and cross-border malaria transmission is frequent, 2) high transmission areas with a characteristic transient low immunity following cessation or interruption of massive insecticide spraying programmes, leaving these populations unprotected and at a higher risk of developing antimalarial resistance [35–37].

While most of South Africa is considered malaria-free, approximately 5 million South Africans (10% of the country's population) reside in the malaria-endemic areas in Mpumalanga, Limpopo, and KwaZulu-Natal provinces [38]. Almost all malaria infections in South Africa are caused by *P. falciparum*, with local transmission primarily occurring in low-altitude areas [39] near international borders shared with Mozambique and Zimbabwe [39]. Transmission of malaria in South Africa is seasonal, mainly during the summer rainy season (September to April)[40].

South Africa is one of the 2025-malaria-elimination (E-2025) countries and is on target to achieve a 75% reduction in incidence by 2025, as identified by the 2023 WHO Malaria Report [2]. South Africa was estimated to have 7,820 cases in 2022, with a reduction of indigenous transmission by 31.3% from 2012 to 2022 [2]. Most of the malaria transmission in South Africa is imported, with the government estimating this to be as high as 83% [41, 42]. In the past three decades, South Africa has experienced spikes in malaria, such as in 2000 and 2017, when over 60,000 and 30,000 malaria cases were notified, respectively [43, 44]. The former

change was attributed to SP resistance, increased migration along the borders and insecticide resistance. In contrast, the latter was attributed to migration and changes in climatic conditions favouring the multiplication of mosquitoes [45]. By 2023, only a few South African districts had achieved zero indigenous cases, and these still need to sustain this status for the three years required for sub-national certification.

A new national strategic plan for malaria elimination is currently being drafted for 2024 – 2028 (Personal communication, Barnes KI, co-chair South African Malaria Elimination Committee). A key aspect of this and the previous strategic plan is to sustain and strengthen the surveillance system, ensuring that all malaria cases are reported to the malaria information system within 24 hours by 2020 [46]. In alignment with the South African malaria treatment guidelines, malaria diagnosis is conducted using the *P falciparum* histidine-rich protein 2 (*Pfhrp2*)-based rapid diagnostic test (mRDT) or confirmed by microscopy; to date, no infections have been detected in South Africa with dual *Pfhrp2* and *Pfhrp3* deletions that would result in false negative *Pfhrp2*-based rapid diagnostic tests (Personal communication J Raman, National Institute for Communicable Diseases). Individuals identified with asymptomatic or uncomplicated malaria through proactive, active or passive case detection are treated with the WHO-recommended weight-based 3-day Artemether-Lumefantrine (AL) regimen, the only ACT currently available in South Africa, with a single low dose of primaquine recommended in non-pregnant patients over 1 year of age to limit onward *P. falciparum* malaria transmission [47]. Given the low to very low transmission intensity (defined as <5 and <1 malaria case per 1000 population, respectively) of malaria in South Africa and the potential emergence of antimalarial drug resistance, the South Africa Malaria Elimination Strategy also recommended continuously monitoring antimalarial drug efficacy through routine surveillance for MMR [46].

This thesis used iterative, sequential, explanatory, mixed-methods study designs to strengthen evidence on P. falciparum antimalarial drug resistance in Asia and South Africa. The thesis developed impactful policymaker-friendly tools to support best practices in detecting, reporting and responding to antimalarial drug resistance. It also highlighted important processes, barriers and enablers in rolling out an early warning system for antimalarial drug resistance in South Africa.

1 Chapter 1

Mapping Genetic Markers of Artemisinin Resistance in *Plasmodium falciparum* Malaria in Asia: a Systematic Review and Spatiotemporal Analysis

Frank M. Kagoro [1,2,3,4,5], Karen I. Barnes[2,5], Kevin Marsh[4], Nattwut Ekapirat[1], Chris Erwin G. Mercado[1], Ipsita Sinha [1,4], Georgina Humphreys [2,4], Mehul Dhorda[1,2,3,4], Philippe J. Guerin[2,3,4], Richard J. Maude[1, 4, 6, 7]

[1] Mahidol Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

[2] WorldWide Antimalarial Resistance Network (WWARN), Oxford, UK

[3] Infectious Diseases Data Observatory (IDDO), Oxford, UK

[4] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

[5] Collaborating Centre for Optimising Antimalarial Therapy (CCOAT), Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa,

[6] Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA

[7] The Open University, Milton Keynes, UK

Keywords: Artemisinin resistance, Kelch-13, *Plasmodium falciparum*, Asia, Spatial-temporal model, malaria

1.1 Abstract

1.1.1 Background

The increase in artemisinin resistance threatens malaria elimination in Asia by the target date of 2030 and could derail control efforts in other endemic regions. This study aimed to develop up-to-date spatial distribution visualisations of the *kelch13* (*K13*) gene markers of artemisinin resistance in *Plasmodium falciparum* for policymakers.

1.1.2 Methods

In this systematic review and spatiotemporal analysis we used the WorldWide Antimalarial Resistance Network (WWARN) surveyor molecular markers of artemisinin resistance database. We updated the database by searching PubMed and SCOPUS for studies published between January 1, 1990, and March 31, 2021. Articles were included if they contained data on K13 markers of artemisinin resistance from patients' samples in Asia and articles already included in the WWARN database were excluded. Data were extracted from the published articles and authors were contacted when information was missing. We used the lowest administrative unit levels for the sampling locations of all the K13 data to describe the spatiotemporal distribution. The numbers of samples tested and those with each molecular marker in each administrative unit level were aggregated by year to calculate the marker prevalence over time.

1.1.3 Findings

Data were collated from 72 studies comprising K13 markers from 16,613 blood samples collected from 1991 to 2020 from 18 countries. Most samples were from Myanmar (3,842 [23·1%]), Cambodia (3,804 [22·9%]), and Vietnam (2,663 [16·0%]). The median time between data collection and publication was 3·6 years (range 0·9–25·0, IQR 2·7 [2·5–5·2]). There was a steady increase in the prevalence of WHO-validated K13 markers, with the lowest of 4·3% in 2005 (n=47) and the highest of 62·9% in 2018 (n=264). Overall, the prevalence of Cys580Tyr mutation increased from 48·9% in 2002 to 84·9% in 2018.

1.1.4 Interpretation

From 2002 to 2018, there has been a steady increase in geographical locations and the proportion of infected people with validated artemisinin resistance markers. More consistent data collection, over more extended periods in the same areas with the rapid sharing of data are needed to map the spread and evolution of resistance to better inform policy decisions. Data in the literature are reported in a heterogeneous way leading to difficulties in pooling and interpretation. We propose here a tool with a set of minimum criteria for reporting future studies.

1.1.5 Funding

This research was funded in part by the Wellcome Trust.

1.2 Introduction

Malaria has been declining globally with a 50% reduction of reported cases and an 84% reduction in deaths during the Millennium Development Goals era (2000–15); however, no significant progress in reducing the number of global malaria cases has been made since 2015. Malaria case incidence declined by 27% between 2000 and 2015 and has been increasing since 2015[48]. Due to the remaining high morbidity and mortality caused by malaria worldwide, global mobilisation efforts have been made for its elimination. The WHO Global Technical Strategy for Malaria 2016–2030 emphasises the goal of eliminating malaria in 35 countries and reducing malaria cases by 90% in malaria-endemic countries compared with 2015 [4]. Effective interventions such as insecticide-treated bednets, indoor residual spraying, rapid diagnostic tests, and more effective artemisinin-based antimalarial treatments coupled with evidence-based targeting have been prioritised [49]. However, the emergence and spread of artemisinin and artemisinin-based combination therapy partner drug resistance in the Greater Mekong subregion (GMS) pose a substantial threat to these efforts with the potential to spread or emerge further afield, including into India, Africa, and Latin America [1, 11, 50, 51].

Effectively managing artemisinin resistance requires tools to monitor its spread over time and space to guide interventions to control or ideally eliminate resistant parasites [52]. The identification of mutations in the propeller region of the *kelch13 (K13)* gene associated with artemisinin resistance (with the phenotype of slower parasite clearance) in 2014 has enabled monitoring for artemisinin resistance in research studies and increasingly in routine surveillance [53–58].

Some of the detected mutations are associated with a higher degree of resistance measured as a slower parasite clearance and have a wider geographical distribution than others; for instance, Cys580Tyr is predominant in much of the southeastern GMS [57]. According to WHO, artemisinin resistance should be suspected in a population if more than 10% of patients are still carrying parasites 3 days after the start of artemisinin or artemisinin-based combination treatment and is confirmed when there is a concurrent validated *K13*-propeller domain mutation present[58]. The former requires follow-up and retesting of patients; however, this cannot be done in many settings outside of therapeutic efficacy studies in sentinel sites or as part of research studies, all of which are resource- intensive. A practical alternative that is increasingly being used is to collect blood samples as part of routine surveillance to monitor the prevalence of validated K13 mutations over a large area. Such results can then be used

to inform therapeutic efficacy study site selection or trigger a further investigation for studies to identify the resistant phenotype [55].

There is a range of current initiatives monitoring antimalarial resistance using this method, with the main output being maps of resistance marker prevalence [28, 59, 60]. These maps are intended for policymakers to convey information on the geographical distribution and temporal changes in resistance; however, the maps are usually produced separately from different projects with patchy information and varied formats designed by the scientists who generated the data. There is a risk of policymakers misinterpreting the data or not using the maps if the data presented are not clear, understandable, and relevant. Therefore, the maps produced must be comprehensive, reliable, timely, and user-friendly for their use as monitoring tools and for their impact on malaria strategy to be maximised [13, 61].

This study aimed to determine the spatial and temporal distribution of the genetic markers of artemisinin resistance in Asia using data from the published literature on K13 markers, then present this evidence of artemisinin-resistant *Plasmodium falciparum* malaria in a policy maker-friendly format to help guide the planning of malaria control and elimination strategies.

1.3 Methods

1.3.1 Study design

This study used a sequential mixed-methods design; here, we present the quantitative part that includes a systematic review and descriptive cross-sectional spatiotemporal analysis. The following part used a qualitative end-user usability assessment that comprised interviews and feedback from national malaria programme staff to optimise map presentation for policymakers, which will be published separately. Therefore, this Article presents only the spatiotemporal analysis findings and maps with optimised formats.

1.3.2 Search strategy and selection criteria

The previously established database of the molecular markers of artemisinin resistance in the WorldWide Antimalarial Resistance Network (WWARN) surveyor (up to July 31, 2019) was combined with a systematic review of all published research papers in PubMed and Scopus using the search terms “artemisinin”, “kelch”, “kelch13”, and “k13” along with geographical terms as shown in Table S1 (p 99) from January 1, 1990, to March 31, 2021.

Citations were downloaded and screened using Mendeley citation software (version 1.19.4). R software (version 3.6), stringr, and tidytext libraries were used to scan the article titles and abstracts to remove duplicates, non-malaria, non-artemisinin related articles, studies that were not using blood samples taken from patients, and citations that were already contained in the WWARN database [62]. FMK and RJM reviewed the full articles, scanned to assess the article

eligibility (article contained Asia-related K13 markers of artemisinin resistance from patients' samples), and, if eligible, were included for data extraction into an updated K13 marker dataset. In case of conflicts a third reviewer (KIB) was invited to review and their decision would be final.

1.3.3 Data analysis

We examined the full texts of the obtained articles to identify the lowest administrative unit levels for the sampling locations. For the papers with this information missing (n=2) we contacted the authors by email to request these details. Where information was provided on imported cases, the probable location of transmission was used. This process made it possible to aggregate data from multiple studies at least at administrative level 1 (provinces or regions) for all countries. We took a consensus decision to aggregate data to a level that can achieve geographically comparable sizes of subnational administrative unit levels between countries. Therefore, studies in China, India, Pakistan, and Myanmar (with large administrative level 1 units) had data aggregated at administrative level 2 (ie, districts in China, India, Pakistan, and townships in Myanmar). In each study and year of data collection, we identified and extracted all evaluated K13 MMR; this included whether K13 markers were present or absent and the total number of tested samples. The numbers of samples tested and those with each molecular marker in each subnational level were aggregated by year to calculate the marker prevalence over time. The month and year of sample collection were those reported as the end of sample collection for that administrative unit in the relevant paper where this was available (n=47). For those studies without this administrative unit's information, we used the month of the end of the sample collection period (n=11). For those where only the year was provided, we assumed the month to be December (n=8) to give the most conservative (shortest) estimates of the time from sample collection to publication. Validity and risk of bias between studies and in overall data extracted were mitigated by adhering to the study methods (eligibility, data extraction, and analysis).

The final dataset of aggregated molecular marker prevalence was imported for curation into the RStudio development environment (RStudio, Boston, MA, USA) and geocoded to match The Database of Global Administrative Areas (GADM version 3.6) [58, 63–65]. For location names that could not be matched to those in GADM, Google Maps was used as an additional source of geographical information. This spatial dataset was imported to ArcGIS version 10.6.1 (ESRI, Redlands, CA, USA) to produce maps of the spatial and temporal distribution of K13 markers.

A descriptive spatiotemporal analysis was done to produce thematic maps of the distribution of drug resistance markers and their trends by year. Firstly, we grouped K13 mutations by their

level of evidence for *P falciparum* artemisinin resistance using the WHO classification (2020), with the categories of WHO validated or confirmed, WHO associated or candidate, WWARN associated, not associated, and wild type (Table S2 p 100) [11]. WWARN-associated mutations are additional single nucleotide polymorphisms (SNPs) associated with prolonging parasite clearance identified in an individual patient data meta-analysis[64]. We added an unevaluated category to represent K13 SNPs reported in publications but not yet considered to be validated or associated mutations in the WHO or WWARN categories [64]. Secondly, we used line plots and Loess regression to evaluate trends at different levels for temporal analysis[65]. Trends explored included K13 SNPs and their various classification groups for all of Asia, for the GMS only, and for individual WHO validated markers. For the trend analysis, we only included study areas with more than one timepoint. Thirdly, for spatial analysis, we used aggregated prevalence by administrative unit levels by year to produce thematic maps of drug resistance markers.

1.3.4 Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

1.4 Results

We obtained 11,132 published papers from the PubMed and Scopus search. 8,640 articles were excluded because they were duplicates of articles in the WWARN database, had titles that did not include either malaria-related or *P falciparum*-related information, or did not use blood samples taken from patients. The titles and abstracts of the remaining 2,492 articles were screened and 2,380 were excluded because they did not mention artemisinin antimalarials, did not have identifying K13 markers, included samples from asymptomatic patients, and samples collected outside of Asia. Thus, we identified 112 manuscripts for full article review; of these, 92 were excluded (45 re-analysed previously reported data, 23 reported data from returning travellers from outside of Asia, 18 were reviews or opinion articles, and six were non-clinical studies) and 20 were eligible for analysis along with 52 articles in the WWARN database (Figure 1). These 72 articles contained data obtained from the analysis of blood samples collected between 1991 and 2020 from both prospective and retrospective (using stored samples) studies. Most of these studies were published between 2015 and 2019, with a mean of 6.2 publications per year (Table S3 p 101). Over 53% of samples were collected from 2012 to 2015 (9.8% from 2012, 14.4% from 2013, 16.6% from 2014, and 9.2% from 2015). 54 (82.3%) studies were in a single country and 12 were in multiple countries. All studies evaluated molecular markers of artemisinin resistance with

some also quantifying therapeutic efficacy using clinical treatment failure rates (n=28), parasite clearance times or rates (n=13), or in vitro phenotype (n=12).

A total of 16,613 samples were collected in 18 different countries in Asia (Figure 2). Five studies also evaluated samples from patients who had visited other malaria-endemic countries and were classed as imported cases (n=251). Most of the samples came from the GMS (13,440 [80.9%]), with most from Myanmar (3,842 [23.1%]), Cambodia (3,804 [22.9%]), Vietnam (2,663 [16.0%]), and Thailand (2,124 [12.8%]).

The highest numbers of samples analysed by year were in 2014 (2,750 [16.6%]) and 2013 (2,384 [14.4%]), and few samples were included from before 2009 (Figure S1 p 100). The samples originated from a total of 125 different administrative units (level 2 for China, India, Pakistan, and Myanmar, and level 1 for all other countries). Of these, 60 (48%) administrative units were in the GMS.

The median number of samples collected per administrative unit for all years combined was 42 (range 1–2,052), with 40 (32%) administrative units having less than 20 samples and 33 administrative units (26.4%) having more than 100 samples.

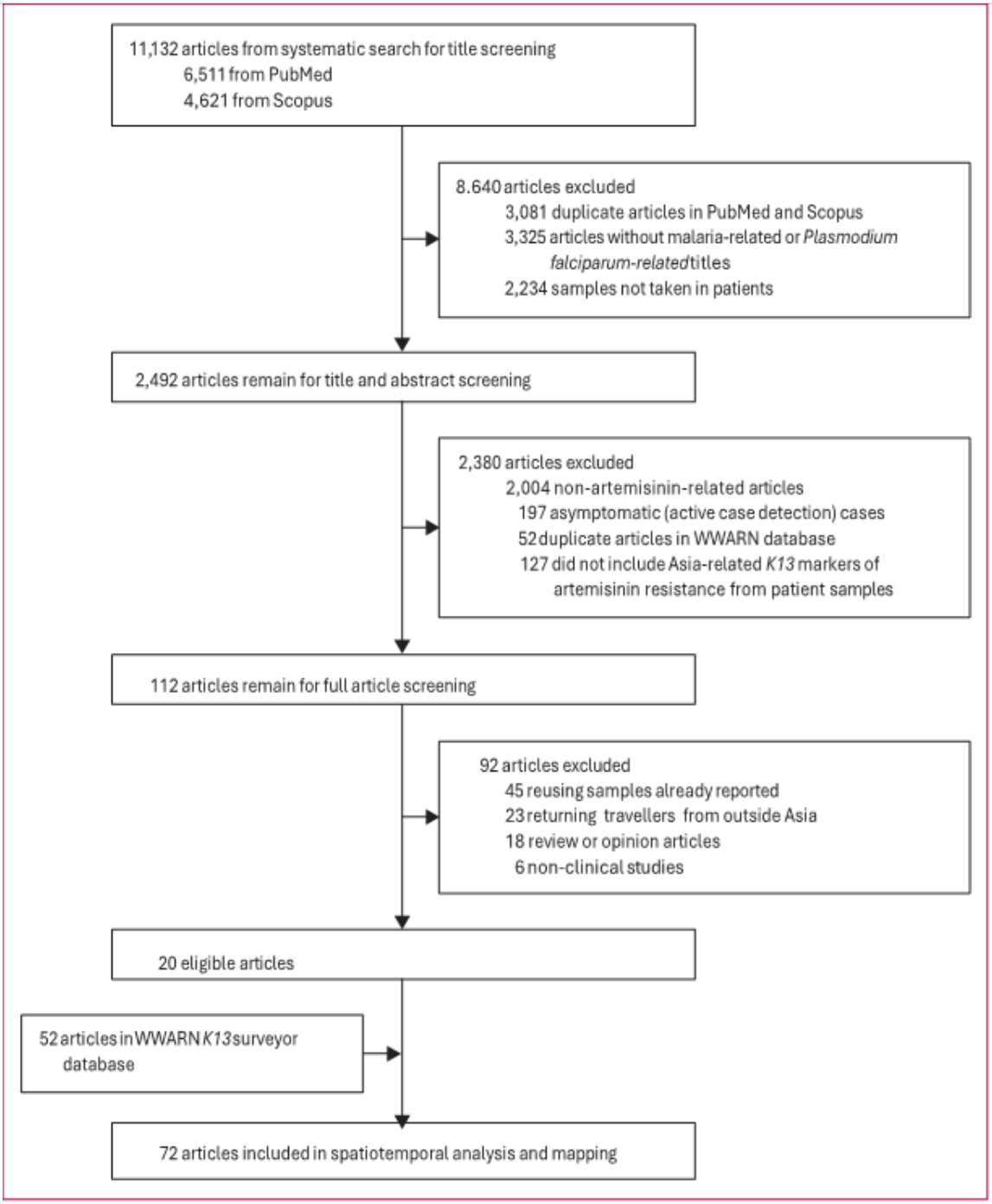


Figure 1: Study selection

K13=kelch13. WWARN=WorldWide Antimalarial Resistance Network.

The median time from sample collection to publication by the administrative unit was 3.6 years (range 0.9 – 25.0; IQR 2.7 [2.5–5.2]; Figure S2 and S3 p 102). Two studies retrospectively evaluated samples collected in 1991 (n=38) and 1997 (n=36) and were published in 2016. We divided the samples into two groups depending on whether samples were collected before or after the discovery of the K13 marker in 2014 [66]. For samples collected before the discovery,

there was a median lag of 4.5 years (range 1.0–25.0; IQR 3.8 [3.5–7.2]). For samples collected after the discovery, this lag was 2.6 years (range 0.9–5.7, IQR 1.0 [2.4–3.4]).

In Asia there were 173 unique K13 mutations reported (Table S5 p 111). We categorised these mutations using 2020 WHO criteria: ten solitary SNPs were classified as validated, 13 isolated and two polyclonal SNPs were classified as associated, and one SNP (Ala578Ser) was classified as not associated and wild type [11]. The other 91 SNPs were considered as unevaluated because they have not yet been classified by WHO or included in the WWARN's list.

Most of the WHO-validated markers of artemisinin resistance were confined to the GMS with the highest prevalence in Cambodia, northeast Thailand, southern Laos, and central Vietnam. These mutations were also identified in three districts in eastern India, one region in Papua New Guinea, and one province in Saudi Arabia. The WHO-associated K13 markers were found in Cambodia, China, Myanmar, Thailand, and Vietnam. Only wild-type K13 markers were found in the other locations studied (Figure 3 and S4 p 103).

The two most prevalent validated molecular markers were Cys580Tyr and Phe446Ile (Figure S5 p 104). Most of the cases with Cys580Tyr mutations were found in the southeastern GMS, including Cambodia, Laos, Thailand, and Vietnam. The Phe446Ile mutation was found in the northern GMS, especially at the borders between China and Myanmar, and Thailand and Myanmar. Phe44GIle has been dominant throughout Myanmar except along the border between Myanmar and Thailand, whereby Cys580Tyr was dominant until 2015, following which Phe446Ile predominated. Cys580Tyr was also reported in 2014 and 2017 in Papua New Guinea.

Our study found that samples from the GMS were collected in more years than outside the GMS (Figure S1 p 100). The GMS had the most samples by location with most malaria-endemic areas being covered (Figure S6 p 105). Validated K13 markers were widely distributed across all the GMS countries with the highest proportions (81–100%) in northeast Thailand, western Cambodia, and central Vietnam. Only associated markers were found in western Myanmar.

Of the 60 administrative units with data in the GMS, 41 (68%) had data from multiple years (Table S5 p 111). The prevalence of validated markers increased in these 41 locations from 48% to 65% overall from 2002 to 2018. Eight (19.5%) of 41 of these locations included more recent data (from 2015, 2016, 2017, or 2018).

Disaggregated data by country showed Cambodia, Laos, Thailand, and Vietnam to have a clear trend of increased validated markers over time (Figure S6 p 105; video). These trends in China and Myanmar were less clear.

Most bordering regions shared similar proportions of molecular markers**.** We observed the highest prevalence of molecular markers of artemisinin resistance in the eastern parts of the GMS (Figure S7 p 106). The administrative units along international borders had more data and showed varied levels of increase in the prevalence of validated markers over time. This trend was most evident in western Cambodia, eastern Thailand, and southern Myanmar (Figure S7 p 106).

There was a sparse distribution of artemisinin resistance data in India, Afghanistan, Bangladesh, Nepal, and Pakistan (Figure S9 p 108). Although sample locations in India were widely distributed geographically and temporally, with collection years from 2010 to 2019, the number of samples in each district per year was generally lower than in the GMS. Validated K13 molecular markers were only detected in 2012 and 2015 in India. These markers were found in the districts of Bankura (Arg539Thr; 2015; 8.3% prevalence), Changlang (Arg561His or Arg561Cys; 2012; 4.2% prevalence), and Kolkata (Phe446Ile and Arg539Thr; 2015; 3.6% prevalence). In 2016 and 2019, only K13 wild-type parasites were reported from 16 districts in India, and in Afghanistan, Bangladesh, and Nepal.

Overall, we found an increase in the prevalence of WHO-validated and WHO-associated markers from a mean of 18.6% for samples collected before 2011 (the minimum of 6.7% in 2003 and maximum of 30.9% in 2010; n=3,324) to a mean of 52.9% for 2011–18 (minimum of 34.5% in 2013 and maximum of 87.4% in 2016; n=12,577; Figure 4). The increase in the prevalence of WWARN-associated markers had a mean of 20% before 2011 and 52.5% between 2011 and 2018 for the same samples. The prevalence of the validated markers increased consistently throughout the study period, with the lowest of 4.3% in 2005 (n=47) and the highest of 62.9% in 2018 (n=264). Only two studies had samples for 2019 (India and Pakistan) and one for 2020 (Saudi Arabia). All the 2019 samples had wild-type parasites (n=202), and 2020 samples had one WHO-validated mutation (Met476Ile), unevaluated markers, and wild-type parasites. All of the 2020 samples were reported in Jizan Province in Saudi Arabia (n=80). None of the GMS administrative units had samples after 2018.

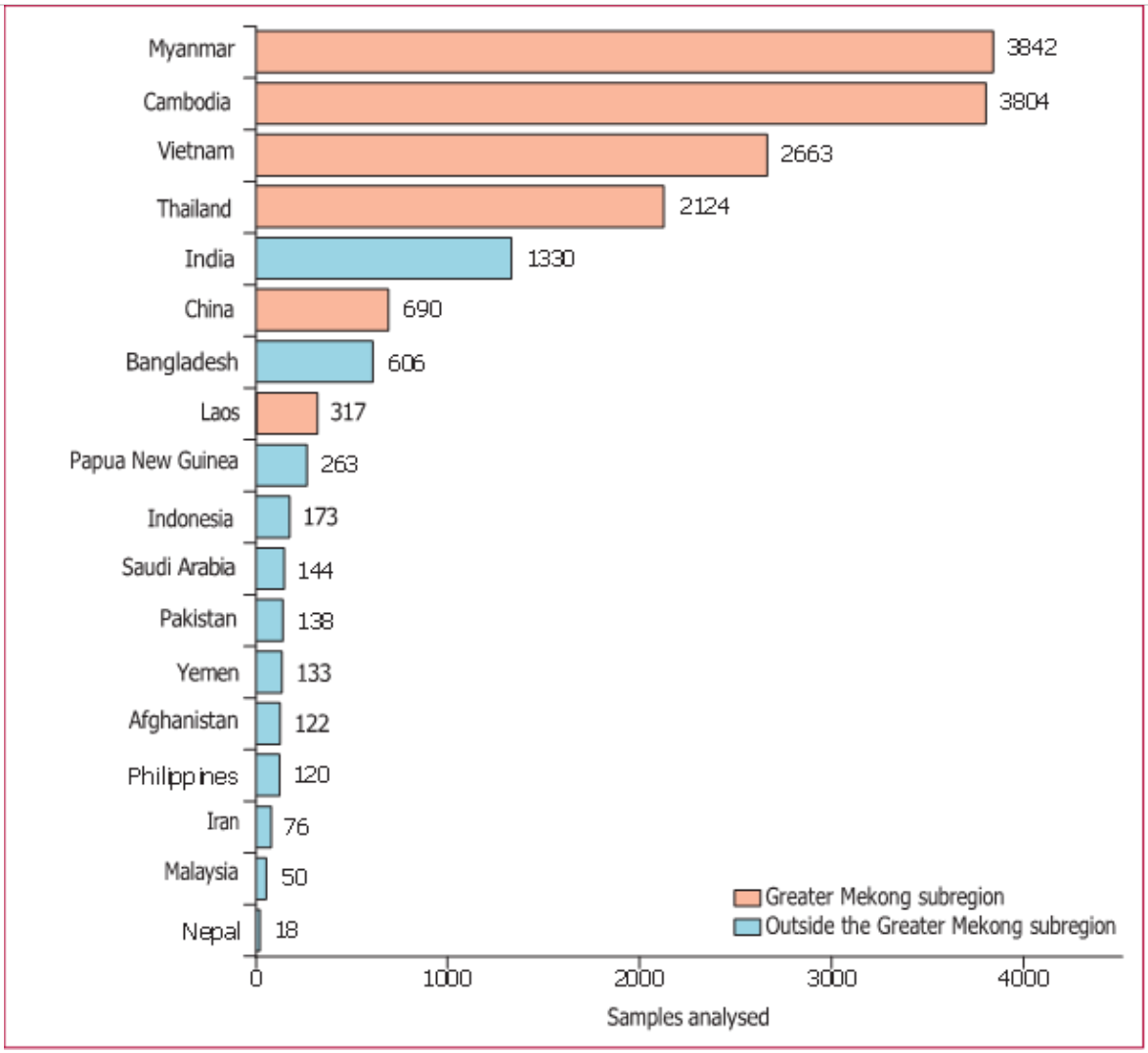


Figure 2: Distribution of samples by country

All samples (n=16,613) obtained from the published K13 studies (n=72) and their distribution, with 13,440 samples from within the Greater Mekong subregion and 3,173 samples from outside the Greater Mekong subregion.

K13=kelch13

In the GMS, proportions of cases with validated K13 mutations increased from 2002 to 2018 and followed a similar pattern over time (Figure S8 p 107). For associated and unevaluated markers, 34 (57%) of the 60 administrative units collected samples in only 1 year. The median of the number of years with samples from the same administrative unit was 1·0 year (range 1·0–14·0; Table S4 p 103). Only 52 (41.6%) of all administrative units in Asia (including 13 157 [79.2%] of all samples collected and 41 [68.3%] of 60 administrative units with data in the GMS) had samples from more than 1 year so could be included in trend analyses.

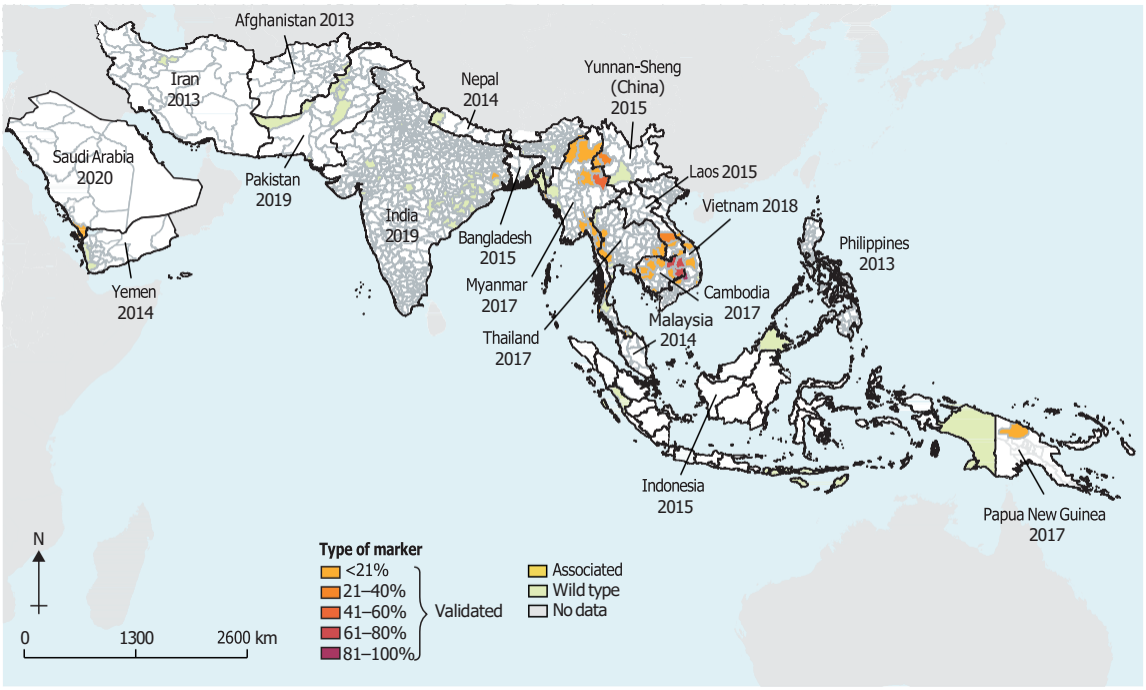


Figure 3: Spatial distribution of K13 markers in Asia

Distribution of the prevalence of K13 markers and the year of the most recent sample collection for each administrative unit. All molecular markers in that year were aggregated in each administrative unit level 1 (Afghanistan, Bangladesh, Cambodia, Indonesia, Laos, Iran, Malaysia, Nepal, Thailand, Vietnam, and Yemen) and administrative level 2 (China, India, Pakistan, and Myanmar). Validated and associated markers were only found in India and the Greater Mekong subregion. K13= kelch13

Overall, the prevalence of WHO-validated molecular markers has increased in the GMS. This increase in prevalence was slow before 2008, levelled off from 2011 to 2014, and then accelerated from 2015 onwards (Figure S8 p 107). The amount of data varied over time, with fewer geographical locations covered before 2010 and in 2018. The changes over time in the WHO-validated K13 marker category was similar to the WWARN- associated markers presented in the WWARN individual patient data meta-analysis; there was also a slight increase of WHO-associated and unevaluated markers from 2010 to 2015 (Figure S8 p 107) [64].

Figure 4: Prevalence of K13 markers by year

All K13 molecular markers were grouped by category and year independently using two classifications (WHO and WWARN) across all countries. The overall prevalence of each category of molecular markers by year was evaluated. Except for 2019 and 2020, there was a consistent increase of WHO-validated, WHO-associated, and WWARN-associated markers over time. Samples for 2019 (from India and Pakistan) showed wild type parasites, and 2020 samples (from Saudi Arabia) had one WHO-validated mutation (Met476Ile), unevaluated markers, and wild type parasites. WWARN=WorldWide Antimalarial Resistance Network. K13= kelch13

Relatively fewer samples were published for 2019 and 2020 than for other years, no validated mutations were reported in 2019, and only one sample had the Met476Ile marker (n=80). Before 2019, the Cys580Tyr marker was detected every year, except in 2003 and 2005 (Figure 5). Some with Cys580Tyr were listed as Cys580Tyr/Cys, which indicates parasites with both Cys580Tyr and wild type (ie, polyclonal infection). There was an increase in samples with Cys580Tyr over time, accounting for most samples in all years except for 2003 and 2005.

There was an increase in the overall proportion of samples with Cys580Tyr or Cys580Tyr/Cys, or both, from an mean of 42.4% between 2002–06 to 71.8% of samples between 2014–18. The prevalence of Cys580Tyr mutation increased from 48.9% in 2002 to 84.9% in 2018.

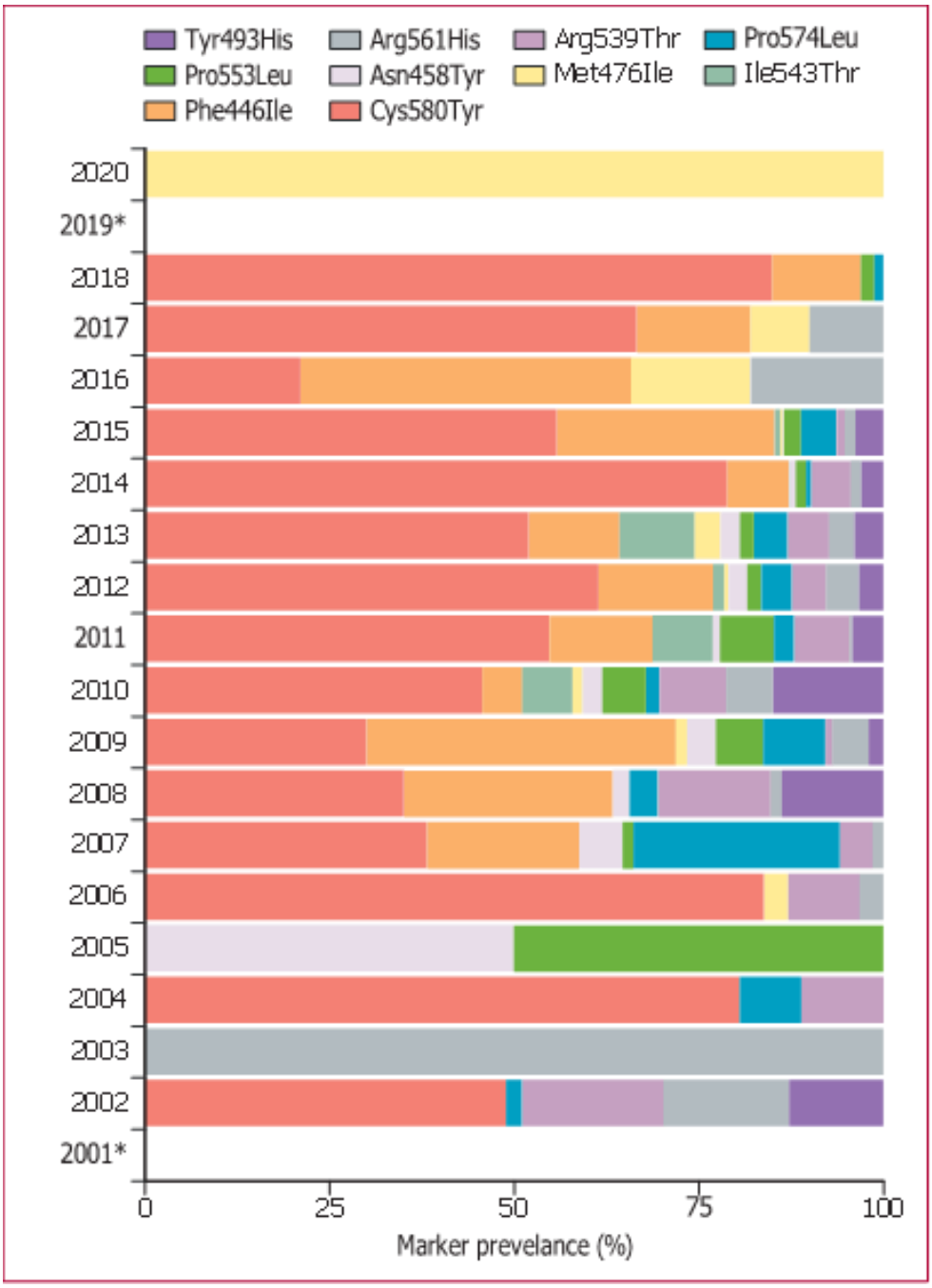


Figure 5: Temporal trends of individual WHO-validated markers

*All samples with single nucleotide polymorphisms categorised as WHO validated pooled together by year and their overall proportions. The 2019 and 2020 samples all came from outside the Greater Mekong subregion, with the result for 2020 being from one (1%) of 80 blood samples from Saudi Arabia. Excluding 2019 and 2020, the Cys580Tyr mutation was the most common WHO-validated mutation in almost every year except for 2003 and 2005. *No validated markers reported.*

1.5 Discussion

We have presented a description of all currently available information on changes in the geographical distribution of genetic markers of *P falciparum* artemisinin resistance markers

(K13 mutations) over time in Asia, including the most recent published data and a subset of unpublished data (up to July, 2020). To do this analysis, we searched citations from the PubMed database and Scopus to update the WWARN K13 dataset. We added additional spatial and temporal details to produce a dataset of K13 markers containing 72 citations. The *P falciparum K13* propeller mutations were used to approximate the spatiotemporal distribution of artemisinin resistance, with an increase in resistance markers by type and geographical extent shown in a range of thematic maps. Over time, we observed a consistent reporting of an increase in the prevalence of artemisinin resistance validated and associated markers in Asia. These mutations were first identified along the border between Cambodia and Thailand in the Battambang and Pailin provinces[26, 67]. By 2009, validated and associated mutations had only been identified in three countries, Cambodia, Myanmar, and Thailand. By 2018, this number had increased to include six additional countries (China, India, Laos, Papua New Guinea, Saudi Arabia, and Vietnam). Thus, increases in the prevalence and extent of WHO-validated or WHO-associated markers over time were seen in Myanmar, Thailand, Cambodia, Vietnam, Laos and, to a lesser extent, in China. Except for India and Papua New Guinea, most of the locations with marker data were along international borders, particularly in the GMS. These border areas are generally where *P falciparum* malaria is most prevalent in these countries[68].

In the present study, we found that although the Cys580Tyr mutation occurs in all the GMS countries, the most frequent validated mutations in the eastern GMS (Cambodia, Laos, east Thailand, and Vietnam) were Cys580Tyr, Arg539Thr, Tyr493His, and Ile543Thr. In western GMS (China, Myanmar, and west Thailand), the most prevalent validated mutations were Phe446Ile, Asn458Tyr, Pro574Leu, and Arg561His. This distribution is consistent with previously published studies, showing localisation of mutations in specific geographical areas, such as Asn458Tyr and Arg561His in the eastern and northern GMS, Arg539Thr in southern and east GMS, and Ile543Thr and Tyr493His in east GMS[28]. Such distribution supports the theory of independent emergence of artemisinin resistance in several locations, with Cys580Tyr sweeping through the region[69, 70]. On the border between Thailand and Myanmar, Cys580Tyr predominated until 2016, when Phe446Ile became more prevalent. This change coincided with an intense effort to eliminate *P falciparum*, which resulted in a significant decrease in cases[71]. Treatment regimens might need to be adjusted for locations with a high prevalence of the subset of K13 markers that are known to be associated with markedly slower parasite clearance and of markers of resistance to artemisinin and artemisinin-based combination therapy partner drugs[72].

Our analysis found a long lag time between sample collection and publication (median 3.6 years). Part of this lag was due to many studies retrospectively analysed samples that had

already been collected before the discovery of the K13 marker in 2014. For studies only analysing samples collected after the discovery of K13, the lag was still substantial at 2.6 years, despite using conservative assumptions for studies in which detailed time information was not available. This lag limits the usefulness and relevance of this marker data for guiding policy decisions. The relative lack of published studies reporting the prevalence of K13 markers in samples collected in 2019 and 2020 is likely to be due to this lag between sample collection and publication. The absence of validated or associated K13 markers in those years is therefore a consequence of the published data being from only a few sites in Pakistan, India, and Saudi Arabia. Large scale sample collections have been ongoing in the GMS during these years, which will contribute more K13 distribution data in the near future[73].

This study has several limitations. Samples were pooled from different studies with different study designs, durations, and sample sizes. Our analysis was limited to published studies and a subset of unpublished data shared and displayed on the WWARN Artemisinin Molecular Surveyor. There were inevitable differences in the level of detail reported and available for each study, particularly concerning which K13 mutations were investigated (and which were not) and when and where the samples were collected. This disparity required us to either make assumptions or constrain the possible spatial and temporal resolution of the analysis. Because of these limitations, we propose a set of reporting criteria for studies that collect K13 molecular marker data, and we have developed a tool that can be used to collate data from published studies and generate a score for these minimal criteria (Tool S1 p 109).

The high disparity of data across the region over time and across geographical locations limited our ability to describe the spatial and temporal trends fully. Only 41.6% of the 125 administrative units included had samples from at least two different timepoints. Thus, it was not possible to assess the trend in resistance marker prevalence for more than half of the administrative units and seven entire countries (Afghanistan, Iran, Malaysia, Nepal, Pakistan, Philippines, and Yemen) with data. Although the *P falciparum* malaria-endemic areas of the GMS were relatively well represented, with 84.6% of samples collected in administrative units, with repeated sampling, the data from other countries were sparser both in space and time. The lack of published K13 markers from some areas might have been due to fewer studies being done in those areas, delayed reporting, or no reporting. We also noted that the K13 markers from the GMS area were only present until 2018 as there was no published study with samples collected after that year.

Prompt reporting of routine surveillance or research activities with a consistent, repeated collection of molecular markers in the exact location (sentinel sites) would allow for more accurate and informative spatial and temporal analysis. The present study's findings can help

identify those areas that would be most suitable for such surveillance activities. However, unless sentinel sites have broad coverage, detecting the emergence of resistance might also be delayed. This study used the K13 propeller mutations to describe artemisinin resistance; however, a 2017 study suggests other K13 mutations apart from propeller mutations (eg, Glu252Gln, Asp281Val, and Arg239Gln) might also be associated with artemisinin resistance[35]. Because the evidence for these markers is absent or scarce due to them rarely being tested for, K13 propeller mutations remain the preferred marker for surveillance of *P falciparum* artemisinin resistance. Our study found a considerable number of unevaluated SNPs that have not yet been classified or graded by WHO or included in the WWARN individual patient data meta-analysis. Such markers could potentially influence the artemisinin efficacy in the geographical areas analysed in this study. Delays in evaluating the association of molecular markers with delayed parasite clearance contribute to the increase of unevaluated SNPs; to date there has been a 3-year gap between WHO evaluations (2014, 2017, and 2020) and only a single evaluation by WWARN in 2017.

This study combined data from all available published sources to define the increase in prevalence and geographical extent of artemisinin resistance markers in Asia. More consistent data collection is needed from the exact locations, from a wider geographical area and over more extended periods to map the spread and evolution of resistance as it continues to unfold. This study also highlights the need for more rapid dissemination of molecular marker data so it is available in time to guide policy decisions.

Contributors

FMK and RJM designed the study and analysed and interpreted the data. FMK and RJM drafted the manuscript. KIB, KM, PJG, NE, GH, IS, MD, and CEGM provided critical review of intellectual content. All authors reviewed the manuscript. FMK and RJM accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The complete and updated datasets, plots, and maps generated and analysed during this study are publicly available on the WWARN website (https://www.wwarn.org/tracking-resistance/artemisinin-molecular- surveyor).

Acknowledgments

This research was funded in part by the Wellcome Trust (grant number 220211). We would like to acknowledge all authors who, through the publication of their data, have compiled a dataset for this study.

We have included the list of articles used in this study in the (pp 16–38). This publication uses data from the GenRe-Mekong Project [73], which is funded by the Bill & Melinda Gates Foundation (OPP111881GG, OPP12042G8) and the SpotMalaria Project coordinated by the MalariaGEN Resource Centre with funding from Wellcome (20G194, 090770). The authors would like to thank the staff of Wellcome Sanger Institute sample management, genotyping, sequencing, and informatics teams for their contribution. We would like to acknowledge Francois Nosten for contributing data into the WWARN dataset and to Sabina Dahlström Otienoburu for compiling the WWARN dataset.

2 Chapter 2

Absence of Kelch13 Artemisinin Resistance Markers but Strong Selection for Lumefantrine-Tolerance Molecular Markers Following 18 Years of Artemisinin-Based Combination Therapy Use in Mpumalanga Province, South Africa (2001–2018)

Jaishree Raman[1,2,3] Frank M. Kagoro[4,5], Aaron Mabuza[4], Gillian Malatje[6], Anthony Reid[7], John Frean[1,2] and Karen I. Barnes[4]

[1]Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, A Division of the National Health Laboratory Service, Sandringham, Johannesburg, Gauteng, South Africa.

[2]Wits Research Institute for Malaria, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa.

[3]UP Institute for Sustainable Malaria Control, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa.

[4]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa. [5]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand. [6]Mpumalanga Provincial Malaria Elimination Programme, Nelspruit, Mpumalanga, South Africa.

[7]Operational Research Unit, Médecins Sans Frontières, Operational Centre, Brussels, Luxembourg.

Keywords: Malaria, *Plasmodium falciparum*, Mutations, Mpumalanga Province, South Africa, ACT, dhfr, dhps, crt76, mdr86, kelch13, Resistance

2.1 Abstract

2.1.1 Background

The ability of *Plasmodium falciparum* parasites to develop resistance to widely used antimalarials threatens malaria control and elimination efforts. Regular drug efficacy monitoring is essential for ensuring effective treatment policies. In low transmission settings where therapeutic efficacy studies are often not feasible, routine surveillance for molecular markers associated with antimalarial resistance provides an alternative for the early detection of emerging resistance. Such a longitudinal survey of changes in the prevalence of selected molecular markers of resistance was conducted in the malaria-endemic regions of Mpumalanga Province, South Africa, where malaria elimination at a district level is being pursued.

2.1.2 Methods

Molecular analyses to determine the prevalence of alleles associated with resistance to lumefantrine (*mdr*86N, *crt*76K and *mdr1* copy number variation) and sulfadoxine–pyrimethamine (*dhfr* triple, *dhps* double, SP quintuple) were conducted between 2001 and 2018, while artemisinin resistance markers (*kelch13* mutations) were assessed only in 2018.

2.1.3 Results

Parasite DNA was successfully amplified from 1667/2393 (70%) of malaria-positive rapid diagnostic tests routinely collected at primary health care facilities. No artemisinin resistance-associated *kelch13* mutations nor amplification of the *mdr1* gene copy number associated with lumefantrine resistance were observed. However, prevalence of both the *mdr*86N and *crt*76K alleles increased markedly over the study period, with all isolates collected in 2018 carrying these markers. SP quintuple mutation prevalence increased steadily from 14% in 2001 to 96% in 2018. Mixed alleles at any of the codons assessed were rare by 2018.

2.1.4 Conclusion

No *kelch13* mutations confirmed or suspected to be associated with artemisinin resistance were identified in 2018. Although parasites carrying the *mdr*86N and *crt*76K alleles associated with reduced lumefantrine susceptibility were strongly selected for over the study period, nearing fixation by 2018, the marker for lumefantrine resistance, namely increased *mdr1* copy number, was not observed in this study. The increase in *mdr*86N and *crt*76K allele prevalence together with intense regional artemether–lumefantrine drug pressure, raises concern regarding the sustained artemether-lumefantrine efficacy. Regular, rigorous antimalarial resistance marker surveillance across all three South African malaria-endemic provinces to inform case management is recommended.

Keywords: Malaria, *Plasmodium falciparum*, Mutations, Mpumalanga Province, South Africa, ACT, *dhfr*, *dhps*, *crt*76, *mdr*86, *kelch13*, Resistance

2.2 Background

Although the global malaria burden has declined markedly since 2000, the disease remains a major cause of morbidity and mortality in Africa. In 2017, Africa accounted for 92% of the estimated 219 million malaria cases and 93% of all malaria deaths [74]. One of the major obstacles to effective malaria control and elimination remains the emergence and spread of antimalarial drug resistance [75]. To increase antimalarial efficacy and delay resistance, the World Health Organization (WHO) recommended artemisinin-based combination therapy (ACT) as the first-line treatment for uncomplicated malaria [76]. South Africa was the first African country to deploy an ACT as first line in 2001 [77]. Artemether–lumefantrine replaced the failing sulfadoxine-pyrimethamine combination (SP) in KwaZulu-Natal (Figure 6), one of South Africa's three malaria-endemic provinces, in 2001 [77], with ACT introduced in the remaining two malaria-endemic provinces, Mpumalanga and Limpopo (Figure 6) in 2003 and 2004, respectively [78].

Figure 6: Map of South Africa showing the endemic provinces.

This map shows the endemic provinces and three municipal districts in Mpumalanga Province.

By 2010, all sub-Saharan malaria-endemic African countries had adopted ACT [75]. Studies have since shown that ACT does not prevent the selection of molecular markers associated with resistance to the partner drugs, particularly if resistance to a partner drug had previously been described in the region [79, 80]. Even more concerning has been the confirmation of artemisinin-resistant parasites along the Thai-Cambodia border [81], the historic epicentre of antimalarial drug resistance. Despite containment efforts, artemisinin-resistant parasites have spread rapidly across at least six countries in the Greater Mekong sub-region [36, 58, 82], with artemisinin resistance most recently also reported in eastern India [83]. While there have been isolated reports of artemisinin-resistant parasites from sub-Saharan Africa [84–86], artemisinin-resistant parasites have not yet become established on the continent [87], where their emergence and spread would severely threaten Africa's malaria control efforts. Following over a decade of impressive gains in controlling malaria and advancing malaria elimination across southern Africa, the region has experienced malaria outbreaks during the last three malaria-transmission seasons [88]. This raised concerns that antimalarial resistance may be contributing to the sharp increases in malaria case numbers, as had been observed previously with both chloroquine (CQ) and SP resistance [89].

To ensure efficacious ACT is in place, it is imperative that regular, rigorous antimalarial drug efficacy/resistance monitoring occurs. The gold standard for assessing drug efficacy, in vivo therapeutic efficacy studies, are resource-intensive, and require a minimum of 50 patients [76]. This is often not feasible in low-transmission settings where few malaria cases are seen at each health facility, and most malaria occurs in highly mobile, migrant populations in whom follow-up for the required 28 to 42 days is challenging [90]. A more feasible, cost-effective method is assessing the prevalence of molecular markers associated with antimalarial drug resistance and treatment failure [91]. Molecular markers associated with therapeutic efficacy of artemisinin, lumefantrine, SP, CQ, and amodiaquine (AQ) have been identified and validated [66, 92–94].

Sustained implementation of effective interventions targeting both the malaria vector and parasite, following the 1999/2000 malaria epidemic, substantially reduced South Africa's malaria burden, allowing the country to transition from malaria control (> 5 malaria cases/1000 population at risk) towards malaria elimination (< 1 malaria case/1000 population at risk) in 2012 [95]. As this low transmission intensity meant that adequately powered in vivo therapeutic efficacy studies were not feasible, the prevalence of molecular markers of antimalarial resistance was used as a proxy for monitoring antimalarial efficacy. This routine surveillance aimed to determine the prevalence and temporal changes of molecular markers associated with artemisinin, lumefantrine and SP resistance in *Plasmodium falciparum* isolates extracted from malaria-positive rapid diagnostic tests (RDTs) obtained from primary health care (PHC)

facilities in Mpumalanga Province (Figure 6), South Africa (2001–2018), with a goal of ensuring that effective antimalarial treatment policies are in place.

2.3 Methods

2.3.1 Country setting

Malaria in South Africa is currently restricted to the low-altitude border regions of three provinces: Limpopo, Mpumalanga and KwaZulu-Natal [40] (Figure 6), with approximately 10% (4.9 million) of the country's total population residing in malaria-risk areas [41]. The predominant malaria vector in South Africa is Anopheles arabiensis [96], with *P. falciparum* parasites as the causative agent in most confirmed infections [96]. In line with South Africa's guidelines for the treatment of malaria [47], all fever cases presenting at PHC facilities within a malaria-endemic district must be tested for malaria using a *P. falciparum*-specific RDT. Patients who are RDT malaria-positive are treated in accordance with the guidelines [47].

2.3.2 Study setting

Mpumalanga Province encompasses an area of 76,500 sq km with an approximate population of 4,040,000 [38]. The province comprises three districts (Figure 6), with Ehlanzeni District (that shares a border with Mozambique and Eswatini) most affected by malaria [97]. Malaria transmission is seasonal but unstable, occurring during the rainy summer months from September to May, generally peaking in January and April, coinciding with the peaks in people moving across the country's border with Mozambique [98]. Despite sharp declines in locally acquired malaria cases, imported malaria case numbers continue to increase, accounting for 87% of the province's reported cases by 2012 [98]. However, recent region-wide malaria epidemics reversed these gains, resulting in an increase in total case numbers and locally acquired infections [88], with the proportion of imported cases decreasing to 68% during the 2017/18 malaria season (Figure 7).

SP replaced CQ as the drug of choice in Mpumalanga in 1997 [99], following a marked increase in CQ treatment failures [100]. This was followed relatively soon by a sharp increase in markers associated with SP treatment failures, which was associated with increased gametocyte carriage, prompting the Mpumalanga Provincial Department of Health to implement an ACT policy in 2003, initially using artesunate plus SP, given cure rates above 90% with SP monotherapy [101]. However, the continued selection for SP resistance markers following artesunate plus SP deployment in Mpumalanga [101, 102]and neighbouring southern Mozambique [80] supported the policy change to artemether-lumefantrine in 2006.

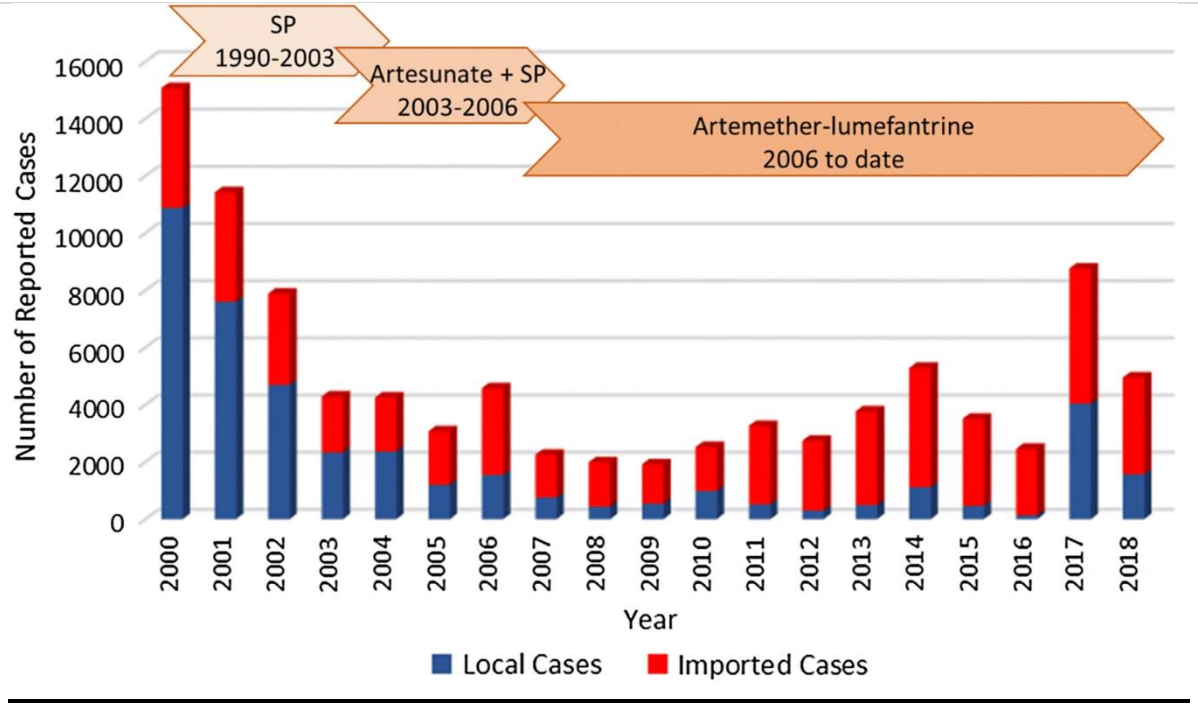


Figure 7: Number of local and imported cases reported in Mpumalanga Province.

The arrows indicate the first-line antimalarial treatments deployed over the reporting period (2000–2018). (Source: South African National Department of Health)

2.3.3 Study design and data collection

The Malaria Molecular Laboratory of the South African Medical Research Council (SAMRC) partnered with the Mpumalanga Provincial Malaria Control Programme to conduct the antimalarial resistance marker analysis using malaria-positive RDTs from PHC facilities, until the closure of the Malaria Laboratory in 2013 as part of the SAMRC restructuring. The surveillance programme was revived by the Laboratory for Antimalarial Resistance Monitoring and Malaria Operational Research of the National Institute for Communicable Diseases (NICD) and the Mpumalanga Provincial Malaria Elimination Programme during the 2017/2018 malaria season. This cross-sectional, antimalarial resistance marker prevalence study was conducted between 2001 and 2018 using malaria-positive RDTs collected from various PHC facilities within the malaria-endemic districts of Mpumalanga Province. The collected malaria-positive RDTs were transported to the SAMRC on an ad hoc basis but were couriered weekly to the NICD.

2.3.4 Molecular analysis

In the laboratory, parasite DNA was extracted from the positive RDTs (ICT™, Global Diagnostics, Cape Town, South Africa; SD Bioline, SD, Korea; First Response, Premier Technologies, India) using a modified Chelex method [103] from 2001 until 2011 and the Qiagen DNA mini extraction kit (Qiagen, Germany) in 2018. Once confirmed as *P. falciparum*

positive by either qPCR [104] or multiplex PCR [105], polymorphism analysis of *dhfr*, *dhps*, *crt*, and *mdr*1 genes was conducted. Molecular markers associated with SP resistance were assessed in all study years using all available DNA isolates. Budget constraints limited the analysis of lumefantrine tolerance/resistance markers in the mdr1 (mdrN86Y and mdr1 copy number variations) and *crt* (*crt*K76T) genes, to 2001, 2011 and 2018, with an additional assessment of the *mdr*1 markers conducted in 2009 (Table 1). As the kelch13 markers associated with artemisinin resistance were identified in 2014, these were only assayed in the samples collected in 2018.

Primers, PCR amplification conditions and restriction endonucleases used to detect polymorphisms in the dhfr (codons 51, 59, 108, 164), dhps (codons 436, 437, 540 and 581), mdr1 (codon 86), and crt (codon 76) genes have been described previously [79, 106, 107]. Digestion products were separated on a 2% agarose gel using electrophoresis, then visualised and photographed using either a MiniBIS™ (BioSystematica, UK) or Omega Fluor™ (Gel Company, USA) documentation system. Codons were classified as either wild-type, mutant or mixed (both mutant and wild-type genotypes present in an individual sample). Genotyping assays were run in duplicate, with a third assay performed on any discordant results. When calculating the overall prevalence of infections with mutant genotypes, codons with mixed genotypes were grouped with pure mutant codons.

Copy number of the *mdr1* gene was assessed using the qPCR method, primers, probes, and qPCR cycling conditions previously described by Price et al. [108]. Every qPCR run contained three reference DNA samples from D10 and Fac8 clones, having an *mdr1* copy number of one and three, respectively, as well as a no-template control. Assays were repeated if the threshold cycle values were greater than 35. The propeller domain of the *kelch13* gene was amplified using the protocol of Talundzic et al. [109]. The amplified products were sent to Inqaba Biotechnologists (Pretoria, South Africa) for Sanger sequencing. Sequences were then aligned against a reference *P. falciparum kelch13* gene (XM_001350122.1) using a BLAST search and BioEdit Software to detect poly-morphisms after codon 400 of the *kelch13* gene, the genetic region containing the mutations associated with delayed parasite clearance in Southeast Asia [64, 66].

Year	Number of mRDTs collected	DNA successfully extracted (%)	Number of parasite isolates analysed				
			SP resistance markers[a](%)	Lumefantrine tolerance/resistance markers			Artemisinin resistance markers[e] (%)
				*mdr*N86Y[b] (%)	*mdr*1 copy number[c] (%)	*crt*K76T[d]	
2001	195	93 (48)	93 (100)	14 (15)	12 (13)	22 (24)	–
2008	190	57 (30)	57 (100)	–	–	–	–
2009	190	81 (42)	81 (100)	81 (100)	73 (90)	–	–
2010	95	58 (61)	58 (100)	–	–	–	–
2011	663	596 (90)	596 (100)	558 (94)	390 (65)	333 (56)	–
2012	97	97 (100)	97 (100)	–	–	–	–
2018	963	686 (71)	655 (96)	514 (75)	482 (70)	452 (66)	532 (78)
Total	2393	1667 (70)	1637 (98)	1167 (70)	957 (57)	807 (48)	532 (32)

Table 1: Number of parasite isolates analysed by year and mutation marker in Mpumalanga Province, South Africa (2001–2018)

RDTs rapid diagnostic tests, DNA deoxyribose nucleic acid, SP sulfadoxine–pyrimethamine
[a] Mutations at codons dhfr51, dhfr59, dhfr108, dhfr164 of the dihydrofolate reductase (dhfr) gene and dhps436, dhps437, dhps540 and dhps581 of the dihydropteroate synthetase (dhps) genes were assessed
[b] Mutations at codon mdr86 of the multidrug resistance 1 (mdr1) gene were assessed
[c] Variations in the mdr1 gene copy number were assessed
[d] Mutations at codon crt76 of the chloroquine resistance transporter (crt) gene were assessed
[e] Mutations at 25 codons in the propeller domain of the kelch13 gene were assessed

2.3.5 Spatial data exploration and curation

A dataset of molecular markers with clinic names was imported for cleaning and analysis into R Studio version 3.5.2. Coordinates and location information was secondarily added by linking the molecular dataset with a facility and localities location dataset maintained at the NICD that contained facility coordinates.

Provincial malaria control programme information officers assisted with the identification of health facilities/localities data that did not match in the NICD facility database and provision of missing coordinates information. A few facility/locality observations (9%) lacked adequate information to allow for proper identification.

For verification of the coordinates, all the matched locations were further explored using Google Maps. Two locations that fell outside the study area were removed, resulting in a final

dataset comprising 90 locations and 1658 (73%) observations from the molecular marker dataset.

2.3.6 Spatial analysis

Using ArcMap 10.6.1, the molecular markers dataset was linked to the curated location coordinates to produce the spatial dataset. All country and sub-level shapefiles were obtained from an open-source platform of the latest Database of Global Administrative Areas (GADM version 3.6 released on 6 May 2018) [110]. All coordinates were assumed to have been based on the WGS 1984 coordinate system, and the Esri Display XY dialogue was used to integrate longitudes and latitudes of the localities on the maps.

Four important themes in defining molecular markers dictated the choices of colours and legend, namely tolerant, mixed, sensitive or being absent. Colour-friendly choices were picked from the colour brewer's toolkit [111]. Graduated symbols of equal proportions were also used throughout the maps for denoting the sample size of the markers involved for each locality to enhance interpret- ability [112].

2.3.7 Ethics approval

Approval for this study was obtained from the Mpumalanga Provincial Department of Health (MP_2015RP53_229), and the University of Witwa tersrand Human Research Ethics Committee: Medical (M160229). It also met the criteria for studies of routinely collected data of the Ethics Review Board of Médecins Sans Frontières.

2.4 Results

2.4.1 Plasmodium DNA isolates

The number of malaria-positive RDTs submitted for analysis increased over the study period, from under 200 per year between 2001 and 2010, to 663 in 2011 and 963 in 2018 (Table 1). Overall, parasite DNA was successfully extracted and amplified from 70% (1667/2393) of the malaria-positive RDTs received for analysis. Between 2001 and 2009, DNA was successfully extracted from 40% of the RDTs received, increasing to 61% in 2010 and consistently over 70% between 2011 and 2018. Method of DNA extraction did not appear to influence the success of DNA extraction.

2.4.2 Artemisinin resistance marker prevalence

Presence of the kelch13 artemisinin resistance markers could be determined in 78% (532/686) of the samples from which parasite DNA was extracted in 2018. Not one of the 25 polymorphisms confirmed or suspected to be associated with delayed parasite clearance in Southeast Asia was detected in these samples (Figure 8j).

2.4.3 Lumefantrine tolerance marker prevalence

Prevalence of the pure mdr86N wild-type allele (associated with lumefantrine tolerance but CQ and AQ sensitivity) increased significantly over the study period ($p < 0.0001$), from 57% (8/14) in 2001 to 59% (48/81) in 2009 and 91% in 2011, reaching fixation (100%, 514/514) by 2018 (Figures. 8a–c and 9a). Although the prevalence of the pure mdr86N wild-type allele was similar in 2001 and 2009, there was a sharp increase in mixed mdrN86Y alleles from 7% (1/14) to 39.5% (31/81) over this period (Figure 9b). Thereafter the prevalence of the mixed mdrN86Y alleles decreased markedly, with no mixed alleles detected in the 2018 samples (Figures 8c and 9b). No variation in mdr1 gene copy number was observed in any sample analysed over the study period.

At baseline (2001), only 18% (4/22) of the samples analysed carried the *crt*76K wildtype allele (Figures 8d and 9a) associated with lumefantrine tolerance. However, prevalence of this allele increased significantly to 75.7% (252/333) in 2011 (Figures 8e and 9a) and reached fixation, being present on all 452 samples analysed in 2018 ($p < 0.001$; Figures 8f and 9a). Mixed *crt*76 alleles were rare, only detected in 2011 (Figures 5e, f and 9c). Over the study period isolates carrying the *crt*76K wild-type allele were over 10 times more likely to carry the *mdr*86N allele (OR: 10.67; 95% CI 5.5–20.7; $p < 0.0001$), with all 452 samples assayed for the *crt*76 mutation in 2018 carrying the wildtype *mdr*86 allele.

2.4.4 SP resistance marker prevalence

The *dhfr* triple haplotype (codons *dhfr*51I, *dhfr*59R and *dhfr*108N) associated with pyrimethamine resistance increased significantly ($p < 0.0001$) over the study period, from 80% (74/92) in 2001 to 99% (653/658) by 2018 (Figure 10a). This paved the way for parasites carrying the *dhps* double mutation to increase more steeply during the study ($p < 0.001$) from 14% (13/93) in 2001 to 97% (635/655) in 2018, which was mirrored by the SP quintuple mutation increasing from 14% (13/93) in 2001 to 96% (630/655) in 2018 (Figures 8g–i, 10a); p-values < 0.001 for both. Mixed *dhps*437 and *dhps*540 alleles were seldom detected at the start of the study, with most isolates carrying the *dhps*437A and *dhps*540K wild-type alleles (Figure 10b, c). Over the study period the prevalence of both the mixed, as well as mutant *dhps*437 and *dhps*540, alleles increased (Figures 10b, c). Mixed *dhps*437 alleles peaked at 41% in 2011 but declined to 30% by 2012 (Figure 10b). In contrast, mixed *dhps*540 alleles continued to increase over the study period, constituting 38% of all *dhps*540 alleles analysed in 2012 (Figure 10c). However, by 2018 mixed alleles were extremely rare with over 97% of the samples analysed carrying pure mutant *dhps*437 and *dhps*540 alleles (Figure 10b, c). Mutations at codons *dhfr*164 and *dhps*581 were not detected in any of the samples tested.

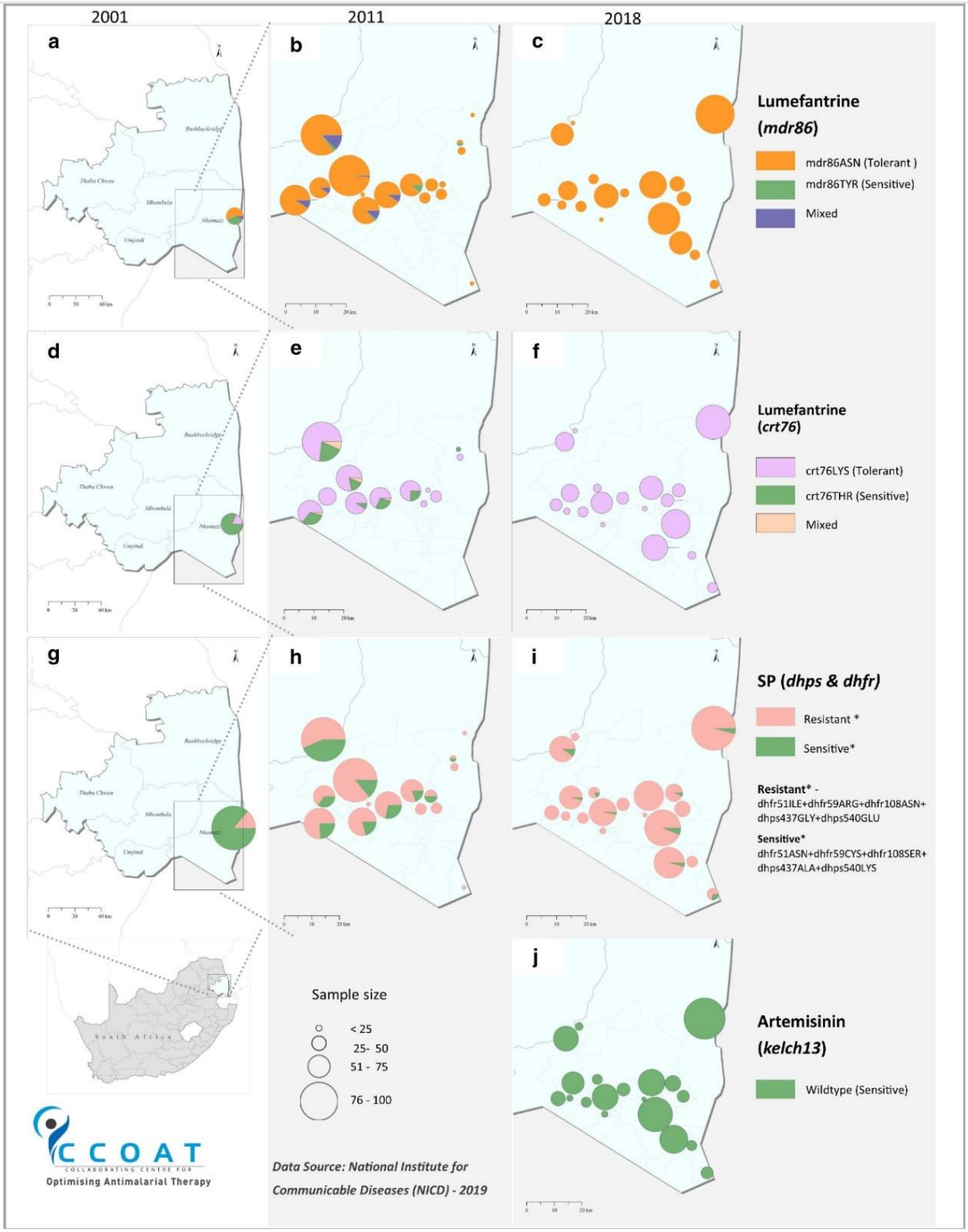
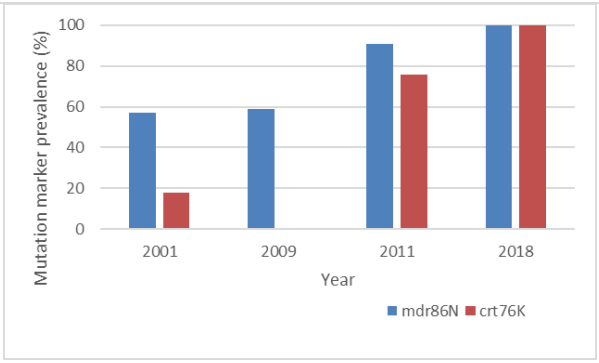


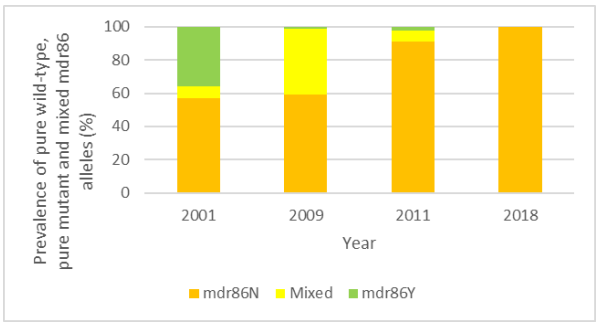
Figure 8: Spatial and temporal changes in the prevalence.

a–c mdr86ASN lumefantrine tolerance marker, d–f crt76LYS lumefantrine tolerance marker, g–i the quintuple SP resistance marker and k the kelch13 markers in Ehlanzeni District, Mpumalanga Province, South Africa.

a a



b



c

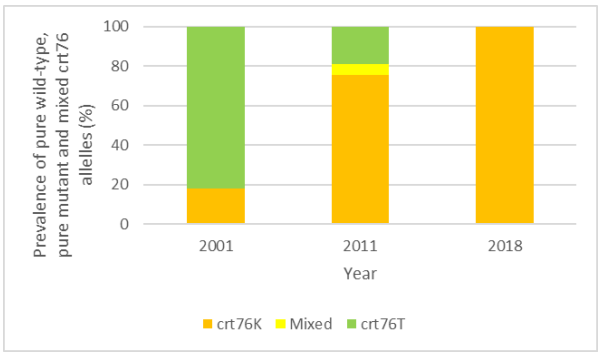
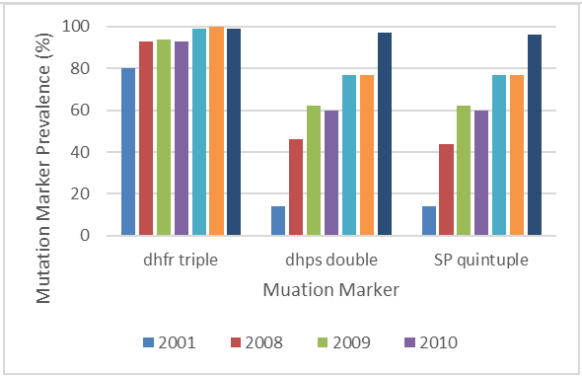
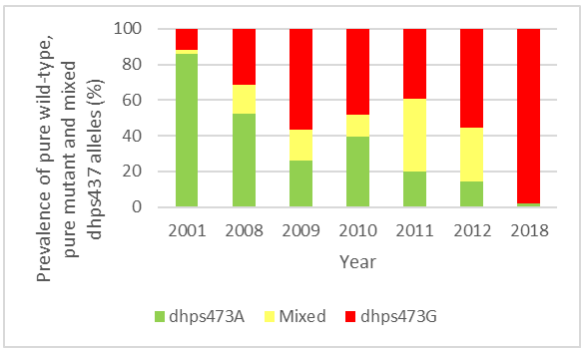


Figure 9: Prevalence of mdr86Y, mdr86N, mdrN86Y, crt76T, crt76K and crtK76T

a: Prevalence of the mdr86Y and crt76T mutations in Plasmodium falciparum isolates from Ehlanzeni District, Mpumalanga Province, South Africa, by year (2001–2018). Mutations at both codons were assessed in 2001, 2011 and 2018, with mutations in the mdr1 gene also assessed in 2009, b changes in the prevalence of pure wild-type mdr86N, pure mutant mdr86Y and mixed mdrN86Y alleles (2001–2018) and c prevalence of pure wild-type crt76K, pure mutant crt76T and mixed crtK76T alleles (2001–2018).



b



c

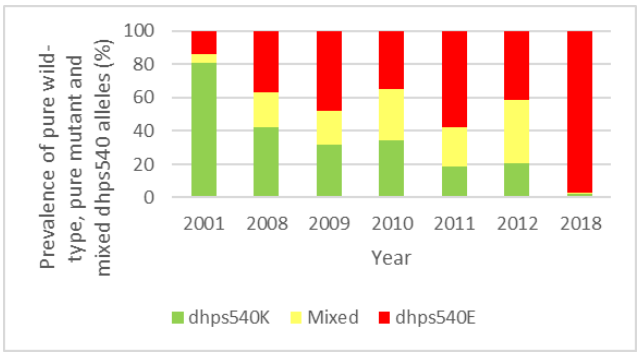


Figure 10: Prevalence of dhfr triple, dhps double and SP quintuple mutations

*a. Prevalence of dhfr triple, dhps double and SP quintuple mutations in Plasmodium falciparum isolates from Mpumalanga Province, South Africa, by year (2001–2018), b changes in the prevalence of pure wild dhps437A, mutant dhps437G and mixed dhpsA437G alleles (2001–2018) and **c** pure wild dhps540K, mutant dhps540E and mixed dhpsK540E alleles (2001–2018).*

2.5 Discussion

The rapid selection of malaria parasites resistant to first-line antimalarials is of great concern to the affected communities, clinicians, malaria researchers, and malaria control specialists. Regular drug efficacy monitoring using therapeutic efficacy studies or molecular resistance marking has been recommended by the WHO to enable early detection of emerging resistance and facilitate prompt policy changes before therapeutic efficacy falls below 90% [75]. Data presented here describe the first long-term study in Mpumalanga Province, South Africa, assessing temporal trends in antimalarial resistance marker prevalence.

Over the 18-year study period, parasites carrying the mdr86N and *crt*76K wild-type alleles associated with lumefantrine tolerance were strongly selected for, with all parasites analysed in 2018 carrying these wildtypes alleles. Similar selection for lumefantrine tolerance has been observed across Africa, particularly where artemether–lumefantrine is first-line treatment [113]. Parasites with the *mdr*86N wild-type allele have been shown to be more likely to recrudesce after artemether-lumefantrine treatment compared to parasites with the *mdr*86Y mutant allele [114] and are more able to survive exposure to considerably higher lumefantrine concentrations if they also carry the *mdr*184F and *mdr*1246D alleles [115]. Despite the increased wild-type *mdr*86N allele prevalence, amplification of *mdr*1 gene copy number, linked to artemether–lumefantrine treatment failures in Southeast Asia [108], was not observed in this study and is rare in Africa [113], suggesting an alternative mechanism may be associated with lumefantrine resistance in Africa. It is possible that the strong selection for *mdr*86N and *crt*76K wildtype alleles was driven by a reduction in CQ drug-pressure, as previously seen in Malawi [116]. However, as CQ has not been used in Mpumalanga since 1997 [99] and the significant increases in *mdr*86N and *crt*76K wildtype haplotypes were only observed after artemether–lumefantrine had been deployed in the province, the selection for these alleles in Mpumalanga is most likely driven by lumefantrine drug pressure.

In spite of the increased pressure on the artemisinin component, given reduced lumefantrine susceptibility and artemisinin-resistant parasites now being reported from India [83] as well as six other countries in Greater Mekong sub-region [36], artemisinin resistance has not yet been established in Africa. However, there have been reports of decreased artemether-lumefantrine efficacy from certain African countries [117, 118], raising concerns over the therapeutic longevity of artemether-lumefantrine, the most widely recommended ACT in Africa and first-line antimalarial treatment in all southern African countries [74]. Artemether-lumefantrine therapeutic efficacy data from a multi-year, multi-centre study assessing the safety of single low-dose primaquine in Mpumalanga Province reported a 100% PCR-corrected adequate clinical and parasitological response [119]. However, the majority of the study participants

were adult Mozambicans, who most likely had acquired some immunity to malaria due to the higher transmission intensity in that country. It is plausible that this acquired immunity contributed in part to the high cure rate, highlighting the need for regular drug efficacy monitoring in South Africa and other low transmission countries, where acquired immunity in locally transmitted cases is unlikely.

Concurrently with selection for lumefantrine tolerance, was a strong selection for parasites carrying the SP quintuple mutation associated with SP treatment failure. Molecular resistance studies from South Africa [120], Mozambique [80, 91] and Malawi [121] have confirmed that ACT (artesunate plus SP and artemether-lumefantrine) deployment has not halted the selection of molecular markers associated with SP treatment failures. In Gaza Province, Mozambique, which borders Mpumalanga Province, SP quintuple mutation prevalence neared 80% in 2010 despite the ACT, artemether-lumefantrine, being first-line treatment in that country since 2008 [91]. A similar pattern was observed in Malawi, where almost all parasites analysed carried the SP quintuple mutation 5 years after SP had been replaced by an ACT as the antimalarial of choice [120]. Possible reasons for the continued selection of SP resistance markers include sustained regional drug pressure due to the continued use of SP for intermittent preventive treatment (IPT) primarily in pregnancy in many southern African countries with higher intensity malaria transmission [74], and/or cross-resistance resulting from the widespread use of cotrimoxazole, an antifolate-sulfonamide drug combination similar to SP, as prophylaxis against opportunistic infections in people living with HIV/AIDS [122].

Strengthening of the malaria surveillance system in Mpumalanga Province since 2010 has positively impacted the quantity and, more importantly, quality of the RDTs received for analysis. Regular refresher training on administration and interpretation of RDTs results, together with the implementation of guidelines for the packaging (packaged in zip-lock packets with desiccant) and transportation (routine scheduled sub-mission) of used RDTs as part of this system strengthening has resulted in a significant increase in parasite DNA successfully extracted from the RDTs. Although other researchers in Africa have previously used RDTs as source of parasite DNA for antimalarial resistance detection [123–125], this is one of the first studies to use RDTs from a programmatic and operational level for routine antimalarial resistance marker surveillance. This study, therefore, re-enforces the usefulness of RDTs as a source of parasite DNA in resource-limited rural settings where collection and appropriate storage of blood samples may not be feasible.

Unfortunately, as the archived RDTs used in this study contained no patient identifiers it was not possible to link haplotype to a clinical outcome and/or patient characteristics, limiting the immediate clinical impact of the resistance marker data generated. This shortcoming is being

addressed with the roll-out of the smart surveillance for malaria elimination initiative in Mpumalanga, where resistance data will be linked to anonymised patient data in almost real-time. In line with the revised WHO surveillance guidelines [126], the provincial malaria control teams attempt to follow up all notified malaria cases to ensure cure and drug compliance. However, the majority of malaria cases occur in the large mobile and migrant populations on the border with Mozambique. This, together with well over 1000 cases annually, precludes the integrated therapeutic efficacy studies recommended by the WHO [126] for use in low-transmission, pre-elimination settings.

Maps displaying the prevalence and spatial–temporal distribution for resistance markers will be regularly generated to help inform policy in the province. More importantly, containment efforts can be rapidly targeted at the individual and appropriate community level (based on residential and source location) should the first case of artemisinin resistance be identified in this part of southern Africa. To ensure South Africa is able to respond rapidly to any emerging antimalarial resistance parasites, routine surveillance using RDTs should be expanded to the other two malaria-endemic provinces as a matter of urgency.

2.6 Conclusion

This study highlights the feasibility and suitability of using RDTs as a source of parasite DNA for routine antimalarial resistance surveillance particularly in rural, low-prevalence, resource-strained settings with malaria occurring mostly in mobile and migrant populations. The regionwide sustained deployment of artemether-lumefantrine has conferred a strong selective advantage to lumefantrine-tolerant parasites (carrying the wild-type mdr86N and crt76K alleles), enabling them to become the dominant parasite-type circulating within the southern African region. This rise in lumefantrine tolerance has increased the burden on the artemisinin component to clear the parasite load, which has the potential to increase the risk of artemisinin resistance and threaten the sustained efficacy of artemether-lumefantrine. Sustained, rigorous surveillance for molecular markers of antimalarial resistance is recommended to allow for the early detection of resistance, informing treatment policy and facilitating prompt containment efforts should any case of artemisinin resistance be identified. This is essential, given the devastating impact both CQ and SP resistance have had historically in southern Africa, and the malaria epidemiological similarities between this region and the areas in the Greater Mekong sub-region where resistance to widely used antimalarials, including artemisinins, first emerged.

Abbreviations

ACT: artemisinin-based combination therapy; AQ: amodiaquine; crt76: codon 76 of the P. falciparum chloroquine resistance transporter gene; CQ: chloroquine; dhfr: dihydrofolate reductase; dhfr51: codon 51 of the dihydrofolate reductase gene; dhfr59: codon 59 of the dihydrofolate reductase gene; dhfr108: codon 108 of the dihydrofolate reductase gene; dhfr triple mutation: presence of mutations at dhfr codons 51, 59 and 58 of the dihydrofolate

reductase gene; dhps: dihydropteroate synthetase; dhps436: codon 436 of the dihydropteroate synthetase gene; dhps437: codon 437 of the dihydropteroate synthetase gene; dhps540: codon 540 of the dihydropteroate synthetase gene; dhps581: codon 581 of the dihydropteroate synthetase gene; dhps double: presence of mutations at codons 437 and 540 of the dihydropteroate synthetase gene; DNA: deoxyribose nucleic acid; mdr186: codon 86 of the P. falciparum multi-drug resistance gene 1; NICD: National Institute for Communicable Diseases; PCR: polymerase chain reaction; PHC: primary health care facilities; qPCR: quantitative polymerase chain reaction; RDT: rapid diagnostic test; SAMRC: South African Medical Research Council; SP: sulfadoxine– pyrimethamine; SP quintuple mutation: presence of both the dhfr triple and dhps double mutations.

Acknowledgements

The authors thank the Mpumalanga Provincial Malaria Control/Elimination Programme and PHC facility staff for assistance with sample collection, the laboratory staff of the Malaria Unit of the SAMRC and Laboratory for Antima- larial Resistance Monitoring and Malaria Operational Research of the NICD for assistance with generation of molecular data, and both the SAMRC and NICD for financial support to cover laboratory costs. Preliminary data analysis was conducted through the Structured Operational Research and Training Initiative (SORT IT), a global partnership led by the Special Programme for Research and Training in Tropical Diseases at the World Health Organization

(WHO/TDR). SORT IT Programmes include a teaching component developed jointly by the International Union Against Tuberculosis and Lung Disease (The Union) and Médecins Sans Frontières. The specific SORT IT programme, which assisted in the drafting of this publication, was implemented by: WHO/TDR; WHO Global Malaria Programme (GMP); WHO/AFRO; the Operational Research Unit (LuxOR), Médecins Sans Frontières, Brussels Operational Center, Luxem- bourg; the Centre for Operational Research, The Union, France; The University of Nairobi, Kenya; Global AIDS Interfaith Alliance, California, USA; Academic Model Providing Access to Healthcare (AMPATH), Kenya; and Johns Hopkins University, Baltimore, Maryland, USA.

Authors' contributors

JR, AM, KIB conceived and designed the study; JR generated the molecular data, analysed the data, and drafted the manuscript; KIB conducted the statistical analyses; AM, GM coordinated collection and transportation of samples from the field to the laboratory; FMK conducted the spatial analyses; AR, JF contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Funding

Funds from the SAMRC (Grants: Research Incentive) and NICD covered the costs associated with sample collection and mutational analysis, while funds from WHO/TDR, GMP and WHO/AFRO assisted with publication costs. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

The datasets used and analysed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Approval for this study was obtained from the Mpumalanga Provincial Depart- ment of Health (MP_2015RP53_229), and the University of Witwatersrand Human Research Ethics Committee: Medical (M160229). It also met the criteria for studies of routinely-collected data of the Ethics Review Board of Médecins Sans Frontières.

Consent for publication

Permission for publication was received from the Manager of the Mpuma- langa Provincial Malaria Elimination Programme.

Competing interests

The authors declare that they have no competing interests.

3 Chapter 3

Making Data Map-worthy - Enhancing Routine Malaria Data to Support Surveillance and Mapping of *Plasmodium falciparum* Anti-Malarial Resistance in A Pre-Elimination Sub-Saharan African Setting: A Molecular and Spatiotemporal Epidemiology Study.

Frank M. Kagoro [1 2,3,4,5], Elizabeth Allen [1,3,4], Aaron Mabuza [1,3], Lesley Workman [1,3,4], Ray Magagula [6], Gerdalize Kok [6], Craig Davies[7], Gillian Malatje [6], Philippe J Guerin [3,4,5], Mehul Dhorda [2,4,5], Richard J Maude [2,5,8,9], ǂJaishree Raman [10,11,12] & ǂKaren I Barnes [1,3,4]

[1]Collaborating Centre for Optimising Antimalarial Therapy (CCOAT), Division of Clinical Pharmacology, Department of Medicine, University of Cape Town (UCT), Cape Town, South Africa

[2]Mahidol Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

[3]WorldWide Antimalarial Resistance Network (WWARN), Southern African Regional hub, UCT, South Africa

[4]Infectious Diseases Data Observatory (IDDO), Oxford, UK

[5]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

[6]Mpumalanga Provincial Malaria Elimination Programme, Mpumalanga, South Africa

[7]Malaria Programme, Clinton Health Access Initiative, Pretoria, South Africa.

[8]Harvard TH Chan School of Public Health, Harvard University, Boston, MA, USA

[9]The Open University, Milton Keynes, UK

[10]Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Disease, Sandringham, Gauteng, South Africa

[11]Wits Research Institute for Malaria, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

[12]UP Institute for Sustainable Malaria Control, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

ǂ These authors contributed equally

3.1 Abstract

3.1.1 Background

Independent emergence and spread of artemisinin-resistant *Plasmodium falciparum* malaria have recently been confirmed in Africa, with molecular markers associated with artemisinin resistance increasingly detected. Surveillance to promptly detect and effectively respond to anti-malarial resistance is generally suboptimal in Africa, especially in low transmission settings where therapeutic efficacy studies are often not feasible due to recruitment challenges. However, these communities may be at higher risk of anti-malarial resistance.

3.1.2 Methods

From March 2018 to February 2020, a sequential mixed-methods study was conducted to evaluate the feasibility of the near-real-time linkage of individual patient anti-malarial resistance profiles with their case notifications and treatment response reports, and map these to fine scales in Nkomazi sub-district, Mpumalanga, a pre-elimination area in South Africa.

3.1.3 Results

Plasmodium falciparum molecular marker resistance profiles were linked to 55.1% (2636/4787) of notified malaria cases, 85% (2240/2636) of which were mapped to healthcare facility, ward and locality levels. Over time, linkage of individual malaria case demographic and molecular data increased to 75.1%. No artemisinin resistance validated/associated kelch13 mutations were detected in the 2385 PCR positive samples. Almost all 2812 samples assessed for lumefantrine susceptibility carried the wildtype mdr86ASN and crt76LYS alleles, potentially associated with decreased lumefantrine susceptibility.

3.1.4 Conclusion

Routine near-real-time mapping of molecular markers associated with anti-malarial drug resistance on a fine spatial scale provides a rapid and efficient early warning system for emerging resistance. The lessons learnt here could inform scale-up to provincial, national and regional malaria elimination programmes, and may be relevant for other antimicrobial resistance surveillance.

Keywords: Artemisinin resistance, kelch-13, lumefantrine, *Plasmodium falciparum*, Africa, spatiotemporal model, malaria

3.2 Background

Malaria has been declining globally, with a 50% reduction in malaria cases and an 84% reduction in malaria deaths from 2000 to 2015 [127]. Unfortunately, there has been no

significant progress in reducing the global malaria burden since 2015 [128]. The emergence of SARS-CoV-2 threatens to reverse any such progress. The World Health Organization (WHO) estimates an additional 47,000 deaths in 2020 linked to pandemic-related disruptions, with the WHO African region accounting for the majority of these additional cases [129]. The emergence and spread of anti-malarial drug resistance threaten malaria control and elimination efforts, especially in Southeast Asia (SEA), where parasites resistant to artemisinin-based combination therapy (ACT) have been confirmed in at least five countries [130, 131], with resistance markers also reported in China-Myanmar border [132] and eastern India [133]. The majority of SEA countries have low to very low malaria transmission intensities (and thus populations are non-immune), with infections occurring primarily in highly mobile populations along international borders. Several malaria-endemic southern African countries now have similar epidemiological profiles, placing them at increased risk for the emergence of artemisinin (and partner drug) resistance [134, 135]. In sub-Saharan Africa, mutations in the *Plasmodium falciparum kelch 13* gene (*k13*) associated with artemisinin resistance have been identified in Central (Democratic Republic of Congo) [136], Eastern (Kenya, Rwanda and Tanzania) [136–139] and Western Africa (Ghana, Mali and Nigeria) [139, 140], with phenotypic evidence of artemisinin resistance (delayed parasite clearance) recently confirmed in Rwanda [141] and Uganda [142]. Moreover, Cabo Verde, Eritrea and Ghana were identified as having more than 5% K13 mutations. More importantly, the K13 561HIS mutation in Rwanda [141] and the 469TYR and 675VAL mutations in Uganda have been documented in up to 20% of infected individuals [143]. These three mutations have been associated with reduced efficacy to artemisinin both *in-vitro* and *in-vivo* [138, 142, 144]. Recent studies have demonstrated the independent emergence of artemisinin resistance molecular markers in Guyana [145], and the presence of novel K13 mutations in Brazil [146] and Colombia [147]. In their 2021 systematic review of K13 markers frequencies in Africa, Ndwiga et al. highlighted the fact that while many African countries were able to identify the K13 resistance markers using genomic analyses [148], this genomic surveillance was rarely linked to a public health surveillance system.

Robust drug resistance monitoring is a significant challenge, especially in low to moderate malaria transmission settings. While therapeutic efficacy studies (TES) are more feasible in moderate-to-high transmission areas where the required sample sizes can readily be achieved, low and very low transmission settings face recruitment challenges due to fewer malaria cases leading to prolonged study duration, multiple study sites and increased study costs. In such settings, the WHO recommends integrated drug efficacy surveillance (iDES), integrating surveillance of anti-malarial drug efficacy within malaria case-based surveillance [149]. However, resource constraints limit follow up of all malaria cases. This is seldom

feasible for mobile and migrant populations, so many low transmission countries fail to monitor anti-malarial efficacy adequately. As of April, 2022, only China [150] and Thailand [151] had published iDES results since its recommendation in 2018 [152]. As countries move towards malaria elimination, many of those with low to very low case numbers need alternative anti-malarial drug resistance surveillance methods. Surveillance of molecular markers associated with drug resistance collected in different clinical trials and observational research has proved useful. However, these studies are generally short-term and are conducted in a few sites where they are not repeated regularly enough to track resistance trends over longer time periods [153]. Integrating sample collection for monitoring molecular resistance markers into routine malaria case surveillance by national malaria programmes has been suggested as a suitable alternative to provide early warning of emerging resistance [154–156]. Such molecular marker surveillance using routine data could then trigger and target transmission-blocking interventions, such as single low dose (SLD) primaquine and foci clearing, and confirmatory therapeutic efficacy studies needed to inform the effective anti-malarial treatment policies essential for achieving elimination. In a case of imported malaria, the malaria programme at the source of the infection can also be informed to trigger and target similar interventions in the source community.

Health information systems used in malaria, such as “District Health Information System 2” (DHIS2), have functionalities that display maps at the ward, district, province, or national malaria programme levels. Whether thematic or modelled, the accuracy of a map depends on the accuracy and reliability of the source data [19–21]. While the need for maps showing the distribution of parasites (malaria cases), vectors and vector breeding sites and prevalence of insecticide and anti-malarial resistance was realized more than two decades ago, data verification, quality assessment and consideration of malaria programme needs to make these malaria maps user-friendly have rarely been included [157]. 'Human-centred design' is a part of design thinking that incorporates users in the design process [35,36]. The co-designing pathway allows a flow of knowledge to both designers and users from development to deployment [159]. Analysing trends in routine data and reviewing results together with the end-users can help improve the data by informing the co-development of tools and resources needed to appraise and enhance data quality, and "Make Data Map-worthy".

In South Africa, all suspected malaria cases should have a definitive diagnosis confirmed by malaria rapid diagnostic test (RDT) or microscopy before treatment is administered [160]. In pre-elimination settings, the Malaria Elimination Programme (MEP) implements both proactive case detection (screening populations at highest risk, such as migrant and mobile populations), as well as reactive case detection (in households surrounding of an index cases’ residence), in addition to passive case detection of patients presenting to health care facilities

[161]. The Mpumalanga MEP has stratified Nkomazi sub-district as being in the pre-elimination phase, and has piloted two interventions to enhance monitoring of anti-malarial efficacy and advance malaria elimination. The first was Smart Surveillance for Malaria Elimination (SS4ME), which started in February 2018, and comprised the collection of RDTs (and, wherever possible capillary blood filter paper samples) for tracking molecular markers of anti-malarial resistance. The second was the programmatic roll-out of single low dose primaquine recommended by the WHO for malaria transmission blocking [161], in addition to routine treatment of uncomplicated malaria with the artemisinin-based combination therapy, artemether-lumefantrine (AL) [160]; this included WHO recommended follow up of treatment adherence and response during malaria case investigations (from January 2019).

The present study aimed: 1) to map linked patient demographic, clinical and drug resistance profiles in order to identify areas where additional surveillance or containment efforts are needed; 2) to evaluate and quantify the feasibility of this approach, which led to the development of data improvement tools and activities to better meet the needs of the MEP; and 3) to optimise spatial and temporal maps for use by policymakers in local, provincial and national malaria programmes in South(ern) Africa.

3.3 Methods

3.3.1 Design

A sequential explanatory mixed-methods approach, with iterative quantitative and qualitative methods, was used from March 2018 to February 2020 to optimise maps that linked patient demographic, clinical and drug resistance profiles in order to identify areas where additional surveillance and / or containment efforts are needed. The quantitative component was grounded in the post-positivist theory, where a descriptive and exploratory spatiotemporal analysis was conducted, using trend and time-series decomposition analyses [162] to define spatial and temporal patterns for data linkage and mapping [163]. The qualitative component used a pragmatist approach with co-design techniques to innovate and implement tools to bridge gaps identified from the ongoing spatiotemporal activities and analysis. The MEP and study team worked together iteratively to improve the information system, data architecture and maps produced.

The quantitative component included data aggregations, curation, and analyses to generate data visualisations. These visualisations and summaries of the analyses were shared monthly with the MEP. The study team then worked with the MEP to design activities, tools and training to enhance data quality and improve surveillance metrics, including coverage, accuracy and linkage (Figure 11). Data were grouped monthly and quarterly to estimate trends. Monthly

evaluations focused on measuring changes in data from the health information system over time. Quarterly evaluations were used to compare the flow of data and accuracy of GPS coordinate data over time.

Spatiotemporal information in the malaria routine case data was used to produce draft maps of malaria incidence and prevalence of molecular markers of resistance. These maps were then presented to the provincial malaria team to evaluate their 'understandability', using semi-structured interviews and feedback meetings. These understandability assessments fed back iteratively into the analysis and co-design process until the final maps were agreed upon between researchers and the MEP. This optimisation process involved repeatedly deploying and updating the tools and maps produced to enhance routine data and optimise the maps generated.

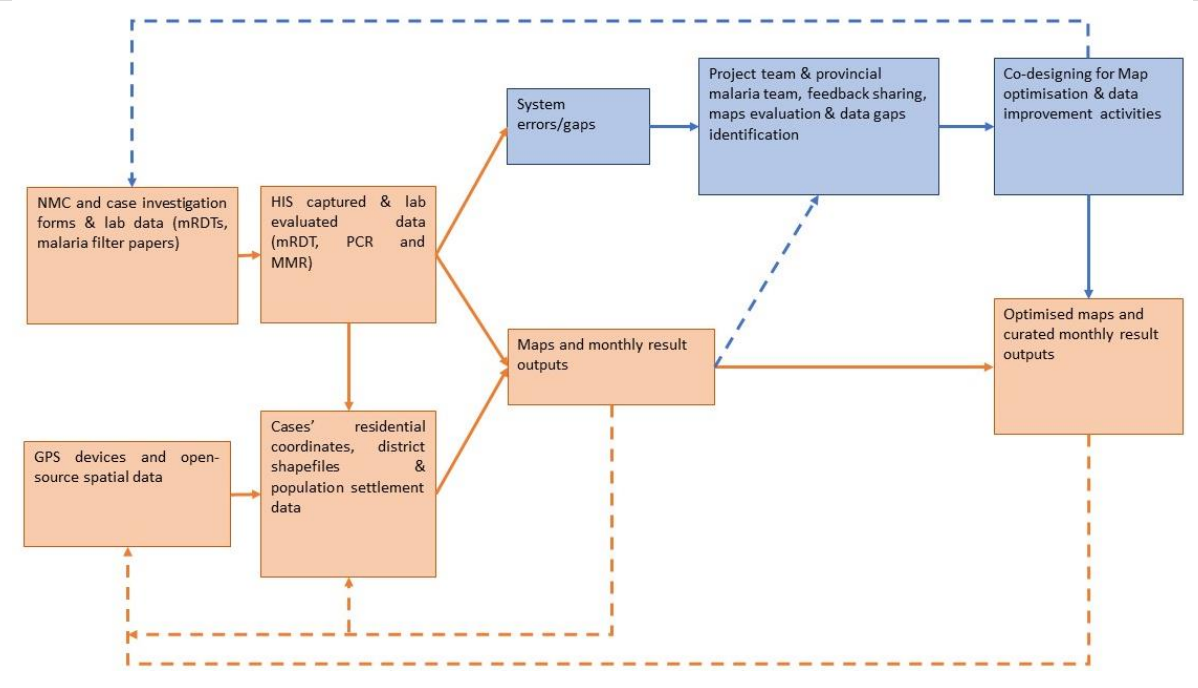


Figure 11: Making data map-worthy study design.

Chart showing different iterations of data curation and map optimisation. Orange and blue colours show quantitative and qualitative methods, respectively. Solid lines indicate analysis and optimisation pathways, while dashed lines show the iteration pathway. NMC notifiable medical condition, HIS health information system, RDT malaria rapid diagnostic test, PCR polymerase chain reaction, MMR molecular markers of resistance.

3.3.2 Study setting

While most of South Africa is considered malaria-free, approximately 5 million South Africans (10% of the country's population) reside in the malaria-endemic areas of Mpumalanga, Limpopo, and KwaZulu-Natal provinces [164]. Most malaria cases in South Africa are imported, with some local transmission occurring in the low-altitude [165] international border

regions shared with Botswana, Eswatini, Mozambique and Zimbabwe. Malaria transmission in South Africa is seasonal, occurring mainly during the summer rainy season (September to April) [166].

This study was conducted in Nkomazi Sub-District, a pre-elimination area in Mpumalanga province, South Africa. All individuals identified using either proactive, active or passive case detection were tested for malaria using a falciparum-specific, histidine-rich protein 2 (HRP2)-based RDT, and positive cases were included whether or not they were symptomatic [160]. As per the national treatment guidelines, those with asymptomatic or uncomplicated malaria are treated with the WHO recommended weight-based 3-day artemether-lumefantrine (AL) (Coartem®) regimen [160]. AL has been used in the study area since 2007 [153, 160, 165]. Additionally, all consenting malaria-positive patients, excluding pregnant women, breastfeeding mothers and children under 10kg or one year of age, are given a single low dose of primaquine (0.25 mg base/kg (15 mg base adult maximal dose) [167–169]. An additional dried blood spot (DBS) on filter paper (Whatman Paper No 1) was collected from RDT malaria positive patients by dabbing the remaining blood at the RDT finger prick site, then labelled, barcoded and sent to the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa, together with its respective positive RDT cassette, for anti-malarial resistance marking [170]. An additional 10% of the negative RDTs were collected and sent to the NICD for quality assurance.

As malaria is a notifiable condition in South Africa, demographic and malaria case information collected at the malaria diagnosis and treatment initiation phase are reported on the Notifiable Medical Condition (NMC) form or mobile application. If reporting is paper-based, forms are collected by a MEP case investigator assigned to that healthcare facility, ideally within 24 hours of diagnosis and delivered to the sub-district malaria office for data quality verification and data capture. Within 24-72 hours of case notification, case investigators should visit the malaria patient's household for in-depth case investigation to assess for the presence of malaria risk factors (e.g., last indoor residual insecticide spraying of that household or nearby mosquito vector breeding sites) and to conduct contact tracing and testing. During these case investigations, the household's GPS location coordinates are recorded, and the case investigation form completed. The malaria treatment adherence and response information (including any side effects) is captured as part of the case investigation using supplementary Guide S1 (p 146). Once completed these forms are submitted to the sub-district malaria office for quality checking and electronic capture into the DHIS2.

3.3.3 Data

3.3.4 Malaria case data

Malaria case data consisted of NMC, case investigation and treatment adherence and response forms as individual case records captured on paper or electronically, and downloaded from the DHIS2.

Geospatial data

The geospatial data consisted of four types. Firstly, each patient's residential address and GPS coordinates were sourced from both MEP android tablets/handheld GPS devices and as recorded in DHIS2. Secondly, population settlement shapefile data were obtained from a) the Ehlanzeni District Municipality, and b) the open-source Global Administrative Areas website for South Africa [48]. Thirdly, a list of locality addresses of malaria cases were obtained from the Mpumalanga MEP office, curated and validated using Google Maps. Lastly, the modelled Facebook population of South Africa density raster was downloaded from The Humanitarian Data Exchange (HDX v1.52.1) [49].

Laboratory data

Parasite DNA was extracted from the RDTs and DBS using the Qiagen DNA mini extraction kit (Qiagen, Germany), according to the manufacturer's instructions. When both the RDT and accompanying DBS were available, DNA was extracted from both sources in a single reaction. The extracted DNA was subjected to multiplex PCR to confirm *Plasmodium* species[50]. To assess possible decreases in lumefantrine susceptibility, samples containing only *P. falciparum* parasites were subjected to conventional PCR and endonuclease cleavage [172] to detect polymorphisms at codons 72-76 of the chloroquine resistance transporter (*crt*) [173] and codon 86 of multi-drug resistance 1 (*mdr1*) genes [174]. Codons were classified as pure sensitive, pure mutant or mixed (both mutant and sensitive genotypes present in an individual patient's sample). Genotyping assays were run in duplicate, with a third assay performed on any discordant results. When calculating overall prevalence of infections with mutant genotypes, codons with mixed genotypes were grouped with pure mutant codons. The copy number of the *mdr1* gene was assessed using quantitative PCR (qPCR), with primers, probes and qPCR cycling conditions previously described [174]. Every qPCR run contained three reference DNA samples from D10 and Fac8 clones, having an *mdr1* copy number of one and three respectively, as well as a no-template control. Assays were repeated if the threshold cycle values were greater than 35. For the assessment of artemisinin resistance, the propeller domain of the K13 gene was amplified as previously described [53]. The amplified products were sent to Inqaba Biotechnologies (South Africa) for Sanger sequencing. Sequences were

then aligned against a reference K13 gene (XM_001350122.1) using a BLAST search and BioEdit Software [54] to detect polymorphisms in 27 codons associated with delayed parasite clearance in South East Asia [177]. Molecular data were compiled monthly and shared with study investigators for further curation and analysis. Results were presented to the Mpumalanga MEP monthly and quarterly for them to take timely action with investigation and response in the event of any significant resistant mutations.

3.3.5 Definition of metrics

Coverage

Four measures of coverage were used: 1) percentages of malaria cases with blood samples taken (RDT/DBS), 2) percentages of cases assigned a correct barcode (necessary for linkage of laboratory results to NMC data), 3) percentage of cases investigated and 4) percentages of investigated cases with GPS coordinates relative to all reported malaria notifications captured in the DHIS2.

Accuracy

Two measures of accuracy used were: 1) percentage of investigated cases with GPS coordinates falling within the study's residential areas, and 2) percentages of notified cases with correctly formatted barcodes, calculated monthly and quarterly.

Linkage

This was measured using the percentage of cases with accurate barcodes linked to the NMC data, the molecular markers results data and accurate GPS coordinates at health facility, ward, locality, and household levels.

3.3.6 Study procedures

The accuracy, coverage and ability to link the malaria notifications to the case investigation, laboratory data and drug report data was evaluated using monthly timelines. Data from DHIS2 was downloaded monthly from the malaria programme and shared with the project team for curation and analysis. A checklist was used to record the settings of each MEP GPS device, and their data was downloaded for further analysis. All data were securely downloaded, encrypted and transferred to the password-protected study computer for further compilation and analysis.

3.4 Analysis

All data analyses were conducted using R programming language (versions 3.6 and 4.0) and Esri ArcGIS ArcMap (version 10.8). The analysis focused on data linkage, spatiotemporal

trends in molecular marker and usability assessments. Coverage, accuracy and linkage metrics were used as units of analysis for temporal trends in the numbers of malaria cases reported, malaria cases investigated, the laboratory received samples, and post-treatment case investigation reports.

3.4.1 Trend analysis

Monthly percentages were calculated using the monthly reported malaria case totals as the denominator. Time-series decomposition was used to evaluate for the non-stationarity of data and account for trend (t), seasonality (s) and random noise (r) [56]. Loess regression was used to obtain the optimum distribution and the 95% confidence margins of the trend (Figures S10(a) p 131, S11(a) p 132 and S12(a) p 133). This time series was further decomposed to evaluate trend, seasonality and random errors using Sen's slope and Mann-Kendall test [39,57]. Seasonality was further explored using box plots.

3.4.2 Molecular markers analysis

The classification of the 27 K13 mutations after codon 400 assessed in this study was guided by the WHO [58,59] and the Worldwide Antimalarial Resistance Network (WWARN) [177]. The 2020 WHO categories of 'validated', 'associated/candidate', 'not associated' or 'wild type' were used [59]. The 'wild type' parasite was renamed to 'sensitive' for further clarity. Mutations at codon 86 of the *mdr1* gene and codon 76 of the *crt* gene together with increases in *mdr1* gene copy number were assessed to determine susceptibility to lumefantrine. Parasites with the *mdr*86ASN and *crt*76LYS alleles but no increase in *mdr1* copy number were categorised as less susceptible (or tolerant) to lumefantrine, while those with an increased *mdr1* copy number considered lumefantrine resistant.

3.4.3 Spatial and usability analysis

All shapefile data and residential coordinates from malaria cases were converted to the HartebeestHoek94 Datum coordinate system and projected to the Universal Transverse Mercator zone 32 [60]. Two draft maps were then drawn to display 1) thematic maps for the distribution of malaria cases by ward and 2) density maps of cases distributed by settlement within their ward boundaries. Twenty-four case investigators, with between 1 and 24 years' experience working in Nkomazi sub-district, reviewed both maps to identify and label the Nkomazi wards (administrative level four). Feedback obtained from malaria case investigators was used to develop malaria case distribution maps and evaluate the shapefiles.

Thematic maps of the distribution of malaria cases by ward were produced using a spatial join tool linking GPS coordinates to the sub-district polygon. All cases falling in the same ward were summed, and an equal-interval scale and continuous colour ramp were used for

displaying the distribution of cases by ward. Density maps of malaria cases per 1000 population were produced using kernel density estimation at 1 x 1 km and 0.5 x 0.5 km grids with a buffer around the sub-district polygon of 1 km and 0.5 km, respectively. The two grids were purposely selected for comparison. The Kernel density estimation used Quartic implementation as per the formula below:

$$Density = \frac{1}{(radius)^2}\sum_{i=1}^{n}\left[\frac{3}{\pi}\cdot pop_i\left(1-\left(\frac{dist_i}{radius}\right)^2\right)^2\right]$$

where:

- *i = 1,…,n* are the input points. The sum of points was used if they were within the radius distance of the (x,y) location.
- pop_i is the population value of point i.
- $dist_i$ is the distance between point i and the (x,y) location [163].

Feedback was obtained from the case investigators using semi-structured interviews to assess if the maps were well understood and whether the distribution of malaria cases corresponded with their local knowledge. A case-based orientation was used to optimize the maps to arrive at the most correct and easily understood versions.

Descriptive exploratory proximity analysis was further conducted on residential coordinates to ascertain the probability of these locations falling in the actual residential area (within 0.5 x 0.5 km) at a given time (t). Two types of analyses were used. Firstly, for identifying the progress of the accuracy and distribution of the malaria case residential coordinates over time, a time-series line using Loess regression was plotted, as were maps to assess the distribution of the coordinates. Secondly, the quadrats of the observed malaria cases at 0.5 km radius compared to expected cases were analysed by a Poisson process using the known malaria incidence and population settlement data to obtain likelihood ratios and Chi-square test. To assess the sparsity of malaria case residential data, average nearest neighbour analysis was used to explore for precision in the GPS coordinate dataset.

3.5 Results

3.5.1 Malaria notification, case investigation, drug adherence and response reports

From 1 March 2018 to 28 February 2020, 4787 malaria cases were notified in Nkomazi sub-district. All cases were definitively diagnosed using RDTs, with 98% (n=4673) treated as outpatients. The probable source of infection for 73% (n=3486) of the cases could be identified, with 96% of these classified as imported cases (i.e. source of infection outside South

Africa). Of the 2531 cases with a reported date of diagnosis, the majority (80%) presented at health facilities within two days of symptom onset. Altogether, 78.5% (n=3758) of cases were investigated and GPS coordinates captured (Figure 10). Treatment adherence and response reports were introduced from January 2019 (Figure S10 p 131), whereafter, of 2464 cases investigated, 61% (n=1510) had the treatment adherence and response report completed. Overall, 72% (1793/2507) of cases were investigated within 24 hours (and 75%, 81%, 87%, and 92% within 3, 7, 14 and 30 days, respectively).

Data linkage was performed at three levels, the first two at household level and the third at locality level. The first group linked case investigation data with accurate household GPS coordinates (n=1053) and RDT barcodes (n=2527), allowing 89% (n=2002) of investigated cases to be linked to their molecular markers of resistance results. The second group included treatment adherence and response reports (Guide S1 p 146), where only 50% (n=1413, Figure S10 p 131) of case investigations since its introduction in January 2019 could be linked to molecular markers of resistance results. The third group used GPS coordinates of locality addresses (n=2255) as an anonymised proxy for residential coordinates, where 85% (n=2240) of cases investigated could be linked to their individual molecular markers of resistance results (Figure 12).

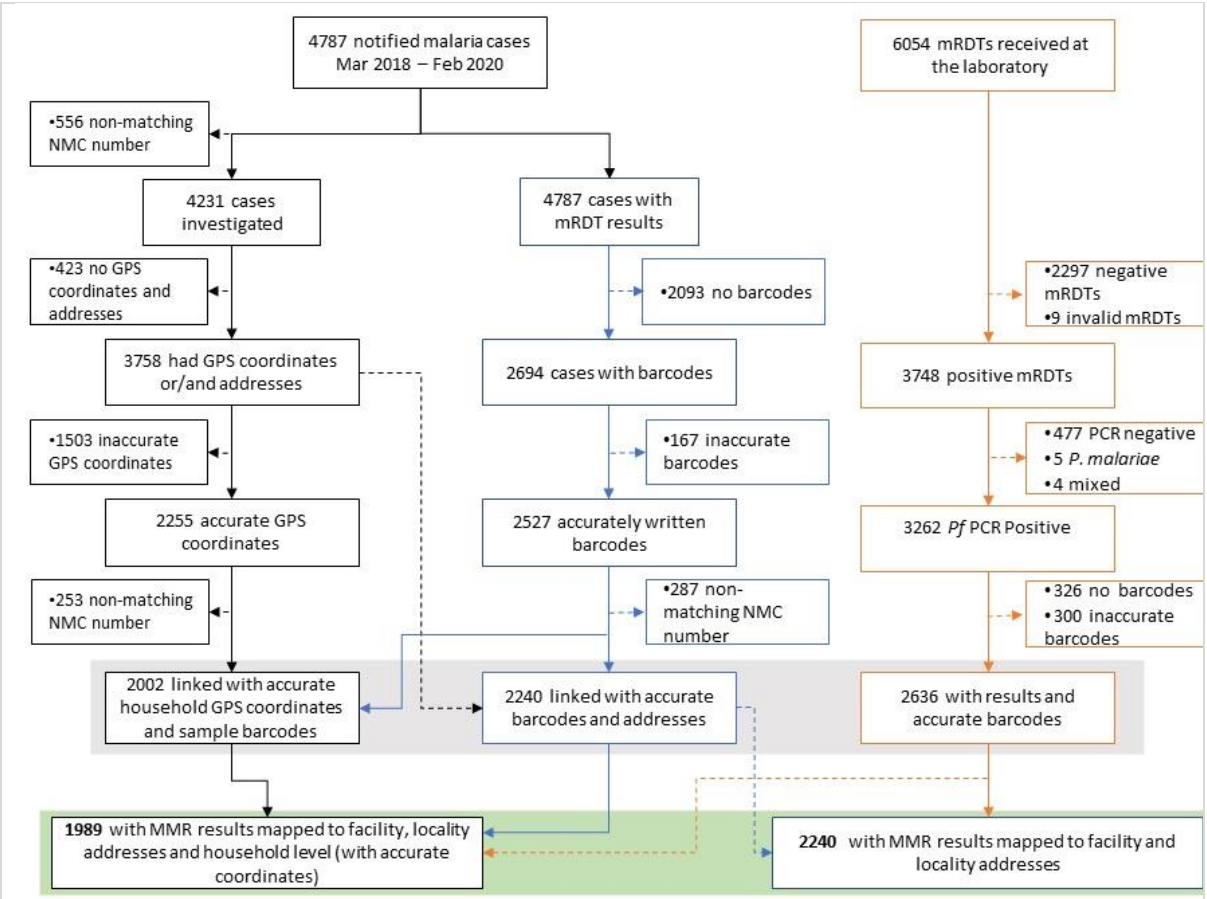


Figure 12: Making Data Map-worthy data flow chart.

A chart showing data flow from the DHIS2 (consisting of malaria notification data captured on the notifiable medical condition (NMC) forms and case investigation data captured on case investigation forms), and molecular laboratory data on molecular markers of resistance from filter paper dried blood spots of RDT positive malaria patients. MMR molecular markers of resistance results, RDT malaria rapid diagnostic test, Pf Plasmodium falciparum).

Of the 6054 RDTs received by the national laboratory, 61.6% (n=3748) were reported as *P. falciparum* positive by the Mpumalanga MEP; the remainder were negative RDTs sent for quality control purposes. Parasite DNA was extracted, and PCR amplified from these positive RDTs and their corresponding filter paper dried blood spots (DBS), with 3340 (88%) found to be *P. falciparum* positive by PCR. Only samples with *P. falciparum* mono-infections [98% (n=3262)] were assessed for molecular markers of drug resistance, of which 80.8% (n=2636) had barcodes for linkage.

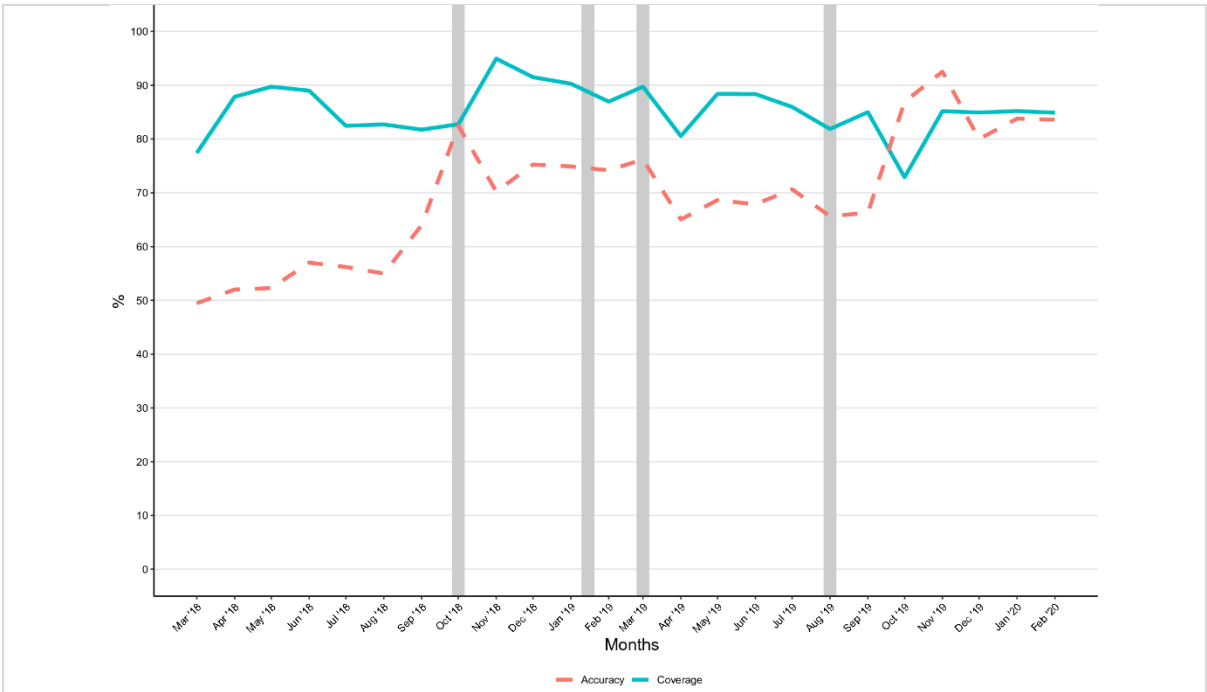


Figure 13: GPS coordinate coverage and accuracy.

The coverage and accuracy of the GPS coordinates were assessed over the two-year study period (March 2018–February 2020). The grey bars indicate when training was conducted

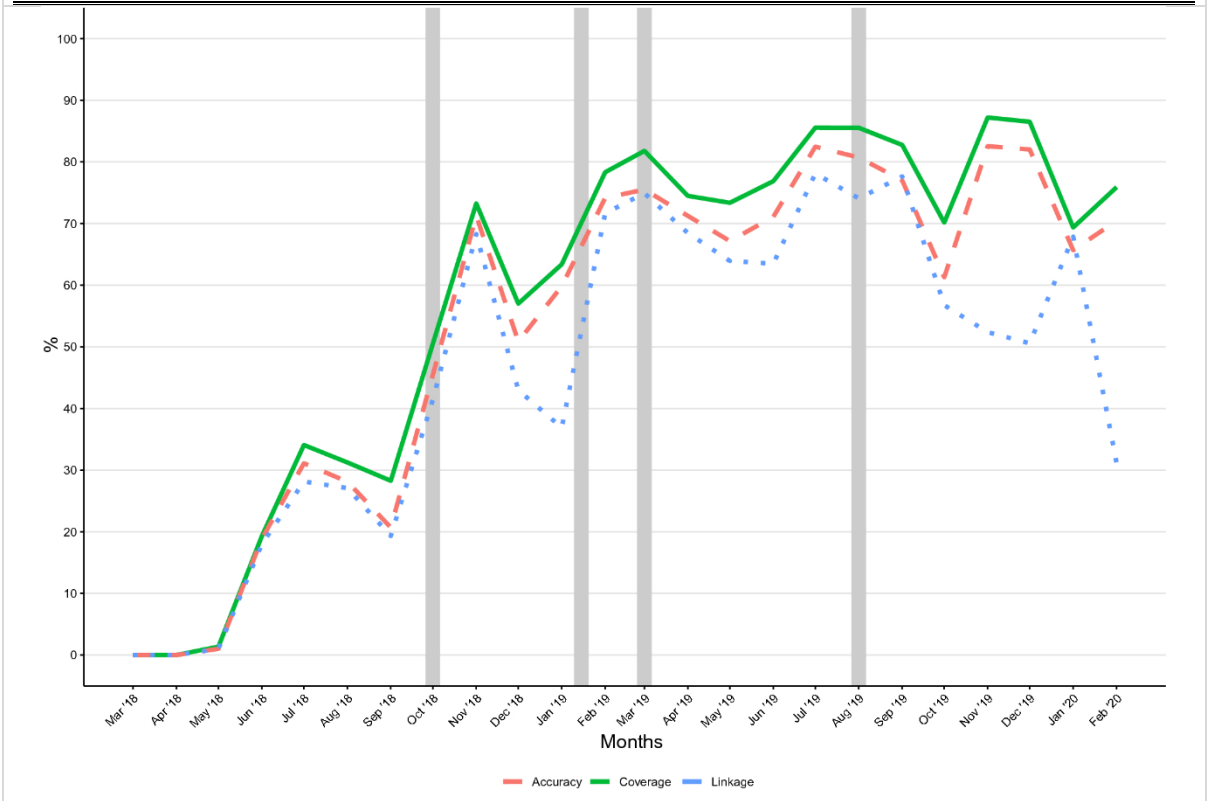


Figure 14: Barcode coverage, accuracy and linkage.

The coverage, accuracy and linkage of the barcodes were assessed over the two-year study period (March 2018–February 2020). The grey bars show when training was conducted

Linkage of the molecular markers of resistance results and case notification data increased to 72% (95%CI: 60 - 82%) at the end of the second quarter of 2019 before dropping to 47% (95% CI: 38-60%) in quarter one of 2020 (Figure S15 p 136). Molecular marker results could be linked to 99% (n=1989) of the notified cases with accurate barcodes and residential coordinates, and 2240 cases with accurate locality addresses and barcodes (Figures 13 and 14).

3.5.2 Temporal trends of the selected surveillance metrics

As shown in Figure 12 (overall) and Figure 15 (longitudinal analysis by semester), linkage data increased from 12% at baseline to 54% in the final quarter. Barcoded case notification forms increased from 38% to 97% overall, while of RDT samples received at the NICD laboratory, those barcoded increased from 19% to 85% (Figure 14). The household GPS coordinate accuracy increased from 48% to 76% over the study period (Figure 13).

GPS coordinate coverage and accuracy

Although the proportion of households with GPS coordinates ("coverage") remained high throughout the study, spikes in coverage that corresponded with the months after on-site training were noted except following the third training. However, the accuracy of these coordinates increased from 48% at baseline to 89% in November 2019, with high levels of accuracy sustained until February 2020 (Figures 13 and S11 p 132).

Barcode coverage and accuracy

Over the course of the study, there was a steady increase in the percentage of accurately barcoded samples. Coverage and accuracy increased from an average of 5% in the first quarter to 80% in the last quarter, again with peaks in the months after on-site visits and training, except for the third training (Figures 14 and S12 p 133). Over the two study years, there was a steady increase in barcode recording accuracy, reaching 75% (95% CI: 64 - 85%) by the last quarter of 2019; however, this dropped to 64% (95% CI: 59 - 85%) in the first quarter of 2020 (Figure S13 p 134).

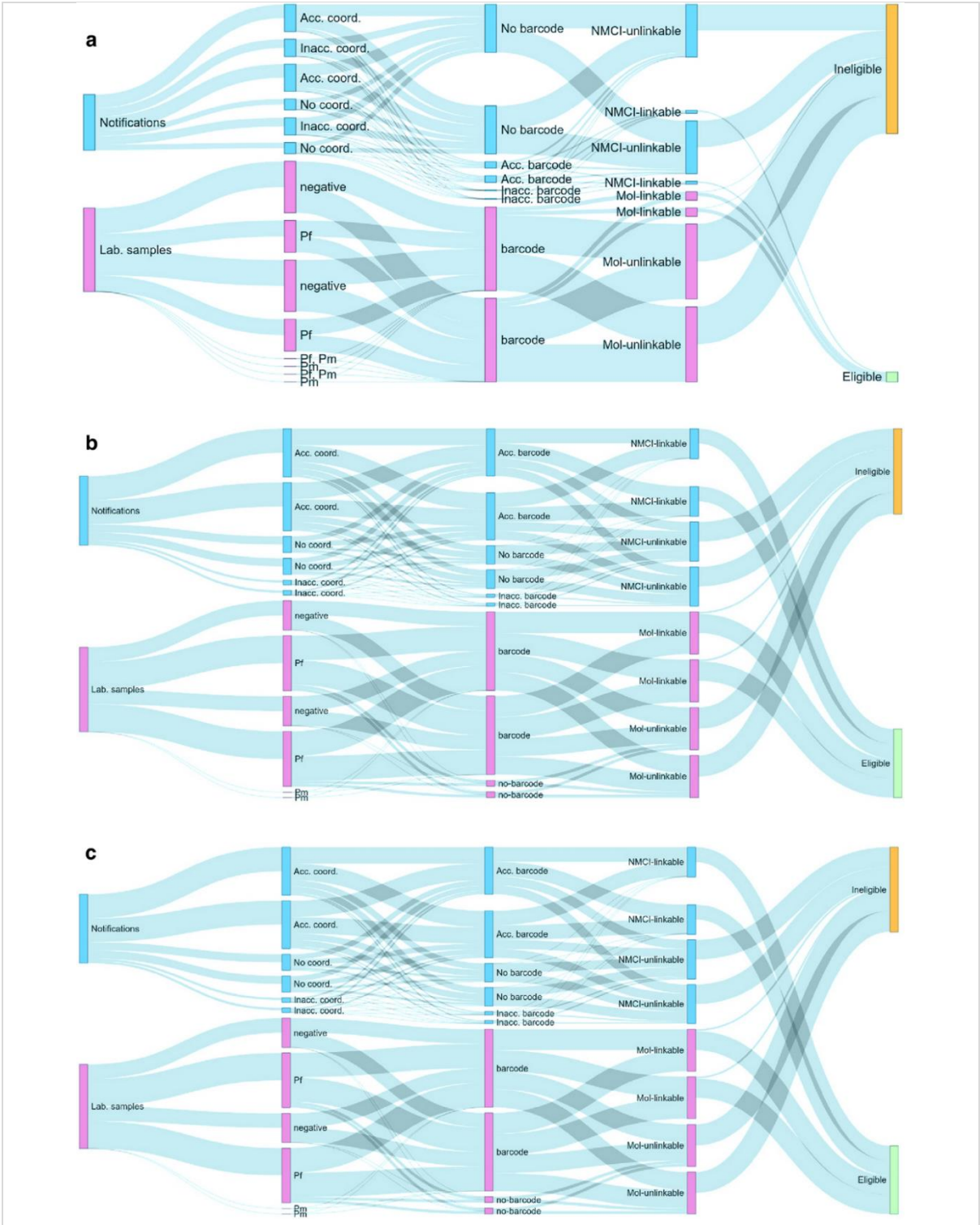


Figure 15: The longitudinal flow of data over the study period (March 2018–February 2020).

*Making Data Map-worthy (MDM) data flow over time from malaria case notification and laboratory data. The coloured bars show the totals, while the flows in grey illustrate the proportions of data that corresponded to the destination bar for the period. Over time, coverage, accuracy and linkage increased, illustrated by increased sizes of the corresponding bars for (**a**) March–August 2018, (**b**) September 2018–June 2019 and (**c**) July 2019–February 2020. (Acc. coord.: accurate residential coordinates, Inacc. Coord.: inaccurate residential coordinates, NMCI notifiable medical condition notification and case investigation data linkable / unlinkable, Mol molecular marker of resistance data linkable/unlinkable, Pf Plasmodium falciparum, Pm Plasmodium malariae*

3.5.3 Spatial analysis and semi-structured evaluation of the spatial data

Widely dispersed household coordinates were obtained in the first three-quarters of the study, including positive and negative coordinates (hence some coordinates in the northern hemisphere or ocean), as illustrated in Figure 16. Twenty-eight GPS collection devices used by Mpumalanga MEP case investigators were assessed. All 19 Android device GPS capturing applications had degrees and decimal minutes (DDD° MM.MM'), while 5/9 handheld Garmin eTrex-10 devices had decimal degrees and decimal minutes (DDD.DDDD°) and the remaining four in the format of degrees, minutes and seconds (DDD° MM' SS.S"). Standard operating procedures (Tool S2 p 138) were developed, which included how to set devices to decimal degrees, and four workshops were conducted (November 2018, January, March and July 2019) to train case investigators on best practices for the collection of GPS data.

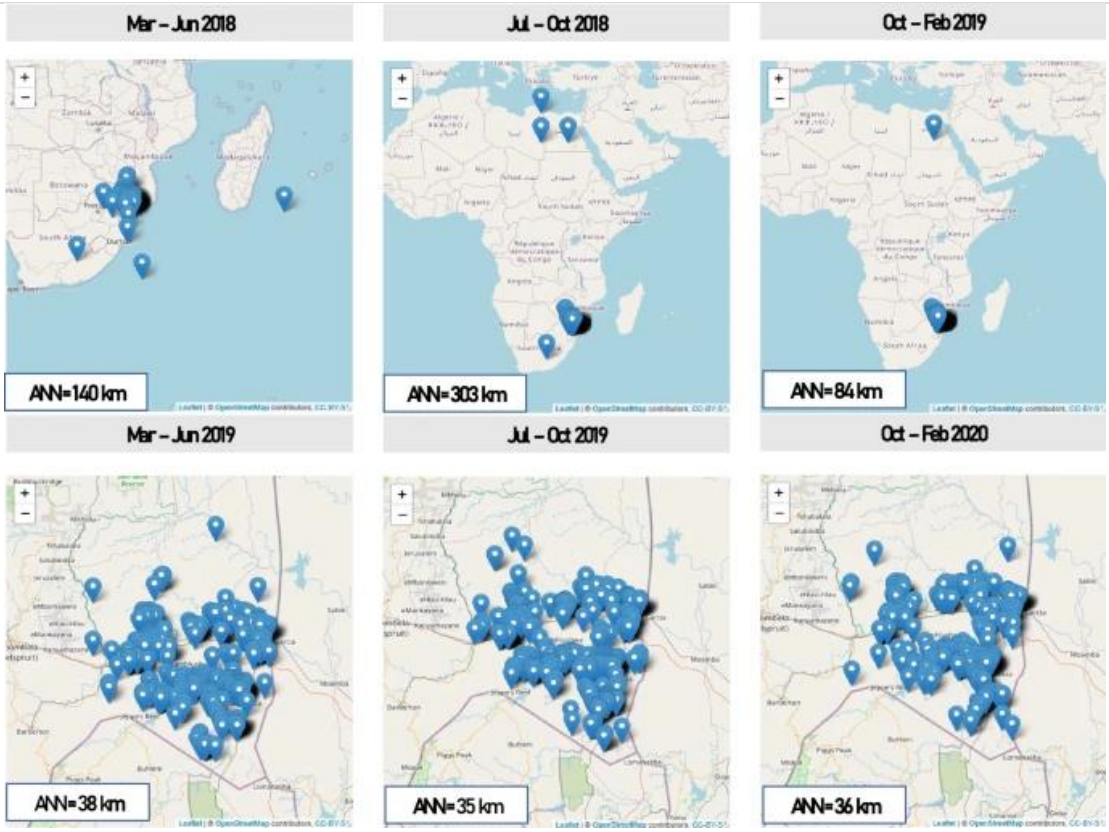


Figure 16: GPS coordinates of the malaria case residential locations collected during case investigation by quarter (2018–2020).

Distribution of GPS coordinates in the six quarters evaluated. The top three maps show some highly dispersed coordinates far away from the study area compared to the subsequent period shown in the bottom three maps. Training using SOPs in Additional file 1: Tool S2 were conducted in October 2018, January, March and August 2019.

Overall, improvement in the accuracy and precision of coordinates was observed over the study period. The average nearest neighbour distance decreased from 330 km in the second

quarter to 35 km by the fifth quarter. The proportion of residential coordinates of a given malaria case falling within the 0.5km x 0.5km area rose from 15% (95% CI: 4 – 48%) in the first quarter to 88% (95% CI: 72 – 96%) by the 5th quarter.

Of the maps generated (Figure S16a p 137), the density map of the distribution of cases by 0.5 x 0.5 km grid was preferred by the 24 MEP staff interviewed. Problems identified in the thematic map (Figure S16b p 137) included colouring of the whole ward polygon while malaria cases are clustered only in certain areas within the wards (other areas are largely unoccupied farmlands or nature reserves), and not residing equally throughout the ward as shown in the choropleth ward map. In addition, non-familiarity with the ward demarcations was demonstrated by case investigators failing to label the respective wards (5/38), with duplicate labelling (7/38) and misplaced labels with no consensus (12/38) reported.

3.5.4 Molecular markers of drug resistance

Of the 3748 malaria-positive RDTs, 13% (n=477) were malaria negative by PCR, five were pure *Plasmodium malariae* and four were mixed infections (*P. malariae* and *P. falciparum*). Of the 2297 RDTs reported as negative by the Mpumalanga MEP, 2% (53) were found to be false negatives by PCR. Of the false-negative RDTs analysed, 96% (51/53) were found to be pure *P. falciparum* infections, with the remaining 4% (2/53) pure *P. malariae* infections by PCR.

	Artemisinin	**Lumefantrine**	
Marker name	*K13*	*Pf mdr186*	*Pf crtK76T*
Samples assayed (n)	2385	2812	2122
Wild type	2385 (100%)	2803 (99.7%)	2121 (99.9%)
Mutant	0(0%)	9 (0.3%)	0 (0%)
Mixed	0(0%)	0 (0%)	1 (0.1%)

Table 2: Prevalence of k13, mdr186 and crt76 mutations in individual patients with P. falciparum infections, Nkomazi Sub-District, Mpumalanga (March 2018–Feb 2020).

Markers showing sensitive parasites include the wild type-k13, mutant-mdr186, and mutant/mixed crtK76T and potentially reduced susceptibility (or tolerant) markers with wild type-mdr186 and crtK76T.

The propeller domain of the *k13* gene was successfully amplified and sequenced from 73% (2385/3262) of the PCR positive falciparum samples (Table 2). All sequenced samples were wildtype at the 27 *k13* single nucleotide polymorphisms (SNPs) known to be associated with artemisinin resistance (delayed parasite clearance).

Almost all the samples in which the *mdr186* and *crt*76 SNPs could be assessed carried wild type *mdr186*ASN (99.7%, 2803/2812) and *crt*76LYS (99.9%, 2121/2122) alleles, respectively (Table 2). No increase in copy number was observed in the 1503 isolates assessed for *mdr1*

copy number. Thus, these samples were classified as potentially having reduced susceptibility (or tolerance) to lumefantrine, but not resistance, as shown in Figure 17.

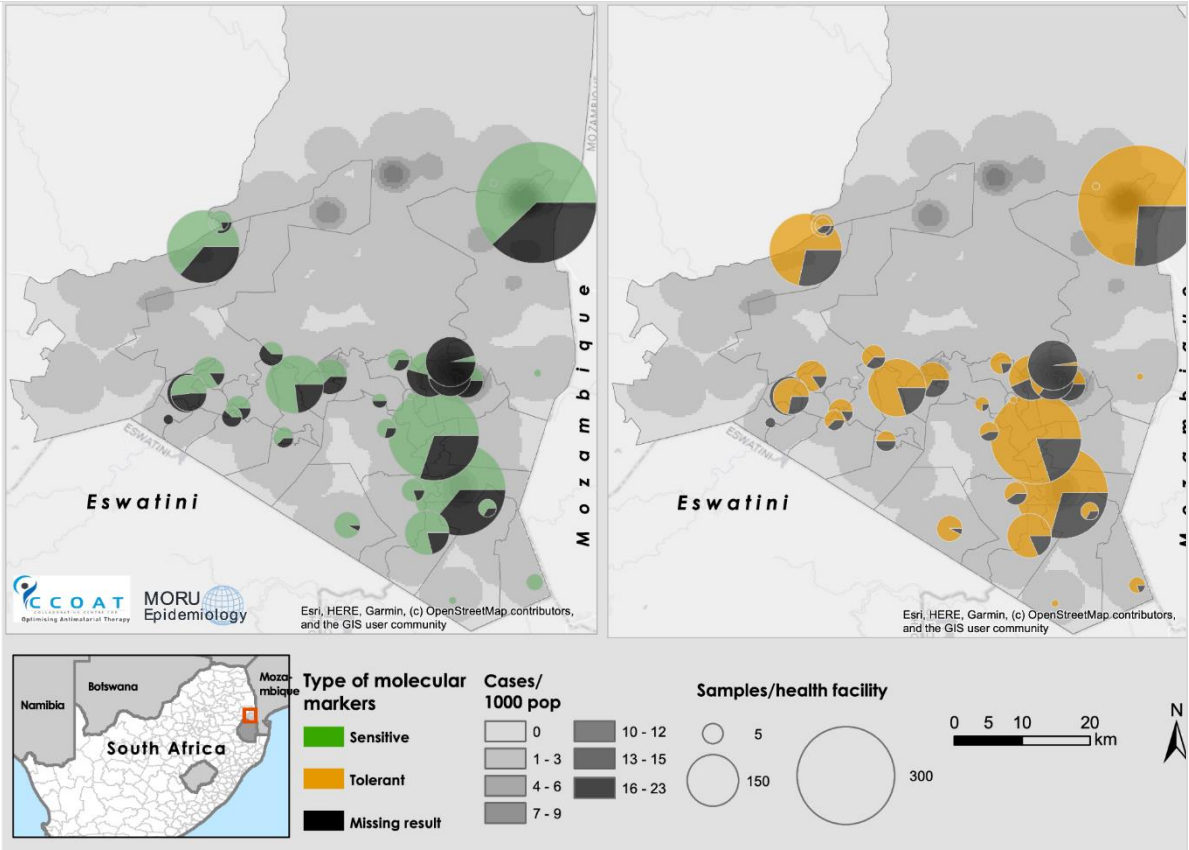


Figure 17: Distribution of confirmed malaria cases and molecular markers of artemisinin and lumefantrine drug "resistance" in Nkomazi sub-district, Mpumalanga (March 2018–February 2020). Distribution of P. falciparum malaria cases by 5 × 5 km grid, cases aggregated by facilities (pie charts), artemisinin Plasmodium falciparum k13 (left) and lumefantrine (right) mdr186ASN/crt76LYS molecular markers of "resistance", denoted by their susceptibility.

3.6 Discussion

Over the course of this study, data was curated from 4787 notified malaria cases and 55.1% of these cases were linked to their individual anti-malarial drug resistance profiles and residential localities in Nkomazi sub-District, a pre-elimination area in Mpumalanga, South Africa. This pilot evaluation used an iterative framework, termed 'Making Data Map-worthy'.

This is the first study utilising routine malaria surveillance data individually linked to molecular surveillance data to create near-real-time maps of anti-malarial drug resistance. The evidence generated by this pilot exemplifies the WHO recommendation to transform surveillance into a core intervention[149]. While most evaluations of molecular markers of artemisinin and partner drug resistance are from clinical trials, routine surveillance has the potential to facilitate the early detection of anti-malarial drug resistance in areas in which clinical trials are not feasible, such as low transmission intensity areas and areas where most malaria occurs in highly mobile and migrant populations [22, 61]. Although the WHO recommends iDES for low malaria transmission settings, there is little evidence of its feasibility in resource-constrained malaria programmes with thousands of malaria cases, particularly if most infections are among mobile and migrant populations. The findings from this study present a possible solution by allowing malaria programmes in such settings to target where and when resource-intensive confirmatory investigations and additional transmission-blocking interventions are needed [61].

Routine malaria surveillance data were collected and assessed over two years to map any spatiotemporal changes in anti-malarial drug resistance molecular markers. A user-centred feedback approach helped to assess data quality and incorporate improvement activities into data collection, analysis and map creation. Understanding malaria programmatic needs to support public health decision-making using an integrative approach, such as user-feedback and co-creation, has previously been shown to assist in the take-up and sustainability of new interventions, especially those that involve new technology adoption [158]. The greatest improvements in the surveillance metrics studied were generally observed following on-site supervision, and were sustained at a moderately high level for seven months after the last on-site supervision visit. Since there are no proposed analysis frameworks for evaluating routine location data from cohorts of infectious diseases patients, metrics were adapted from the latest systematic review and the WHO's Data Quality Review (DQR) framework, an expert proposed framework designed only for the assessment of facility-based data [62, 63]. Malaria surveillance data include off-facility activities such as case investigation home visits to assess treatment adherence and response, while seeking any mosquito vector risk factors.

A 0.5 x 0.5 km malaria density map provided the most user-friendly representation of the distribution of malaria. This finding challenges the most prevalent malaria map designs, namely ward level thematic maps of case distribution, which show that geographical or political boundaries demarcate cases. Density and other modelled maps can show disease distribution beyond uninterrupted land borders, which relates better to how infectious diseases, such as malaria spread. Another advantage of the density maps in this area was the avoidance of large areas used for plantations and a national game reserve that lacked human settlements for

malaria transmission. Density maps displaying cases in a continuous land surface require modelling of the incidence and other key covariates that determine the distribution of cases. This study used the latest available human settlement data and feedback from 24 local malaria case investigators to validate the correct malaria case distribution in their areas.

While only 55.1% of all reported malaria cases could be linked, overall, the iterative analysis, training and feedback improved the precision of collected GPS data from 15% to 88% within 0.5 x 0.5 km grid squares. With 89% (n=2002) of investigated cases having accurate GPS location linked to their individual molecular marker of resistance results, it would be possible to target the correct geolocation of a given case for further investigation and prompt response within that community, should any molecular marker/s of concern be identified.

Almost 45% of individual malaria cases and molecular data could not be linked in this study. To achieve optimum linkage and data curation might only be possible with the iterative analysis of data, identification of gaps and implementation of collaborative surveillance strengthening activities, in order to improve the data collection, capture, analysis and reporting cycle. Although this may be perceived as resource-intensive and challenging to implement in a low resource setting, such an investment is essential for all malaria surveillance objectives to be achieved, not just for promptly detecting, locating and responding to any emerging anti-malarial drug resistance. To avoid straining the already stretched health system, further development of assay methods is needed to obtain an adequate yield from RDTs alone, as filter paper DBS requirements could potentially limit the scalability of this approach. Several studies have proposed the use of RDTs for parasite DNA extraction as a useful alternative to the current methods; however, its applicability at field level is yet to be established [65–68]. Even for next generation sequencing, using DBS is recommended as the DNA obtained from RDTs is generally insufficient and of poor quality [69]. Pooling individual patient DBS samples before performing a genomic analysis has been proven useful for low and high malaria transmission settings [70]; however, no such pooling of samples has been reported yet for RDTs. Morris et al. 2013 noted that when RDTs were used alone, the DNA yield was much lower, allowing for only a “one-shot operation” with no possibility of DNA re-extraction [66]. Thus, each case’s RDT and blood spot were used together in a single reaction to increase the parasite density, if both were present. The DNA yield for RDT vs DBS was not quantified or compared in this study.

Although numerous human and system errors were identified and corrected, especially in the first and second quarters, a significant proportion of the cases could not be linked to their resistance profile or locality. The inability to follow-up patients, particularly those among the highly mobile migrant populations, played a significant role in the low number of household

coordinates collected. Although many of the migrant cases presented at local healthcare clinics, most could not be followed up to geolocate their residential addresses/ward or assess treatment adherence and response. Undocumented migrants may be particularly likely to provide inaccurate contact details for the notification form, and many transit rapidly through endemic areas to reach major cities in non-endemic areas.

Although the proportion of filter paper DBS samples submitted with positive RDTs increased over the study period to 92% in the final quarter, the quality of the DBS collected remained suboptimal. Only 61% of the collected DBS passed the internal quality control screening, in terms of sufficient blood volume and storage conditions to be entered into the laboratory workflow. Despite numerous training rounds, the health facility staff persisted in collecting very low blood volume (less than 10µl) DBS. These low blood volumes decreased the efficiency of both the DNA extraction and downstream PCR analyses, particularly in infections with low parasite densities. It has been shown that DBS with at least 50µl of blood are essential for molecular assays that include next-generation sequencing [72]. More intensive in-person training would be required to improve and sustain progress.

A small proportion (2.2%) of negative RDTs were malaria positive by PCR. This finding could be due to patients with infections that have parasites loads below the detection limit of the RDT (200 parasites per µl blood), but within the detection limit of the more sensitive PCR assay (20 parasites per µl blood). Other possible explanations include inadequate storage conditions of the RDT and/or DBS or the incorrect use of the RDT (addition of too little blood or too much Lysis buffer or reading before the recommended time). Preliminary investigations suggest these false negatives were not due to histidine-rich protein 2 (hrp2) deletions; available evidence suggests hrp2 mutations are currently rare in southern Africa [73]. However, ongoing systematic testing is required to exclude *hrp*2 deletions in this region.

Fortunately, neither 'validated' or 'associated/candidate' K13 mutations associated with artemisinin resistance were found in this study [55,58,59]. tool In Asia, clinical failure rates have been linked to the increase in *mdr1* copy number as compared to the *mdr*86ASN alleles [199]. The increase in *mdr1* copy number has rarely been reported in Africa [52, 80], and this study did not observe any such increase.

This study highlights the need for continued rigorous surveillance, particularly in light of multiple reports of the independent emergence of K13 resistance markers in Central [136], Eastern [136–139], and Western African countries [139, 140]. Despite the absence of validated K13 artemisinin resistance mutations in southern Africa, the decline of ACT clinical efficacy below the WHO threshold of 90% observed in nearby Angola in 2013 and 2015 (Zaire Province) and 2019 (Lunda Sul Province) [81] is of some concern [202], although consecutive

studies in 2017 and 2019 showed adequate parasite clearance rates [201, 203]. The extreme AL drug pressure in sub-Saharan Africa, delayed parasite clearance following AL treatment in Rwanda, and the emergence of clinical artemisinin resistance in Uganda calls for strengthening resistance surveillance across Africa. Such activities will inform efficient targeting of transmission blocking activities (including SLD primaquine and foci clearing) and further investigation of parasite clearance rates and ACT therapeutic efficacy, with prompt changes to treatment policy [143] should treatment failure rates exceed acceptable limits (currently 10%) [181].

Some of the challenges and limitations often seen with the use of routine surveillance data were also encountered in this study, including limited data availability, multiple information/reporting systems and relatively high staff turnover rates. During the course of the study, DHIS2 was being rolled out and updated, while malaria cases notified before the generic notifiable medical condition (NMC) system was introduced in January 2019 were entered in a provincial MS Access-based Malaria Health Information System. The transition between these overlapping systems might have affected the data capturing cycle and impaired harmonization of the two datasets, potentially impacting on data quality. During this transition period, malaria cases could be notified using paper notification forms (later captured into the DHIS2) or on one of two mobile-phone-based systems, a malaria case short messaging service and NMC mobile applications[84]. Ideally, these two systems should feed into the DHIS2 system and remove duplication; however, these two mobile-phone-based databases could not be accessed for confirmation. Insufficient staff and resources may also hamper data quality. During the two years of this study, some of the staff from a collaborative non-governmental organization (who comprise more than half of the malaria case investigators) had to stop working for a few months whilst waiting for the renewal of funding, and this interrupted their surveillance activities. Other studies have also documented similar challenges leading to inconsistencies and inaccuracies in health information systems [85–87]. Such challenges limit the usage of routine data in decision-support systems, especially in low-resource settings. Consequently, countries rely on population-level health surveys, which remain costly, outdated and irregular; for instance, the WHO World Malaria Report still relies on modelling data due to incompleteness and inconsistencies in the malaria routine reporting system [1,3,88–90]. Other factors such as climate, altitude, vectors, or the existing malaria interventions that have been shown to affect the distribution of malaria cases in previous studies were not explored [33, 91], given the focus of this study.

Routine near-real-time mapping of molecular markers of anti-malarial drug resistance data to the healthcare facility, locality and patient household levels offers malaria programmes rapid and efficient monitoring of spatiotemporal changes in anti-malarial drug resistance profiles. By

improving the facility and population-based routine surveillance systems as shown in this study, malaria programmes can identify areas of concern requiring further investigation and conduct targeted therapeutic efficacy trials and transmission limiting activities, hence allocating their resources strategically. This might however be too costly for high transmission settings. Here, molecular markers from a representative sample of malaria cases from a range of health care facilities with optimal geographic and epidemiological coverage could be used to strengthen resistance surveillance and inform programmes of areas where further investigation should be conducted. Such sentinel sites could be linked to a centralized national or regional laboratory, reducing investments and running costs. Low and moderate transmission settings have started implementing centralized genomic surveillance. For instance, Haiti [92], Honduras [213] and South Africa [154] provide examples of national molecular surveillance, while the GenRe-Mekong study provides a model for regional surveillance [94]. However, a feasibility study and cost-effectiveness analysis may be needed to inform the relevance of such a system in high malaria transmission settings. Pragmatic and innovative approaches such as co-design can enable precision mapping, contextualization of analyses and meeting of malaria MEP needs.

Although linking individual patient information and the molecular markers might not directly benefit the patient, the molecular results may be of value in case the patient returns to a healthcare facility with recurrent malaria, as the linked molecular marker data will help differentiate anti-malarial resistance from other causes of treatment failure and thus inform re-treatment strategy. While enhancing the quality of routine data can be a daunting task, identifying, monitoring and improving important surveillance metrics and indicators by MEPs is considered critical to both evaluating progress and achieving malaria elimination targets. This is consistent with the WHO recommendation that surveillance is a core intervention to achieve elimination. Countries that have eliminated malaria have established strong information systems and maintained them to prevent the re-establishment of the disease [149]. Sustainability can be facilitated by researchers and MEPs working collaboratively to develop tools and resources for efficient training and regular supervision that can be cascaded to reach all relevant MEP staff.

3.7 Conclusion

In low malaria transmission settings in sub-Saharan Africa, near-real-time fine-scale mapping of molecular markers of anti-malarial drug resistance can assist in rapidly and efficiently monitoring anti-malarial drug resistance and identifying areas requiring further investigations and interventions. However, the sustainability of such a strategy requires regular training, close supervision and strong programmatic support. More innovation and research are

needed to explore more cost-effective strategies for anti-malarial resistance surveillance systems given current resource constraints, such as sampling at representative sentinel health facilities strategies versus comprehensive sampling, linkage at individual versus health facility levels, particularly in moderate and high transmission settings. The methods piloted, and lessons learnt in this study could inform scale-up to provincial, national and regional malaria control/elimination programme levels in low- and middle-income countries and may be relevant for other antimicrobial resistance surveillance.

Declarations

Ethics approval

This study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (HREC REF Number: 698/2019), including data obtained in the SS4ME project (HREC REF Number: 519/2017). SS4ME was endorsed by the South African Department of Health, the NICD and the Mpumalanga Malaria Elimination Programme. All governmental and non-governmental partners were notified, and their programme staff informed, including malaria elimination programme staff, information officers, clinicians, and data clerks.

Consent for publication

Not applicable.

Data availability

The datasets generated and/or analysed during the current study are available at the WWARN Tracking Resistance website (https://www.wwarn.org/tracking-resistance/artemisinin-molecular-surveyor).

Competing interests

The authors declare that they have no competing interests.

Funding

The Smart Surveillance for Malaria Elimination Pilot in Mpumalanga, South Africa, was co-funded by the South African MRC and the Worldwide Antimalarial Resistance Network (WWARN). WWARN is funded by the Bill and Melinda Gates Foundation and the ExxonMobil Foundation. This research was also, in part, funded by the Wellcome Trust [Grant number

220211]. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission. Funders did not have any influence in the design of the study, data collection, analysis, interpretation of data and or writing the manuscript.

Authors' contributions

FMK, KIB and RJM designed the study. FMK, KIB, AM and JR trained and supervised the field teams. FMK, GK, GM, LW and RM compiled malaria cases data. JR designed the molecular analysis framework, oversaw all molecular laboratory procedures and validated all molecular data. FMK and JR summarized molecular dataset and performed molecular analysis. FMK performed data linkage and curation. FMK and RJM conducted spatiotemporal analysis. FMK and KIB drafted the manuscript. CD, EA, KIB, MD, PJG and RJM provided critical review of the manuscript. All authors reviewed the manuscript.

Acknowledgements

We would like to acknowledge the Mpumalanga Provincial and Nkomazi Sub-district Malaria Elimination Programmes of the National Department of Health of South Africa, Laboratory for Antimalarial Resistance Monitoring and Malaria Operational Research, Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Disease, Clinton Health Access Initiative, South Africa, Humana People to People (HPP), South Africa, all MEP personnel and healthcare facility staff who collaborated in this study for all their support.

4 Chapter 4

Factors Affecting Integration of an Early Warning System for Antimalarial Drug Resistance within a Routine Surveillance System in a Pre-elimination Setting in Sub-Saharan Africa.

(Factors affecting integration of an antimalarial drug resistance early warning system within routine surveillance)

Frank M. Kagoro [1,2,3,4,5], Elizabeth Allen [1,3,4], Jaishree Raman [6,7,8], Aaron Mabuza [1,3], Ray Magagula [9], Gerdalize Kok [9], Gillian Malatje [9], Philippe J. Guerin [3,4,5], Mehul Dhorda [2,3,4,5], Richard J. Maude [2,5,11,12], Karen I. Barnes [1,3,4]

[1]Collaborating Centre for Optimising Antimalarial Therapy (CCOAT), Division of Clinical Pharmacology, Department of Medicine, University of Cape Town (UCT), Cape Town, South Africa

[2]Mahidol Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

[3]WorldWide Antimalarial Resistance Network (WWARN), South-East African Regional Hub, UCT, South Africa

[4]Infectious Diseases Data Observatory (IDDO), Oxford, UK

[5]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

[6]Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Disease, Sandringham, Gauteng, South Africa

[7]Wits Research Institute for Malaria, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

[8]UP Institute for Sustainable Malaria Control, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[9]Mpumalanga Provincial Malaria Elimination Programme, Mpumalanga, South Africa

[10]Malaria Programme, Clinton Health Access Initiative, Pretoria, South Africa.

[11]School of Public Health, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong

[12]The Open University, Milton Keynes, UK

Keywords: Early-warning system, Surveillance, Health Information System, Antimalarial resistance, *Plasmodium falciparum*, Pre-elimination, Africa, Malaria.

4.1 Abstract

To address the current threat of antimalarial resistance, countries need innovative solutions for timely and informed decision-making. Integrating molecular resistance marker surveillance into routine malaria surveillance in pre-elimination contexts offers a potential early warning mechanism to identify resistance signals and target areas for further investigation, including therapeutic efficacy studies. However, there is limited evidence on what influences the performance of such a system in resource-limited settings.

From March 2018 to February 2020, a sequential mixed-methods study was conducted in primary healthcare facilities in a South African pre-elimination setting to explore factors influencing the flow, quality and linkage of malaria case notification and antimalarial molecular resistance marker data. Using a process-oriented framework, we undertook monthly and quarterly data linkage and consistency analysis at different levels of the health system, as well

as a survey, focus group discussions and interviews to identify potential barriers to, and enhancers of, the roll-out and uptake of this integrated information system.

Over two years, 4,787 confirmed malaria cases were notified from 42 primary healthcare facilities in the Nkomazi sub-district, Mpumalanga, South Africa. Of the notified cases, 78.5% (n=3,758) were investigated, and 55.1% (n=2,636) were successfully linked to their *Plasmodium falciparum* molecular resistance marker profiles. Five tangible processes—malaria case detection and notification, sample collection, case investigation, analysis and reporting—were identified within the process-oriented logic model. Workload, training, ease of use, supervision, leadership, and resources were recognized as cross-cutting influencers affecting the program's performance.

Approaching malaria elimination, linking molecular markers of antimalarial resistance to routine malaria surveillance is feasible. However, cross-cutting barriers inherent in the healthcare system can influence its success in a resource-limited setting.

4.2 Introduction

Sub-Saharan Africa bears the largest burden of malaria, with 94% and 95% of the global cases and deaths, respectively [2]. Malaria-endemic countries in Sub-Saharan Africa are now also facing the looming threat of antimalarial drug resistance. Malaria parasites with reduced susceptibility to artemisinin-derivatives are emerging and rapidly spreading, threatening the continent’s control and elimination goals, heightening the need for novel tools and strategies to effectively tackle this threat [33, 34, 215–219]. Such innovations need to be timely, relevant and tailored to existing health systems, particularly in resource-limited settings.

In its strategy to respond to antimalarial drug resistance in Africa, the World Health Organization (WHO) emphasised the need for robust and agile surveillance systems capable of promptly detecting and responding to antimalarial drug resistance and recommends that this surveillance be integrated into routine malaria surveillance systems [220, 221]. This integration is particularly important in pre-elimination areas, where the risk of drug-resistant parasites emerging may be heightened by higher drug pressure and lack of partial immunity [222]. In these settings, the WHO also recommends monitoring the prevalence of molecular markers of antimalarial drug resistance (MMR), as an early warning system to identify

resistance signals and areas requiring further investigation, including therapeutic efficacy studies (TES). Despite TES being the cornerstone of antimalarial drug efficacy monitoring, they are not conducted regularly and do not provide a timely spatial representation of the distribution of antimalarial resistance. Previous evidence has shown that integrating MMR using a routine malaria notification system is feasible in sub-Saharan African settings [223]. However, little is known about the barriers and enhancers to integrating MMR within the routine malaria surveillance system. Understanding such factors is needed by countries adopting or expanding molecular surveillance, for which recent laboratory investments have been extensive [224, 225].

In 2018, the South African National Malaria Programme (NMP) piloted a novel technology to consolidate and enhance malaria surveillance activities and treatment approaches fundamental to achieving malaria elimination. Through the Smart Surveillance for Malaria Elimination (SS4ME) initiative, MMR data were linked to malaria case notifications near real-time in Nkomazi, Mpumalanga, a pre-elimination setting in near real-time [226]. SS4ME included the collection of malaria rapid diagnostic tests (mRDTs) and, wherever possible, dried blood spots (DBS) on filter papers for assaying MMR that can be linked by unique barcodes to individual case notifications.

Developing and evaluating the integration of MMR into routine malaria surveillance required an assessment of the surveillance system's performance, both independently and together with other routine notification components, making for a complex intervention [227]. As per national malaria treatment guidelines, all suspected malaria cases presenting to health facilities should be confirmed by a mRDT or microscopy (passive case detection) before treatment is administered [47]. Additionally, in pre-elimination areas of South Africa, the NMP screens high-risk groups, such as migrant and mobile populations (proactive case detection), and households surrounding the residence of index cases (reactive case detection)[33, 218]. Therefore, integration of MMR into routine malaria surveillance system involved adopting several technologies, activities, and processes into the existing malaria notification system, with regular monitoring and adaptations. For SS4ME, a conceptual framework was developed to explore and guide how the roll-out, adoption and utilisation of new and existing technologies would inform malaria elimination goals. This laid a foundation for benchmarking how SS4ME was received and understood by potential key stakeholders. As with other such conceptual theories, this framework lacked a mechanism to explore the interaction between the different determinants of success and how these interact with the users or beneficiaries. Therefore, this approach needed to be revised and expanded to identify internal, external and interactive factors that could affect the implementation and impact of the intervention. Through the step-by-step depiction of how a program operates and how it leads to the desired outcomes,

process-oriented logic models have been used in programme planning and evaluation to understand its key elements [228].

'Making Data Mapworthy' was a quantitative sub-study linked to the SS4ME pilot that evaluated the feasibility of integrating MMR into the routine malaria surveillance system using coverage, accuracy and linkage of malaria cases in near real-time [223]. However, these metrics alone could not fully examine the flow of data, or users' practices and perceptions. Here, factors influencing the flow, quality and linkage of malaria notification data and associated MMR from different reporting levels are investigated to inform the enhancement and sustainability of this integrated early warning system for antimalarial resistance.

4.3 Materials and Methods

This SS4ME sub-study used an iterative sequential mixed-methods design and included 1) monthly and quarterly quantitative descriptive analyses, 2) a healthcare facility staff survey, and 3) focus group discussions (FGDs) and in-depth interviews (IDIs) with healthcare facility staff involved in malaria case management and NMP staff in Nkomazi sub-district, a pre-elimination area in Mpumalanga province, South Africa.

Individuals identified through proactive, reactive, or passive case detection were screened for malaria using a falciparum-specific histidine-rich protein 2 (HRP2)-based mRDT (First Response™ Malaria Ag P. falciparum HRP2 Detection Rapid Card Test, Premier Medical Corporation Ltd, India) according to the Mpumalanga Malaria Programme tender process. An additional 10% of the negative mRDTs were collected and sent to the National Institute for Communicable Diseases (NICD) for quality assurance. Both symptomatic and asymptomatic positive cases were treated following national treatment guidelines with the WHO-recommended 3-day artemether-lumefantrine regimen, used in the area since 2007. For MMR surveillance, DBS filter papers were collected from all patients with positive mRDTs. Demographic and case data were collected through the Notifiable Medical Condition form or app, and verified for quality within 24 hours at the sub-district NMP office. Case investigators conducted household visits within 24-72 hours of notification, recording Global Positioning System (GPS) coordinates and assessing malaria risk factors. All notifications were quality-checked and electronically captured into the routine District Health Information System II (DHIS2) at the sub-district NMP office [47].

Three levels of notification data were examined: healthcare facilities, the sub-district NMP office serving as a data collection / capturing centre and provincially through DHIS2 submissions. All SS4ME-participating healthcare facilities were enrolled for malaria case data analysis at monthly and quarterly intervals, to gauge coverage and consistency as

performance metrics. The facilities were then categorised as low- or high-performing on the overall data linkage, and two from each level were purposively selected for enhanced data quality assessment.

A paper-based survey (Tool S1) was administered to primary healthcare facility staff treating malaria patients to evaluate their practice, perception, and experience of the integration of MMR into routine malaria surveillance system activities. For FGDs and IDIs, various cadres of staff involved in malaria case management at healthcare facilities and in the NMP were invited to participate by email or phone call. At least one staff member performing any malaria-related activities was invited per healthcare facility. After obtaining verbal consent, interviews were conducted in English using pre-prepared interview guides *(Guides S2 and S3, page 148 and 151)* to maintain consistency and quality. The audio recordings were securely stored on the password-controlled study computer and later transcribed. Two study investigators listened to audio recordings with reference to the transcribed scripts and resolved any interpretation conflicts or transcription errors by consensus.

This study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (HREC REF Number: 698/2019), including for data obtained in the SS4ME study (HREC REF Number: 519/2017). SS4ME was also endorsed by the South African Department of Health, the NICD and the Mpumalanga Malaria Programme. All partners were notified, and their staff were informed of the study, including NMP staff, information officers, clinicians and data clerks.

The descriptive analysis focused on staff survey data and monthly and quarterly DHIS2 data evaluations. The latter summarised notified and investigated cases, mRDT / DBS samples analysed, their linkage, as well as spatiotemporal trends in MMR and usability assessments. All quantitative data analyses were conducted using R programming language (versions 3.6 and 4.0). Further quantitative methods used have been explained elsewhere [223]. For consistency, quarterly aggregates of notified cases and investigated cases from each healthcare facility sampled were compared at three levels (healthcare facility, sub-district data capture centre and provincial DHIS2 records) and the median difference was computed. Consistency was defined as an equal number of cases being reported at different levels, with a difference of +/- 5 cases allowed to account for delayed reporting. Since a consistency benchmark for integrating MMR data into malaria case data had not been described before, this was established by adapting the internal consistency benchmark proposed by the WHO Data Quality Assurance guideline [229]. Data were then explored quantitatively and qualitatively to identify possible causes of inconsistency.

For the qualitative data, each audio recording was transcribed and imported to NVivo 12 before being coded deductively, based on the conceptual framework and interview guides and inductively from other observations. The first coding cycle assigned labels to text excerpts in the transcripts as lowest level 'nodes'. These were then explored for repeated ideas and patterns, which were grouped, organised and categorised as higher-level themes, based on the interview guide sections and new concepts emerging. The prevailing themes and their theories were extracted and further analysed through the framework matrix, and the analytical outputs were evaluated across the range of participants and stakeholder groups.

As a complex intervention, the study used a process-oriented logic model to link the overall qualitative and quantitative data to determine the enablers and barriers that would explain the changing trends. To do so, results were further categorised into processes and logically analysed to identify inputs and activities, intended and unintended outputs, and outcomes. A matrix was constructed to explore the level of influence and performance for each key feature identified from the process logic model.

4.4 Results

4.4.1 Overall results

A total of 4,787 malaria cases were notified in Nkomazi, Mpumalanga with 78.5% (n=3,758) investigated by the NMP (Figure 18) from March 2018 to February 2020. Of the notified cases, 55.1% (n=2,636) were linked to their *Plasmodium falciparum* MMR profiles, with 85% (n=2,240) of the linked cases mapped to healthcare facility, ward and locality levels. Further quantitative results are reported elsewhere [223].

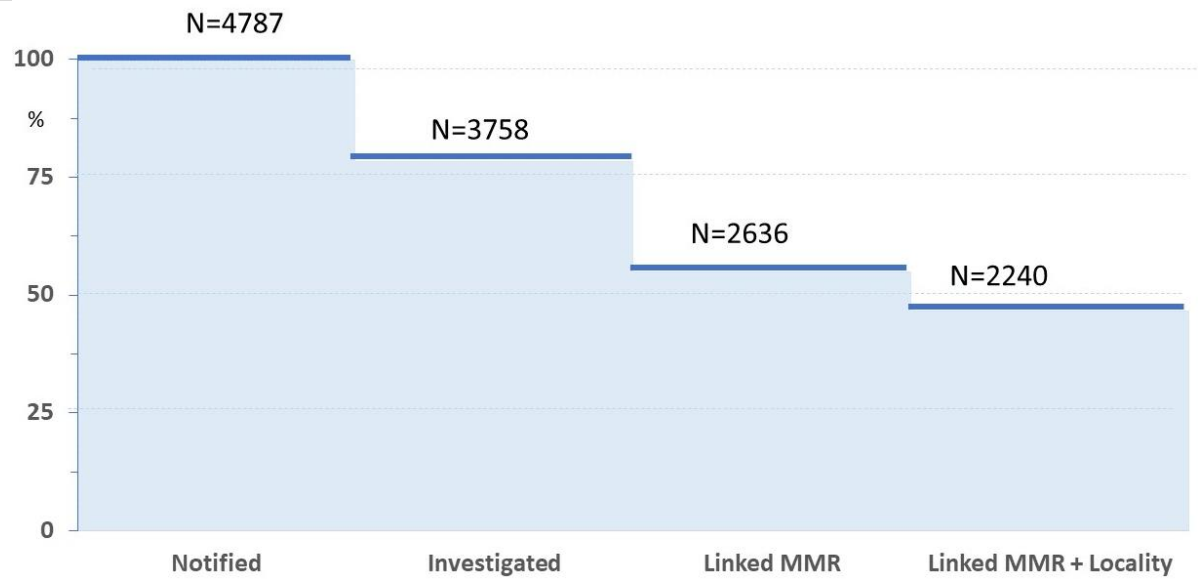


Figure 18: Summary of cases notified, investigated, and linked to MMR and locality from March 2018 to February 2020 in Nkomazi sub-district, Mpumalanga.

A total of 46 healthcare workers participated in five separate FGDs, with group sizes ranging from 8 – 12 participants. Of the participants, 32 were nurses (including professional nurses, occupational nurses and assistant nurses), and 14 were environmental health officers who worked as health promoters or malaria case investigators, data clerks, data capturers and surveillance officers. The majority of the participants were women (n=31). Four IDIs were conducted with staff at the supervisory and decision-making levels performing infection control coordination, district and provincial NMP management, primary healthcare management and malaria surveillance supervision.

The 50 participants involved in the FGDs and IDIs had between 1 and 31 years of experience working with the NMP and were involved in the surveillance data reporting chain. Their responsibilities included field and clinical diagnostics, care of patients, case notification, case investigation, data capture, sample collection, labelling and transport, supervisory, and reporting roles and decision-making. Several themes emerged from discussions on the integration of MMR into the routine malaria surveillance system, which included malaria case notification, sample collection, packaging and transportation, case investigation, data capture and reporting.

A total of 64 nurses from 21 of the 42 healthcare facilities participated in the survey. Respondents were registered / professional nurses (67.1%), enrolled nurses (22%), and occupational nurses (6%). Two respondents did not mention their job titles. Five quarterly assessments were conducted for three healthcare facilities to evaluate consistency. Due to logistical challenges, the team did not manage to enroll the fourth health facility.

In Figure 19 below, prevalent themes in the FGDs and IDIs included ease of use, perceived usefulness of surveillance, staff members' reluctance to adopt new activities, contradictions in best practice definitions, workload, and system support. These themes converged into sub-themes: work commitment, agency and ownership, challenging processes, compromise, staffing needs, training, leadership, and supervision.

Five key processes (Tool S3 A and B, p 157) emerged: malaria case detection and notification, sample collection, case investigation, data capture, analysis and reporting. These themes are described below using the process-oriented logic model outlined in Tool S3 B, linking them to processes, inputs, outputs, and outcomes.

4.4.2 Process I: Malaria case detection and notification

The change introduced during the integration of MMR into the routine malaria surveillance system was the inclusion of barcode stickers on the case notification forms during passive, proactive and reactive case detection. The study also developed activities to enhance the collection of all required malaria case details in the reporting notification form (e.g. contact information and household address / directions). This was to support the linkage of demographic and location data to the MMR. This also resulted in additional workload, which was aggravated by the multiple notification reporting systems. As shown in Figure S17 (p 147), the NMP introduced two new malaria notification systems, leading to three malaria notification systems running concurrently during the study period, namely a) the Notifiable Medical Condition (NMC) book / forms, b) the Malaria Connect mobile application and c) NMC mobile application.

The surveillance team supervisors mentioned that healthcare workers routinely used the Malaria Notification Book for notification only, and notified cases using other systems as an additional option. This was corroborated by healthcare workers themselves who reported using the notification book first and other notification methods depending on the workload, as quoted below:

> *'We do the paper first, the notification book first, the remaining depends on their time or workload'*. ***[Nurse, IDI06].***

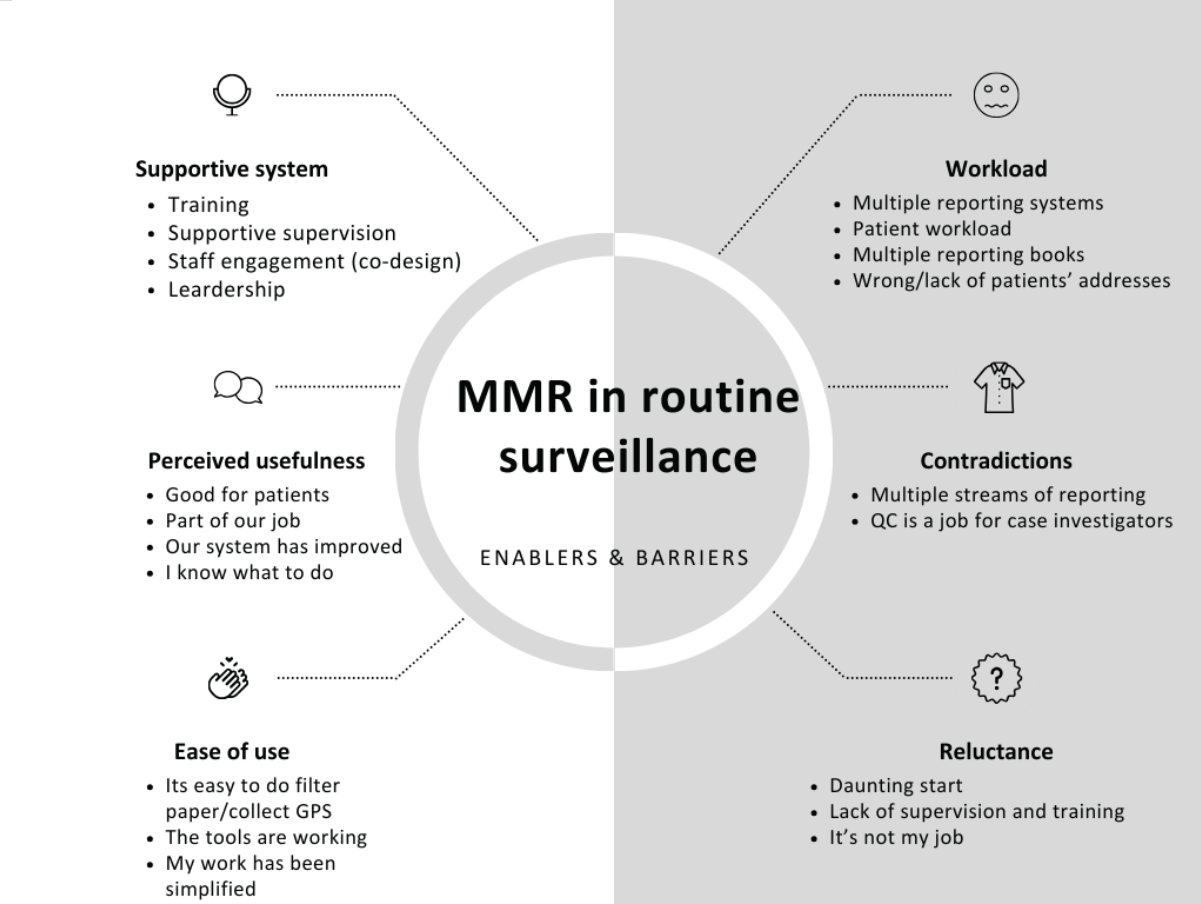


Figure 19: The illustration of factors discovered during focus group discussions and in-depth interviews influencing the implementation of MMR into the routine malaria surveillance system in the Nkomazi sub-district.

Several themes and sub-themes surfaced from these discussions. Initially, participants voiced reluctance and contradictions, leading to a confusion over roles. This uncertainty spawned a challenging start and negatively impinged on the work. More intensive training, committed leadership, and consistent supervision were recommended to counter these issues. In instances where confusion persisted, a supportive system was seen as beneficial to harmonise tasks and provide necessary training and quality control. This tactic successfully alleviated immediate challenges. Lastly, even though the integration process demanded an increased workload, healthcare staff who were willing to learn and perceived the benefits of this integration displayed a heightened commitment to their work. This level of dedication indicates a need for increased staffing or a reorganised work system to provide adequate support.

However, in responding to the survey question on which systems are used frequently for notification, 61/64 participants reported using these interchangeably: Malaria Connect (36, 59%), NMC Notification Book (15, 25%) and NMC Mobile Phone Application (3, 5%). Of those who reported using more than one system, only one (2%) reported using all three, while five (8%) used Malaria Connect and NMC Notification Book only, and one (2%) used both Malaria Connect and NMC Phone App (Figure 3).

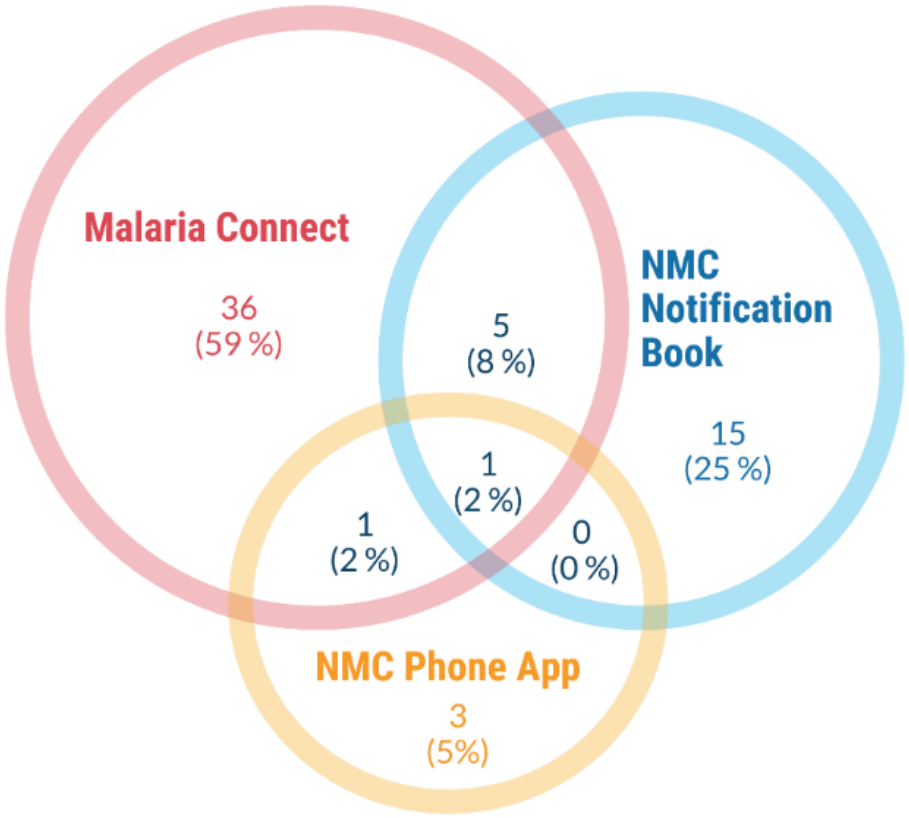


Figure 20: Malaria notification systems used by 61 staff working in 21/42 healthcare facilities in Nkomazi, Mpumalanga, South Africa.

NMC: Notifiable Medical Condition.

Participants reported that the parallel reporting systems introduced an additional burden to their daily work, particularly in the community health centres (CHC), operating 24-hours per day, 7 days a week, where a high number of patients present. For example, one of the nurses from a CHC in the FGDs said:

> '*But the volume you can look at the CHCs [Community Health Centres] and eight-hour [clinics], there are more people coming in because day and night we are working*.' ***[Nurse FGD03].***

4.4.3 Process II: Sample collection

The project introduced the collection of DBS filter papers, with related SOPs at all participating healthcare facilities. Participants expressed reluctance about collecting DBS and capturing patient information on mRDTs. This called for increased programmatic support and training, and with time, the study observed improvement of barcoded mRDT samples received at the NICD laboratory from 19% in the first quarter to 85% in the final quarter, reflecting gradual to acceptance and adaptability. [223]

'Basically, at first it was quite a daunting task because now [I] remember we had facilities that recently introduced the new NMC form and also Malaria Connect and all of that. So when we went to train the health facilities [about notification and DBS sample collection] they were resistant because they feel that it is extra work on their behalf...' ***[Environmental Health Officer, FGD01].***

Not all healthcare workers grasped the procedure for blood collection immediately. Due to difficulty in obtaining enough blood from the initial finger prick, they occasionally had to prick the patient's finger a second time. This caused some resistance among patients, according to the staff:

'Yes, there is not enough [blood from one finger prick]. There is a little, but it is not enough. So, most of the time they [nurses] have to prick maybe twice, or three times sometimes.' ***[Case investigator, FGD01]****.*

However, this also encouraged the healthcare workers to understand the rationale behind filter paper sample collection, to be able to explain this to the patients who were hesitant:

'These activities have helped us to know what we are doing and to help the patients; if they ask me now why we are taking another blood sample, it is for monitoring drug resistance and making sure you are cured. So, it's a very good thing' ***[Nurse, FGD04].***

After DBS sample collection, healthcare workers were asked to label and package samples, ready to be collected and transported each week by the NMP to the national laboratory. The intended output was to obtain accurately barcoded filter papers for molecular analysis and linkage to individual case notifications. However, this again meant an additional workload for the healthcare staff, which might have a negative impact on their other activities. In the first quarter, poor sample collection, packaging and recording of the required patient details, large numbers of samples / notification forms without barcodes and a low number of quality samples shipped to the central lab for molecular analysis were observed. This necessitated frequent refresher training, quality checks and supportive supervision. Despite the slow start, the overall outcomes of the number and quality of samples for molecular marking increased, for instance, the overall linkage of the MMR and case notification rose from 51% in the first quarter to the highest of 75.1% in the third quarter, with participants noting how the activities strengthened the routine antimalarial resistance surveillance [223].

'… remember we went with the supervisor to a couple of clinics when we identified that problem (positive mRDT sample with insufficient or missing DBS) and then we went back and told them that now we no longer just take the RDT. We also need to take the dry blood spots, so we need enough blood for the dry blood because sometimes we

*find that the dry blood is just a tiny bit of the blood and it really cannot be used. So we had to make sure that we go back to them and tell and train them that when you prick, make sure you prick enough just in case it is a positive you can also get more blood. They are currently a lot better' **[Nurse, FGD03].***

As shown in *Table 1*, among the 64 survey participants who performed these tasks, the majority reported that overall, it is easy for them to collect, label, package and ship DBS samples (55.1%). However, 29.5% found it difficult sometimes, and 9.4% found it always difficult.

	Not part of my work (%)	Easy for me (%)	Sometimes difficult (%)	Always difficult (%)
Labelling individual mRDTs	7.8	43.8	39.1	9.4
Collecting and labelling individual filter paper samples (blood spots)	4.7	51.6	34.4	9.4
Packaging positive and negative mRDTs for shipping	4.7	65.6	17.2	12.5
Packaging and shipping DBS samples?	6.3	60.9	26.6	6.3
Overall, how did you feel about the activities?	5.9	55.1	29.5	9.4

Table 3: Experience in collecting, labelling, packaging and shipping dried blood spots (DBS) as reported by 64 staff working in healthcare facilities in Nkomazi, Mpumalanga, South Africa.

4.4.4 Process III: Case investigation

Malaria case investigators aim to trace all confirmed malaria cases, obtain their residential GPS coordinates, identify malaria risk factors and actively screen nearby contacts for malaria (reactive case detection). The additional intended output for the integration of MMR into the routine malaria surveillance system was to enhance residential GPS coordinate data quality using *eTrex-10* GPS devices or Samsung tablets, with training and related SOPs.[230] Overall, 78.5% of malaria cases were successfully investigated (*Fig. 1)*, with the accuracy of GPS coordinates increasing from 48% in the first quarter to 89% in the last quarter [223]. The poor accuracy in the first two quarters was associated with device-related (non-uniform settings, device malfunctioning, lack of enough battery) and human-related (transcription errors, incomplete patient addresses) and system and resource-related factors (lack of enough battery or backup devices, increased workload, formatting during file transfers).

While discussing the issue of missing contact details, participants in FGDs mentioned that many of their patients are immigrants who lack phone numbers, travel far to seek healthcare or do not know how to clearly describe the locations of their recently acquired residences.

Additionally, due to their foreign and, at times, undocumented migrant status, many patients are often hesitant to share their contact details. These missing details lead to case investigation failure, resulting in more missing data.

The case investigators occasionally reach out to the notifying healthcare staff when faced with a case lacking contact information, aiming to gather additional details about the patient. This additional follow-up was received both positively and negatively. Positively, this would reduce the cost of case investigation since they travel substantial distances for case investigation, and a few more details can assist in finding the index case's location more directly.

> '*We drive almost 40 km or more to find a case, then the phone number is wrong and there is only one line for the address. Just looking for one case, you take the whole day while other notifications are waiting.' [**Case investigator, FGD01].***

However, the practice of case investigators contacting clinic staff on their mobile phones was met negatively by some clinic staff, who perceived it as an invasion to their privacy, potentially threatening their working relationship. During a FGD a nurse stated:

> *'Yes, they do so [call] because in the notification book we also write our phone numbers when we notify a patient; if there is something missing they can call. Even if we are not on duty, yah, threatening our working relationship.' **[Nurse, FGD03]***

On the other hand, some clinic staff and case investigators reported that calling each other is not a problem, even after-hours calls, mentioning that it's useful and gives them a sense of duty. They have developed trust, and without phone calls investigators would be travelling long distances to look for cases. One participant in an FGD with the nurses said:

> *'…it is fine because they want information, and they want to know if we have referred the patient [to hospital] or if the patient is going home.' **[Nurse, FGD03]***

Another challenge raised by the NMP staff was administrative inefficiencies resulting in multiple notification books at health facilities. These contribute to some tension. For instance, case investigators reported having collected the notification papers from the wrong notification book (outdated book / not currently in use) at some larger facilities, where several notification books may be used simultaneously. Others failed to access the notification book during the facility visit due to it being misplaced. This is usually resolved by removing the outdated books and leaving the facility with only one notification book and informing the supervisors.

To help address these gaps, the study identified the need for regular refresher training on malaria surveillance with guidance on malaria notification and capturing of case information at the facility level supported with new SOPs and facility guidebooks. The additional hours spent

on refresher training and learning the new SOPs were rewarded when the interviews revealed that these activities resulted not only in an increase in the accuracy of coordinates as stated above, but also in an overall improvement of the NMP's surveillance system functioning. An information officer in a FGD said:

> *'The ownership thing you know when you understand exactly what it is you are capturing and how important it is, then it makes all the difference in terms of making sure it is on file, because how these things usually get presented … it is always just another study that we do not know how it is going to end up, who it is going to benefit. So, I think also that approach of you [the study team] is excellent as we know what this is … and what this is for. This is how important it is, it is ours only. [**Information officer, FG02]***

4.4.5 Process IV: Data capture

Malaria-related data are captured at the sub-district data capture centre daily. Malaria case investigators submit case report forms from both the healthcare facilities and their case investigation visits. The additional data input for the integration of MMR into the routine malaria surveillance system was the capturing of the barcode number into the DHIS2 system. A barcode scanner was introduced to reduce workload and avoid transcription errors. Even after being trained, data clerks experienced challenges using the scanners, leading to inconsistencies in barcode capturing with both manual capturing and scanner use. The barcode scanning was also affected by internet connectivity, as any drop in WiFi signal disconnected the DHIS2 system. Overall, the process increased the workload and affected the perceived usefulness of the scanner, quoting a data clerk interviewed:

> *'Typing is easier than using a barcode scanner'. **[Data clerk, FGD01].***

Furthemore, delays in data capturing could last a few days to weeks. Delay were caused by interruption in network connectivity, increased caseloads and staff turnover:

> *'When it is the rainy days and the cases increase, sometimes it's only two of us capturing data. Therefore, we would be late to capture… Late for a few days to one or two weeks.' **[Data clerk, FGD01].***

In the first two quarters, there were significant missing details on the notification forms. For instance, not all forms had barcodes, coordinates or patients' addresses. Data clerks identified patterns of missing barcodes and help identify whether facilities were underperforming or ran out of barcodes. This was be communicated to the case investigation supervisors who provided supportive supervision to the healthcare workers notifying malaria. Supervisory staff reported that it remained common to find paper notification forms, mRDTs or DBS without

barcode stickers due to forgetfulness, increased workload or reluctance from the clinic staff. Unidentifiable sample forms without barcodes were discarded as no information was provided for identification or linkage. Quoting one of the participants in an FGD:

> *'Yes, that are missing, maybe the facility code or anything like that, then maybe three or four times we have experience that the RDT [referring to mRDT] came alone like just the RDT without the filter paper, without a barcode. So, most of the time we just discard because there is nothing we can do or go back and find this person and do the thing again' [**Surveillance supervisor, FGD02]***

This resulted in low linkability of samples because of inadequately barcoded samples, transcription errors and slow data-capturing processes. However, in the longer term, due to supportive supervision and re-training, cases linked at the household level increased with an increase in willingness and uptake of the programme [223].

4.4.6 Process V: Analysis and reporting

The NMP collates and analyses malaria data at the provincial level on a monthly basis and shares the reports with the healthcare facility, district, provincial and national teams. For the integration of MMR into the routine malaria surveillance system, the additional analyses included malaria case distribution and linkage of individual notifications to their MMR. The NMP information officers and molecular laboratory team downloaded data from the DHIS2 each month and shared it with the research team for further analysis and reporting 1) maps of the distribution of malaria cases; 2) linkage of residential locality and health facilities with MMR and 3) data quality. If any gaps were identified, activities were formulated for further enhancing resistance surveillance.

A total of 4,787 malaria cases were notified, and 78.5% (n = 3,758) of cases were investigated in the study period. However, data did not always match, and since these data came from separate files within the DHIS2 system, they needed to be merged outside the DHIS2 environment, leading to extra workload for information officers with a risk of non-matching, duplication, or deletions of some cases, variables or values. Commenting on the workload and how the DHIS2 framework could lead to non-matching of data and hence linkage failure, an NMP officer said:

> *'…I need to merge the NMC form together with the case investigation form and to take it outside the computer and merge; even if it is Excel, it is prone to changing formats and that could introduce issues that could lead to non-matching within the DHIS2 framework.' **[Information officer, KII03].***

Figures S18(a) and (b) (p 162) compare data from DHIS2 and the two lower levels (health facility and sub-district data capture centre); the notified case counts were only consistent in 40% (6/15) of the notifications and 20% (3/15) for cases investigated. Most of the matching evaluations occurred in the final two study quarters. Looking at the discrepancies, the two secondary levels (reports at the data collection centre vs data in the DHIS2) had less data variability compared to their primary source (health facility). Although the study's quantitative analysis identified substantial duplication, the malaria team did data cleaning before sharing the datasets, as explained by one of the interviewees:

> *'Once notifications were submitted and paper forms were captured in the DHIS2 system, ideally, all cases were merged and duplicates removed. In our analysis and feedback, some cases had duplicates that required further cleaning.'* ***[Information officer, KII03].***

Analysis and reporting activities were part of routine practice before the integration of MMR into the routine malaria surveillance system. However, due to the expansion of reported variables and the monthly downloads, merging and reporting required, this process became more complex and increased workload, especially in the first quarter. However, these extra activities were perceived as useful to the programme. The research team and the programme co-developed a training programme and data curation tools engaging all involved in identifying gaps and improvements that further simplified the data analysis and reporting and improved the overall notification system [230]. This improvement reflected beyond the study and supported improvement for the programme reporting, quoting an IDI participant:

> *'I think another thing that is good, our case investigation has also improved because I remember, when we started our baseline was 35% [for] 48 hours [time between case notification and investigation] and [for] 72 hours it was around 48%. So now on third quarter we reported above 65 (%) which was even above the target we set for this financial year. I think with them making the follow ups, having to go to the facilities to check on the stocks and everything has made them also be involved in going there and investigating the cases because the thing was XXXX and XXXX [SS4ME pilot programme staff names] would be analysing and supporting with feedback'* ***[Supervisory staff, KII02]***

4.5 Discussion

This study identified factors affecting data quality, linkage, and consistency for integrating antimalarial resistance monitoring into routine malaria notification in a pre-elimination malaria setting in sub-Saharan Africa. Healthcare facilities, district and provincial level healthcare staff

in Nkomazi sub-district, Mpumalanga, South Africa were key beneficiaries, and their perspectives spearheaded this integration. Applying a process-oriented logic model, an iterative process for comprehensively analysing various factors that either facilitate or hinder successful integration was established, with end-to-end data flow and interaction with various users, beneficiaries, the existing systems and newly adopted technology. The programme's overall performance was influenced by cross-cutting factors such as workload, training, perception, supervision, leadership and resources – acting as enablers or barriers based on their availability, adequacy and perception.

As reported previously [11], 45% of malaria cases could not be linked to their molecular profiles or localities. Many of these individuals were migrants who could not be followed up due to the absence of an accurate local addresses or their undocumented status, which limited the information they could provide (Figure S19 p 163). Additional factors contributing to this non-linkage included errors in capturing location data, whether due to human mistakes or technical issues with devices, further complicating the reporting process.

Increased workload was identified as a cross-cutting factor for all processes. Adequately trained staff who perceived the activity as useful maintained high-performance levels, delivering better-quality outcomes even during months with a high malaria caseload, reduced staff and multiple reporting systems. In contrast, if staff did not perceive the activity as useful, data quality remained low, irrespective of resources and training provided. Previous studies have shown that a negative perception affects overall performance as well as the adoption and implementation of new technologies [231, 232]. Low morale could lead to reluctance and the other activities being prioritised contributing to poor data quality.

The impact of devices (GPS devices, tablets, barcode scanners) introduced or updated for the integration of MMR into the routine malaria surveillance system depended on the healthcare workers' skills and perception of the devices' usefulness and ease of use. Optimal functionality of devices coupled with adequate training enhanced performance, leading to observable improvements in data quality, especially during and immediately post-training and supervision periods [223]. Conversely, malfunctions in devices, such as internet disruptions, barcode scanner failure to connect to the DHIS2, depleted GPS device batteries, or clinic-level shortages of barcode stickers, significantly impacted team performance, morale, and data quality. Other studies exploring technological adoption in the health sector highlight the crucial need to fully understand end-users' needs and potential useful functionalities to facilitate usage and enhance quality. These studies stress the importance of user-friendly functionalities and overall device performance [232, 233].

This study found sustained supportive supervision and quality assurance tools and resources improved performance. These include adequate financing, such as having long-term appointments without interruptions of contracts, a manageable workload and good collaboration among and between both government and non-governmental organisation staff. On the other hand, multiple parallel reporting systems, communication challenges, inadequate supervision, lack of quality control tools, unmanageable workload, and poor collaboration negatively impacted intervention implementation.

The study highlighted challenges at different operational levels, specifically in the malaria notification and case investigation processes. Although the NMC Notification Book served as the gold standard for reporting malaria cases, the survey results revealed parallel and occasionally interchangeable use of reporting systems. A 2019 review assessing the strengths and challenges of implementing DHIS2 across 11 Low- and Middle-Income Countries (LMICs) highlighted pervasive data quality issues, notably demonstrated in a Nigerian study where reported data in DHIS2 remained incomplete at least 40% of the time [234, 235]. Despite encountering such data quality challenges in this study, more than half of the cases could be linked to MMR data, with clear improvements in data quality observed over the study period [223].

Contrasting responses were observed regarding follow-up phone calls for additional information —some healthcare workers felt it assisted with case investigations while others felt it strained the working relationships, negatively impacting staff morale and performance. This challenge relied primarily on staff perceptions and relationships. However, effective leadership, including the establishment of conducive communication channels, procedural guides, and efficient documentation systems, has successfully addressed similar challenges in comparable contexts [233]. This demonstrated the pivotal role of leadership and management in optimising communication and operations. Previous studies in similar settings have shown similar factors impacting the general adoption and implementation of information systems in health care [231, 236–239]. A high level of perceived workload, data tools not being used as intended, and data quality issues were previously identified as challenges facing DHIS roll-out in Uganda and South Africa [240, 241].

Our study acknowledges several limitations in diagnostic methods, evaluation processes, participant selection, and the analytical approach used for combining qualitative and quantitative data. As this study was embedded in the routine malaria notification system, diagnostics relied on the *hrp2-based* mRDT, which is *P. falciparum* specific. This may have led to non-*P. falciparum* malaria being missed. However, regular sample testing and species detection studies have shown *P. falciparum* to dominate in this area, where other species are

rare [12]. Moreover, quality control measures, such as collecting 10% of negative mRDTs for further monitoring, including surveillance for hrp2/3 deletions, were implemented [12].

No single framework was comprehensive enough for the evaluation of the integration of MMR into a routine malaria notification system. Incorporating diverse technologies such as barcode stickers, filter papers, GPS protocols, and scanners introduced complexity to the evaluation process. The 2021 United Kingdom Medical Research Council framework for developing and evaluating complex interventions offers a comprehensive evaluation spanning a study's lifecycle, from conception to implementation. However, it lacks detailed guidance on assessing individual components independently and primarily serves as a guiding framework without providing specific granularity for evaluation [242]. For the integration of MMR into the routine malaria surveillance system, immediate end users are the healthcare staff and malaria case investigators. However, most determinant frameworks do not include end-users, and research has been scarce for improving the evaluation of how various end-users influence implementation effectiveness. Thus, our study employed an iterative process-oriented logic model.

Our results may be biased as only 42 staff members from the participating healthcare facilities consented to participate in the FGDs and IDIs, while 64 participated in the survey. The study results might not fully reflect the view of all staff in Nkomazi sub-district. However, the study team ensured diversity during participant recruitment to allow for different views and experiences. Most healthcare staff have multiple commitments, and their time is very limited. Hence, the generalisability of this study may be limited. Nonetheless, saturation was achieved with the diversity of perspectives, the depth of information provided, and the quality of dialogue. The latter, underpinned by a strong theoretical background, contributed to achieving high information power in the study. More studies might be needed to establish the extent to which the results from this setting may be applicable in other pre-elimination contexts.

.

4.6 Conclusion and Recommendation

Overall, the factors influencing the integration of MMR into the routine malaria surveillance system in this sub-Saharan pre-elimination setting were rooted in challenges inherent to the surveillance system itself, and were not specific to the integration of MMR. This study underscores the intricate interplay of factors influencing malaria notification data quality and the integration of drug resistance markers within the routine surveillance system. It emphasizes the pivotal role of aligned perceptions, adequate resources, and supportive supervision in bolstering data quality, while also revealing the vulnerabilities stemming from

device malfunctions, conflicting guidance, and disparate reporting systems. The evaluation frameworks used highlighted the need for more comprehensive models that consider the holistic healthcare environment, user perceptions, and nuanced interactions, particularly for complex interventions such as this. To enhance the implementation of such multifaceted interventions, future evaluations should include multiple countries to capture various healthcare settings, individual perceptions, and contextual nuances, enabling a more generalisable assessment and refinement of these interventions within routine healthcare systems.

Acknowledgements

We would like to acknowledge the Mpumalanga Provincial and Nkomazi Sub-district Malaria Control Programmes of the National Department of Health of South Africa, Laboratory for Antimalarial Resistance Monitoring and Malaria Operational Research, Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Disease, Clinton Health Access Initiative, South Africa, Humana People to People (HPP), South Africa, all NMP personnel and healthcare facility staff who collaborated in this study for all their support.

Funding

The Smart Surveillance for Malaria Elimination Pilot in Mpumalanga, South Africa, was co-funded by the South African MRC and the Worldwide Antimalarial Resistance Network (WWARN). WWARN is funded by the Bill and Melinda Gates Foundation and the ExxonMobil Foundation. This research was also, in part, funded by the Wellcome Trust [Grant Number 220211]. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission. Funders did not have any influence in the design of the study, data collection, analysis, interpretation of data and or writing the manuscript.

5 Chapter 5

Discussion

This thesis offers critical insights into the near real-time monitoring of MMR in two distinct settings: the Asian region advancing towards malaria elimination and a sub-Saharan African pre-elimination area (Nkomazi sub-district, Mpumalanga, South Africa). Utilising both

published and routine surveillance data, this research provides new evidence on the spatiotemporal patterns of MMR, providing actionable insights for regional, national and local malaria elimination programmes.

Despite the introduction of two effective malaria vaccines by the WHO Malaria Programme by October 2023 [2], there is still a gap on interventions and tools to accelerate malaria elimination effectively. The stagnation and decline in funding for malaria control and elimination and its research, also pose a significant risk, particularly to less-resourced programmes in sub-Saharan Africa. Coupled with the emergence and spread of antimalarial resistance, these undermine global efforts to reduce the burden and eventually eliminate malaria. Our findings respond to the WHO Global Technical Strategy for Malaria 2016 – 2030[8], which underscores the urgency of integrating advanced tools, such as an early warning system, into routine malaria surveillance to combat drug resistance.

Our comprehensive analysis of 16,613 samples across 18 Asian countries from 2002 to 2018 indicates a persistent rise in artemisinin resistance markers, initially identified in 2002 in the Greater Mekong Subregion. By 2018, these markers had spread to several other countries, highlighting the critical need for enhanced surveillance and international cooperation with prompt evidence-sharing to track MMR and mitigate its impact on antimalarial efficacy. The median delay of 3.6 years from sample collection to publication reinforces the necessity of real-time surveillance methods to provide timely support for local, national and regional response initiatives.

In Africa, where malaria exerts the heaviest toll with over 95% of cases and deaths, our study accentuates the strategic importance of MMR surveillance in supporting NMPs, especially those in pre-elimination and elimination areas. The situation in South Africa, which has made significant strides in transitioning from control to elimination since 2012, exemplifies the need for such ongoing surveillance to mitigate the sporadic surges in malaria cases and assess the efficacy of current antimalarial treatments, the focus of this thesis, as well as insecticides and diagnostics. The three endemic provinces of South Africa share borders with five countries, of which Mozambique experiences the fourth highest burden in Africa [2], putting neighbouring countries at a substantial risk of cross-border transmission. South Africa has recognised the need to determine the prevalence and temporal changes of molecular markers associated with the resistance, towards identifying target areas where TES studies and resistance containment interventions are most needed. Furthermore, this would help inform the new South African National Strategic Plan (2024 – 2028) being drafted to achieve zero malaria transmission [46].

Notably, in Mpumalanga, we observed an 18-year trend of a steady increase in parasites carrying *mdr86ASN* and *crt76LYS* wildtype alleles that are modestly associated with lumefantrine tolerance or reduced susceptibility or reduced susceptibility [45, 46]. Parasites carrying these markers became the dominant parasites by 2018 and remained so from March 2018 to February 2020. Studies have shown variable results regarding the extent to which *crt*76 and *mdr86ASN* are associated with tolerance to AL. A review published in 2017 reported that *mdr86ASN* is associated with 4 to 5-fold increased risk of recrudescence [243]. A study in Uganda also found a substantial PCR-adjusted increase in relative risk of treatment failure [244]. However, an individual patient data meta-analysis of 31 studies from Africa and Asia only found a slight increase in recrudescence and reinfection for parasites carrying *crt*76 and *mdr*86ASN alleles following AL treatment [114]. In northern Uganda, *ex vivo* growth inhibition assays show decreased susceptibility to lumefantrine, and artemether-lumefantrine efficacy fell below the WHO threshold of 90% in two of three study sites [245]. The increase in *mdr1* copy numbers has been more closely linked to increased failure rates than *mdr86ASN* in Asia [114]. Our study did not observe any such increase, and overall, an increase in *mdr1* copy numbers is rarely reported in Africa. Nevertheless, this growing lumefantrine tolerance is expected to exert greater drug pressure on the artemisinin component in ACTs, calls for the diversification of the ACTs used in the region and highlights the necessity for continued vigilance and molecular surveillance for antimalarial resistance.

While our study, fortunately, did not detect K13 artemisinin resistance markers in South Africa, the identification of wild-type alleles linked to lumefantrine tolerance aligns with the broader trends of antimalarial drug resistance observed in Africa, where artemether-lumefantrine PCR corrected efficacy at day 28 has dropped below the WHO's 90% threshold at study sites in at least five countries (Angola, Burkina Faso, Democratic Republic of Congo, Kenya and Uganda [2]. While the recent PCR-corrected efficacy rates of ACTs in Africa have generally been over 90% [246], an increasing frequency of K13 markers associated with artemisinin resistance is of concern, especially in Central Africa (Democratic Republic of Congo) [247], East Africa (Rwanda, South Sudan, Tanzania, and Uganda) and the Horn of Africa (Eritrea, Ethiopia) [246–251]. This underscores the need for African nations to enhance their surveillance capabilities to identify and respond promptly and effectively to emerging antimalarial drug resistance.

More studies in recent years have shown the independent emergence and spread of K13-validated mutations in the East and the Horn of Africa. For example, R561H has increased from 1% in 2014 – 2015 samples in Rwanda to a prevalence of at least 20% in Rwanda and bordering regions of Tanzania and Uganda [250]. The hypothesis surrounding the independent emergence of these markers in Africa points to a variety of transmission dynamics and

treatment practices as key drivers. In areas with low malaria transmission, such as the Horn of Africa, Rwanda, and southern Uganda, the emergence points to low immunity of the population due to low malaria transmission. Conversely, in regions with high malaria transmission, the misuse of artemisinin monotherapies, are hypothesised to complicate the situation further. Practices such as using injectable or rectal artesunate without the necessary addition of ACTs or employing injectable artesunate for uncomplicated malaria are notable examples [37, 252]. Additionally, the resurgence of malaria in populations with lowered immunity—following the cessation of comprehensive indoor residual spraying programmes — may also contribute to the development and spread of drug-resistant malaria strains [37]. These factors combined suggest a complex interplay of biological, environmental, and human behaviour factors influencing the evolution of drug resistance in malaria across different parts of Africa. This independent emergence and the speed of spread of the resistant strains (e.g., R561H mutations in East Africa) bear a close similarity to the sweeping spread of C580Y and its dominance in the GMS [55], hence a dire need of enhanced surveillance.

Moreover, non-K13 MMR have been detected in Africa, with *in-vitro* evidence of conferring resistance to artemisinin. For example, a 2018 *in vitro* study utilising samples from Senegal identified *Pfcoronin* mutations in parasites exhibiting prolonged survival to dihydroartemisinin during the ring-stage survival assay [253]. Furthermore, a study conducted in Israel on returning travellers from Africa detected *Pfcoronin* mutations in patients who received AL treatment in the absence of K13 mutations associated with artemisinin resistance [254]. In an analysis of 336 samples collected from patients returning to Israel between 2009 and 2020, 15 exhibited treatment failure with AL therapy, and 28.6% of those samples harboured *Pfcoronin* without K13 mutations. Moreover, these treatment failure rates had been increasing compared to previous years, suggesting the potential for parasite evolution (0% in 2009-2012, 9.1% in 2013-2016, and 17.4% in 2017-2020). While this Israeli study remains as the major *in vivo* study proposing *Pfcoronin* as an artemisinin MMR, subsequent studies from Senegal did not find *Pfcoronin* mutants[57,58]. Therefore, it remains imperative to strengthen and expand surveillance for molecular markers to identify the spread of K13 markers and identify and confirm the presence of other non-K13 antimalarial resistance markers, integrating them within the early warning system. Although our study exclusively focused on K13 markers, future studies could consider incorporating other potential markers of artemisinin resistance, such as *Pfcoronin*, for a more comprehensive assessment.

From March 2018 to February 2020, this thesis demonstrated the feasibility of integrating real-time surveillance for antimalarial resistance markers with routine malaria notifications in Mpumalanga's Nkomazi sub-district in South Africa. This approach, successfully linking over half of reported malaria cases to their individual MMR and specific health facilities and

locations, proves particularly valuable in areas where implementing traditional therapeutic efficacy studies are challenging. The WHO recommends Integrated Drug Efficacy Surveillance (iDES) in pre-elimination settings, which involves following up on all malaria cases. The latter can be challenging where mobile and migrant populations carry most malaria and require significant resources, so it is only feasible when malaria cases are few. This thesis' findings could prove valuable for enhancing surveillance by incorporating molecular surveillance within routine case notification and investigation, and help identify locations that need further investigation or interventions to limit the impact of antimalarial resistance. Given the increasing reports of antimalarial drug resistance in several African countries [246, 250, 256], it is essential for South Africa to sustain MMR surveillance to promptly detect and respond to antimalarial resistance.

To enhance the fight against malaria, novel antimalarials and approaches are currently in development. However, the earliest any of these will become available would be in 2027 / 2028 and those both rely on sustained efficacy of lumefantrine [257, 258]. There remains insufficient evidence regarding the effectiveness of interventions such as multiple first line treatments other than in mathematical models [259] and single low dose primaquine in mitigating the spread of antimalarial drug resistance in moderate-to-high transmission areas Africa [221]. In 2019, WHO included artesunate-pyronaridine in its recommended ACTs [260–262], with Cameroon, the Democratic Republic of Congo, and Nigeria incorporating it into their national malaria treatment guidelines [2]. Additionally, two vaccines have been recently recommended: the RTS,S vaccine in 2021 and the R21/Matrix-M vaccine in 2023, aimed at preventing malaria morbidity in areas with moderate-to-high transmission rates [263].

These interventions offer hope in the fight against malaria, yet their full potential in real-world settings remains unrealised as they have only recently started to be introduced [264]. Furthermore, neither the vaccines nor the new antimalarial drugs have been piloted in regions with low transmission rates. Despite the recommendation of RTS,S in 2021 and the conclusion of pilot implementations in 2023 (limited to Ghana, Kenya, and Malawi), demand exceeds supply [263] . Implementing these interventions may be delayed despite WHO and GAVI, the Vaccine Alliance, aiming to roll out the R21/Matrix-M vaccine by mid-2024. Questions regarding the slow rollout, lack of evidence in moderate transmission settings at large scale of sub-Saharan Africa, and feasibility analyses are crucial in understanding the real-world impact of these interventions. Consequently, while these practical innovations hold promise in the fight against malaria, and eventually decrease demain for artemisinin-based treatment, they may struggle to keep pace with the spread of artemisinin resistance. Therefore, maintaining vigilance and expanding MMR surveillance is vital for informing and enhancing these programmes.

This thesis emphasises the critical role of robust programmatic support and collaboration between researchers and the malaria programmes. Valuable tools and methodologies were co-designed that have proven beneficial for local malaria programme efforts and have impact not just on antimalarial drug resistance surveillance. This is exemplified by the on-site staff training and supervision for more effective use of GPS devices for spatiotemporal maps essential for targeting all malaria interventions appropriately. Acknowledging user needs and contexts is crucial for selecting suitable visualisations to aid analytics and inform public health decision-making [231, 265]. In Nkomazi, the preference for density maps over thematic maps emphasises the importance of user-driven visualisations in enhancing the effectiveness of antimalarial drug resistance monitoring. For instance, the addition of health facilities to the *P. falciparum* density map in Nkomazi (Figure S 16 p 137), along with the inclusion of K13 artemisinin resistance and *mdr186ASN / crt76LYS* molecular markers of lumefantrine tolerance (Figure 17), enabled the study team to identify areas where samples and/or results were missing, or performance was below expectations. This information assisted the malaria programme to identify areas requiring additional interventions, e.g. malaria transmission hot spots, and health facilities with under-reporting or missing results despite having a relatively higher malaria caseloads.

The use of density maps in this area brought significant benefits, one of which was the ability to bypass extensive regions used for plantations and national game reserves. These regions were devoid of human settlements, making them inaccurate for reflecting malaria incidence, as had been done previously. Density maps also offer a visualisation of malaria cases across a continuous landscape, necessitating the incorporation of models that account for incidence rates and other crucial variables influencing case distribution. To add more local relevance, our research utilised the most up-to-date data on human settlements and incorporated insights from 24 local malaria case investigators. These investigators provided valuable feedback, ensuring the accurate representation of malaria case distribution in their respective areas. Given the focus and time limitations, this study did not incorporate other factors such as climate, altitude, vectors, or the existing malaria interventions that have been shown to affect the distribution of malaria cases in previous studies to further explain the malaria burden in this area[266, 267]. Future studies could explore how such additional data can improve these maps and their understandability to the malaria programmes.

This approach illuminates not only the adoption of molecular resistance surveillance as part of an early warning system and the nurturing of programmatic support and collaboration, but also the employment of user-centric visualisations to identify and respond to antimalarial drug resistance. It highlights the significance of workload, training, supervision, leadership, and resources in integrating MMR surveillance into routine malaria surveillance. This evidence

bears relevance for African countries nearing malaria elimination and / or contending with antimalarial drug resistance, advocating for using molecular resistance surveillance as an early warning method to identify potential areas for further TES investigation and to intensify efforts to reduce transmission. As noted by Assefa et al. 2024, in response to the increasing artemisinin resistance in East Africa and the Horn of Africa, *"there is no need to panic, but it is vital to remain vigilant"*; thus, these countries must adopt an early warning system such as the use of molecular surveillance for antimalarial resistance [268].

In this study, GPS devices / tablets, barcode scanners and procedures like DBS samples on filter papers were introduced to enhance MMR surveillance. Their effectiveness depended on a number of factors. Firstly, the effectiveness of these devices and procedures relied on the skills and perceptions of the malaria staff regarding their usefulness and ease of use. Secondly, optimal device and system functionality significantly enhanced data quality and improved overall system performance. This also included availability of the devices at all times of work, availability of all the applications (e.g. DHIS2 platform which is internet-dependent) or availability of supplies such as filter paper and mRDT reagents whenever needed. Interruptions in electricity or internet connectivity hinder performance of tasks and impact staff performance and the overall perceptions of the usability of the device. Lastly, ensuring the provision of adequate training and supportive supervision was crucial for achieving optimal performance. By evaluating and addressing training needs iteratively, we enhanced staff confidence in using the new devices and procedures. This, in turn, fostered a positive perception of their overall effectiveness in performing their tasks. Our findings align with previous studies on technology adoption in the health sector, which emphasise the significance of meeting the needs of end-users and recognising the value of functionalities to improve overall performance [232, 233].

This thesis presents new evidence and best practices in the integration of molecular surveillance for markers for antimalarial resistance into pre-elimination and elimination settings, employing an iterative framework focusing on high-quality evidence, target audiences, co-design methodologies, and iterative evaluation to significantly impact malaria programmes (Tool S4 p 164). This methodological synergy, increasingly recognised in healthcare technology adoption, fosters user-centred designs and ensures the inclusion of nuances potentially overlooked without audience involvement in project design and implementation phases [269, 270]. As maps have become common-place tools for the visualisation of trends of diseases and intervention coverage, the significant challenge remaining has been ensuring that they remain up-to-date and readily understandable. Many such maps contain technical information that is too detailed for policymakers to understand and use in malaria programmes [23]. The framework generated from this study can guide the

evaluation of user-specific features included in the mapping process, as well as their final products. This is important to guide future research and implementation projects, facilitating the integration of early warning systems into routine malaria surveillance systems.

Additionally, the study introduces tools to bridge identified gaps in current literature and practices, including a mechanism for the standardised reporting of MMR to facilitate data harmonisation and trend analysis (Tool S2 p 138). Moreover, with the implementation of FAIR (Findable, Accessible, Interoperable, and Reusable) data standards, authors have become more willing to share their pre-published data with regional and global repositories such as the WWARN Molecular Surveyors, which call for optimised data reporting tools to avoid inclusion of incomplete data [59]. This study proposed a tool for assessing the minimum essential information required to report MMR for current and future studies (Tool S1 p 109). Through the proposed naming of the geographical administrative units, this tool can help with the proper documentation of study sites and sample sources, thus avoiding unstructured genomic and spatial data. Thus, this tool aims to ensure data harmonisation to facilitate its secondary use and enable the evaluation of spatial and temporal trends. Recently, various efforts have been made to develop genomic surveillance networks to support quick sharing and harmonisation of genomic data, tools, and processes at global and regional scales [271, 272]. However, these initiatives still face significant challenges in building sustainable networks, infrastructure, and workforce, especially in Africa [273, 274]. These molecular data are seldom linked with outcomes of more direct relevance to policymakers, such as increased risk of treatment failures and malaria transmission [274, 275]. Thus, this study encourages further research to explore how outputs and tools from this research can strengthen the utility of genomics in resistance surveillance in Africa.

In addition, specialised tools and guidelines were developed to improve location data accuracy in malaria investigations, utilising both eTrex 10 GPS devices and Android devices. This suite of tools included a GPS training toolkit for malaria case investigators (Tool S2 p 138), a comprehensive training manual for trainers (Tool S2 a p 138) and a detailed standard operating procedure for eTrex 10 devices (Tool S2 b 138), informed by the Health Geolab Collaborative 2018 standard (Tool S2 c p 138) [230]. These guidelines and training materials were shown to ensure the precise collection of GPS coordinates, thereby enhancing the efficiency and accuracy of malaria case investigation and reporting.

This research has developed a comprehensive, process-oriented logic model to effectively oversee the integration of early warning systems into existing surveillance frameworks (Tool S4). This model, informed by an exhaustive literature review [242, 276, 277], serves as a blueprint for monitoring and evaluating new surveillance components, bridging the gap left by

the absence of formal guidelines. It details the mapping of stakeholders, the delineation of inputs, outputs, and outcomes, and identifies potential barriers and facilitators to successful implementation. This provides a robust foundation for the successful integration and optimisation of early warning systems to detect and respond to antimalarial resistance within routine surveillance operations.

Additionally, the framework helps identify and address barriers and enhancers that may affect the integration process. It considers potential obstacles and challenges that may hinder successful implementation, as well as factors that can enhance the effectiveness and efficiency of integrating early warning interventions into existing surveillance systems. Overall, this process-oriented logic model serves as a comprehensive and practical approach to monitor and evaluate the integration of new components within routine surveillance systems. Its iterative nature and evidence-based foundation make it a valuable resource for ensuring successful integration and enhancing the overall effectiveness of both early warning interventions and routine surveillance.

The study on artemisinin resistance markers in Asia encountered data, methodological and logistical challenges, and interrupted timelines, including due to the COVID-19 pandemic. In Asia, the lack of consistent and extensive data collection across similar areas over an extended period restricted my spatial and temporal analysis of antimalarial drug resistance. About 60% of the GMS administrative units had samples from only one timepoint between 2000 and 2018. Thus, the representativeness of the distribution of MMR results observed in Asia may have been limited by the type of data used, as sites likely to detect MMR may have been more likely to be conducting MMR studies, while the absence of MMR of significance may have also discouraged longitudinal studies. Additionally, the heterogeneity and patchy data reporting on artemisinin resistance markers made it difficult to aggregate and assess trends effectively. The long delay between completing sample collection and publishing results, with a median of 3.6 years, made it impossible for malaria programmes to respond timeously. The study was, however, comprehensive in that it included all the available data and contacted study investigators for further data contribution to address information gaps. Moreover, the study identified minimal essential information for reporting MMR to support future research [278].

Similarly, in South Africa, multiple malaria notification and reporting systems imposed burdens on healthcare workers, resulting in fatigue, increased duplication, and missing data, which ultimately compromised the overall quality of data collected. Slow data-capturing processes and data-capturing errors resulted in being unable to link 45% of individual malaria cases to their molecular data. The study addressed these challenges by supportive supervision,

training, and development of tools to support data collection and avoid field and data-capturing errors. Thus, this study highlights the critical importance of investing in ongoing supportive supervision and data curation efforts when piloting early warning systems at regional and local levels. Simultaneously, we emphasise the importance of implementing health information systems optimally to avoid significant disruptions to the performance of surveillance programmes.

This study navigates the complexities of integrating qualitative and quantitative data within the evaluation framework for the surveillance of MMR, acknowledging the need for future research to explore alternative models and theories that can accommodate the multifaceted nature of program implementation, such as the incorporation of diverse technologies – for example, barcode stickers, filter papers, GPS protocols, and scanners – and assess their impact. This exploration is crucial for deepening our understanding of the dynamics at play in integrating MMR surveillance into routine malaria notification systems.

In conclusion, my thesis affirms the critical need to strengthen antimalarial resistance surveillance by confronting data-related challenges, championing robust data management practices, piloting innovative early warning systems, sustaining regular supportive supervision and conducting thorough evaluations to inform policy and practice. Further research can prioritise comprehensive and consistent data collection across similar regions over an extended duration, incorporating additional factors affecting malaria transmission, such as mosquito vector populations, malaria control / elimination interventions, and population movement. This approach will enable a more thorough examination of the spatial and temporal trends in antimalarial drug resistance and provide policymakers with a clearer understanding of these trends and their implications. By spotlighting the necessity of such systems for monitoring artemisinin and partner drug resistance markers, and acknowledging the current methodological limitations, my thesis calls for further research to develop more inclusive and comprehensive frameworks for evaluating complex health program implementations, thereby contributing to the global effort to combat antimalarial drug resistance with evidence-based strategies. Furthermore, my study calls for more caution and further evidence on optimal methods to roll out new health information systems on top of the existing ones to avoid disrupting the current activities.

6 Supplementary Documents

Chapter 1

Table S 1: Search strategy

#1	artemisinin OR kelch OR kelch13 OR k13
#2	Asia OR Greater Mekong OR Bangladesh OR Cambodia OR China OR Indonesia OR Malaysia OR Myanmar OR Lao OR Afghanistan OR Viet Nam OR India OR Iran OR Nepal OR Philippines OR Thailand OR Bhutan OR Oman OR Lebanon OR Jordan OR United Arab Emirates OR Yemen OR Syria OR Kyrgyzstan OR Mongolia OR Iraq OR Saudi Arabia OR Taiwan OR Uzbekistan OR Turkmenistan OR Tajikistan OR Kazakhstan OR Azerbaijan OR Russia OR Armenia
#3	#1 AND #2

Table S 2: Classification of K13 mutations

Categories	SNPs	Meaning
WHO validated/ confirmed	C580Y, F446I, I543T, M476I, N458Y, P553L, P574L, R539T, R561H, Y493H	A SNP with evidence of correlation with artemisinin efficacy decline in clinical studies and in vitro studies and included in the list of WHO validated mutations.
WHO associated	A481V, A675V, C469F, C469Y/F, E252Q, F673I, G449A, G449A/D, G538V, N537I, P441L, P527H, P574L, R515K, V568G	A SNP with evidence of correlation with a therapeutic decline in clinical studies but without evidence of being tested in in vitro studies and included in the WHO candidate/associated mutations.[279]
WWARN associated	*__P553L__, +__P441L__, *__F446I__, +__N537I__, *__P574L__, *__R561H__, *__C580Y__, *__Y493H__, __V568G__, *__I543T__, *__R539T__, __R539R/T__, C580C/Y, +__N525D__, *__N458Y__, *__M476I__, +__G538V__, F614L, E252Q, +__A675V__, +__A481V__, H719N, +__G449A/D__, R561H/C, D584V	A SNP with evidence of correlation with slow parasite clearance using the WWARN in vivo parasite clearance half-life estimator.[177]
Unevaluated	Any reported K13 SNPs to be determined in the review	A reported K13 SNP but not yet included as a validated/associated mutation by WHO or WWARN.
Not associated	A578S	A SNP not associated with a therapeutic decline in either clinical or in vitro studies.
Wild type	Wild type SNPs	A non-mutated allele that has full sensitivity to artemisinins.

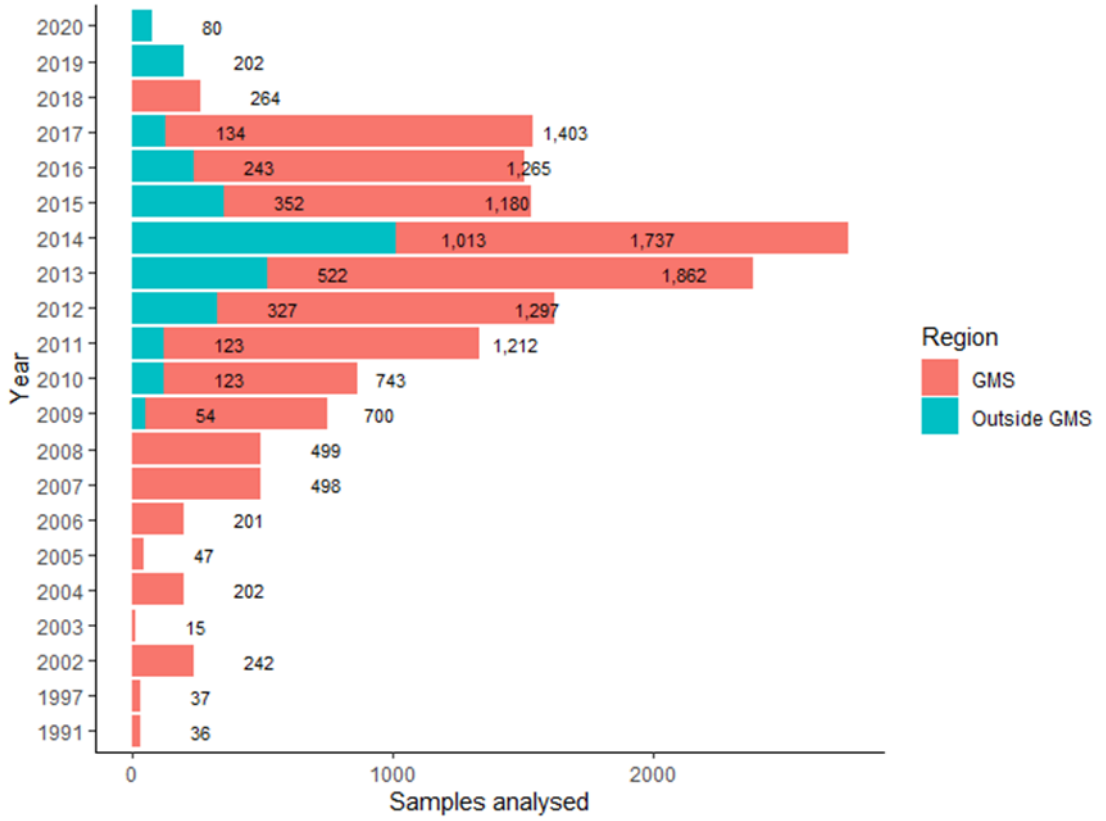
*Grouped Single Nucleotide Polymorphisms (SNPs) based on their association with P. falciparum clearance rate. The grouping is as per the WHO classification and the published WWARN Individual Patient Data (IPD) meta-analysis (Bolded K13 SNPs show where they mirror the *WHO validated and +WHO associated groups). Unevaluated markers will include all new and old SNPs that have not yet been evaluated to be included in the WHO or WWARN lists.*

Table S 3: Included K13 studies by publication year

Year	Total	Cum. Total
2020	8	72
2019	13	64
2018	5	51
2017	14	46
2016	14	32
2015	12	18
2014	5	6
2010	1	1

Table displaying the number of manuscripts included in the review by year of publication. The majority of these studies were published between 2015 and 2019, with an average of 6·2 publications per year overall.

Figure S 1: Distribution of samples by year



All samples (n=16,613) were categorised by year of sample collection and indicating those from the Greater Mekong Subregion (GMS) (n=13,440) and outside the GMS (n=31,73). Of all years, 2014 had the highest number of samples.

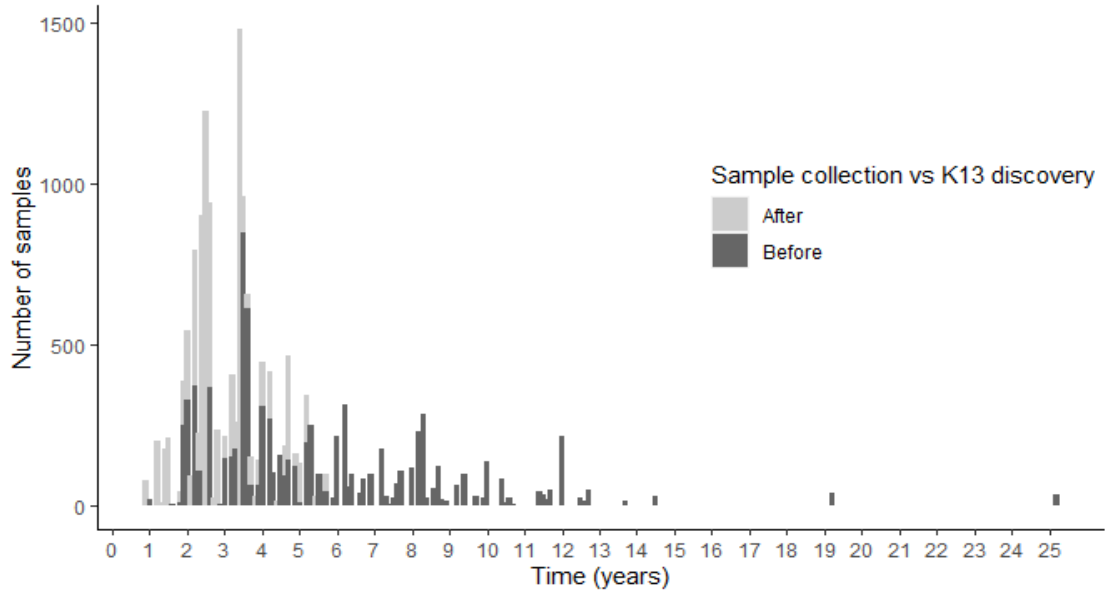
Table S 4: Years of sample collection by administrative units

	All			GMS			Outside GMS		
Number of years	Admin units	%	Cum. %	Admin units	%	Cum. %	Admin units	%	Cum. %
1	73	58·4	58·4	19	31·7	31·7	54	83·1	83·1
2	21	16·8	75·2	15	25	56·7	6	9·2	92·3
3	9	7·2	82·4	5	8·3	65	4	6·2	98·5
4	5	4	86·4	5	8·3	73·3	0	0	0
5	8	6·4	92·8	7	11·7	85	1	1·5	100
6	2	1·6	94·4	2	3·3	88·3	0	0	0
8	1	0·8	95·2	1	1·7	90	0	0	0
9	3	2·4	97·6	3	5	95	0	0	0
10	1	0·8	98·4	1	1·7	96·7	0	0	0
11	1	0·8	99·2	1	1·7	98·4	0	0	0
14	1	0·8	100	1	1·7	100	0	0	0

Number of years with samples by administrative unit overall, in the GMS and outside the GMS (Admin=administrative, Cum=cumulative). Around 60% of the administrative units had data only for one year. Over 68·3% (n=44) of administrative units had samples from more than two years coming from GMS.

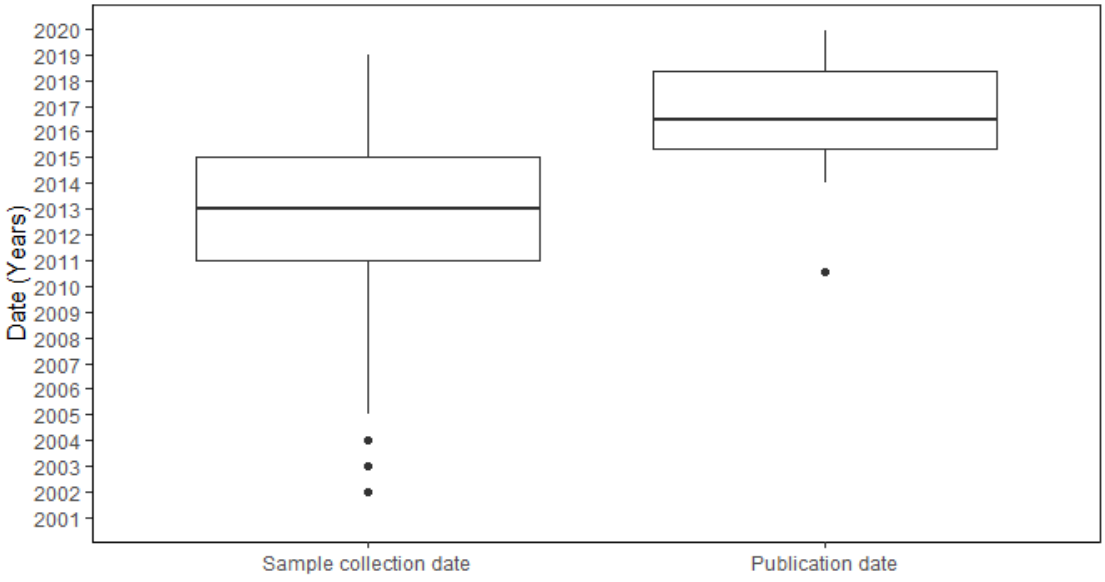
K13 markers publication lag

Figure S 2: Interval between sample collection and publication



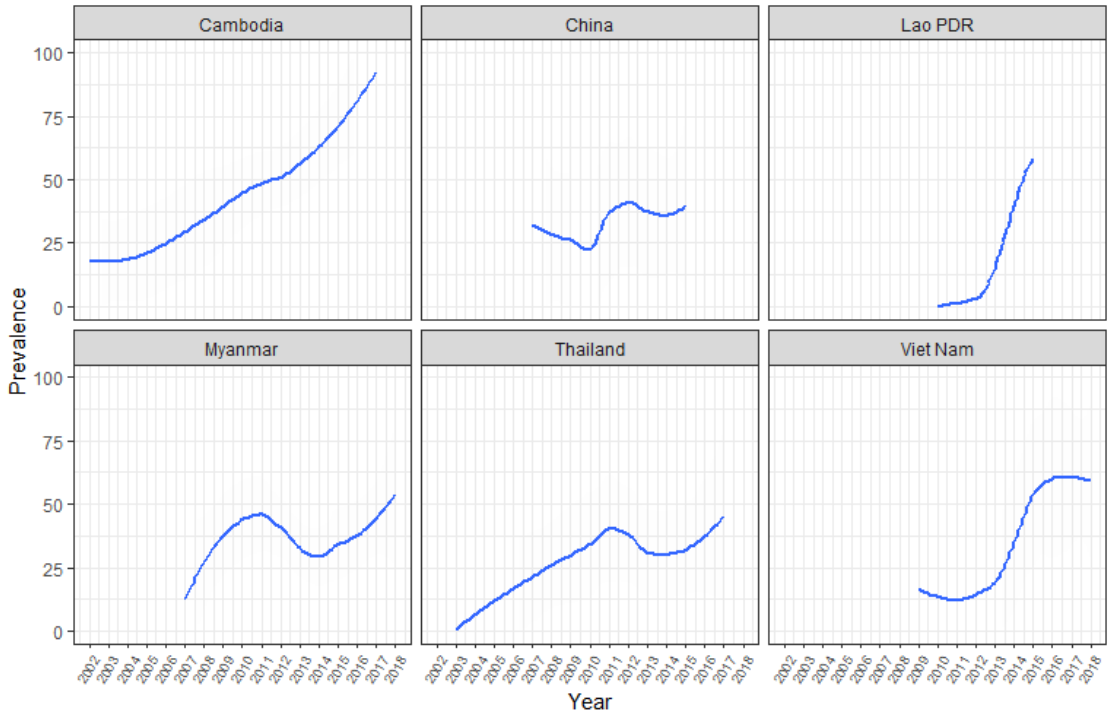
The duration between sample collection and publication calculated as the difference between the two respective dates for each sample. The median (range) time between sample collection and publication was 3·6 (range of 1 to 25 and IQR of 2·7 with the 25th and 75th percentiles of 2·7 and 5·2 respectively) years. Samples collected after the discovery of K13 *had shorter interval compared to the rest of the samples.*

Figure S 3: Difference between sample collection and publication time



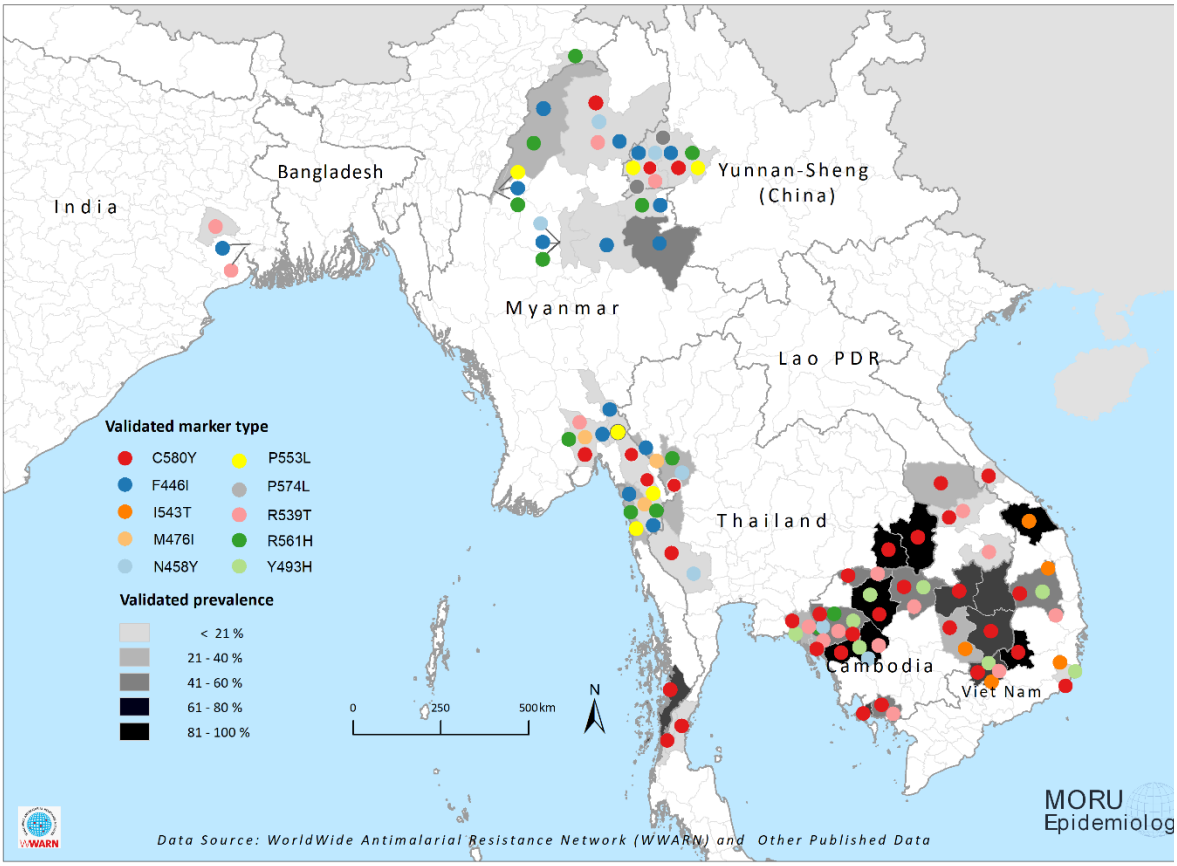
Collection and publication dates, median and interquartile ranges for pooled K13 *sample collection and publication dates. The median time between sample collection and publication was 3·6 (range of 1 to 25 and QR of 2·7 with the 25th and 75th percentiles of 2·7 and 5·2 respectively) years.*

Figure S 4: Temporal trends of K13 markers per country



The overall prevalence of validated molecular markers by administrative unit per country over time from published K13 *studies in the GMS. All countries show an increase in the 'validated'* K13 *markers.*

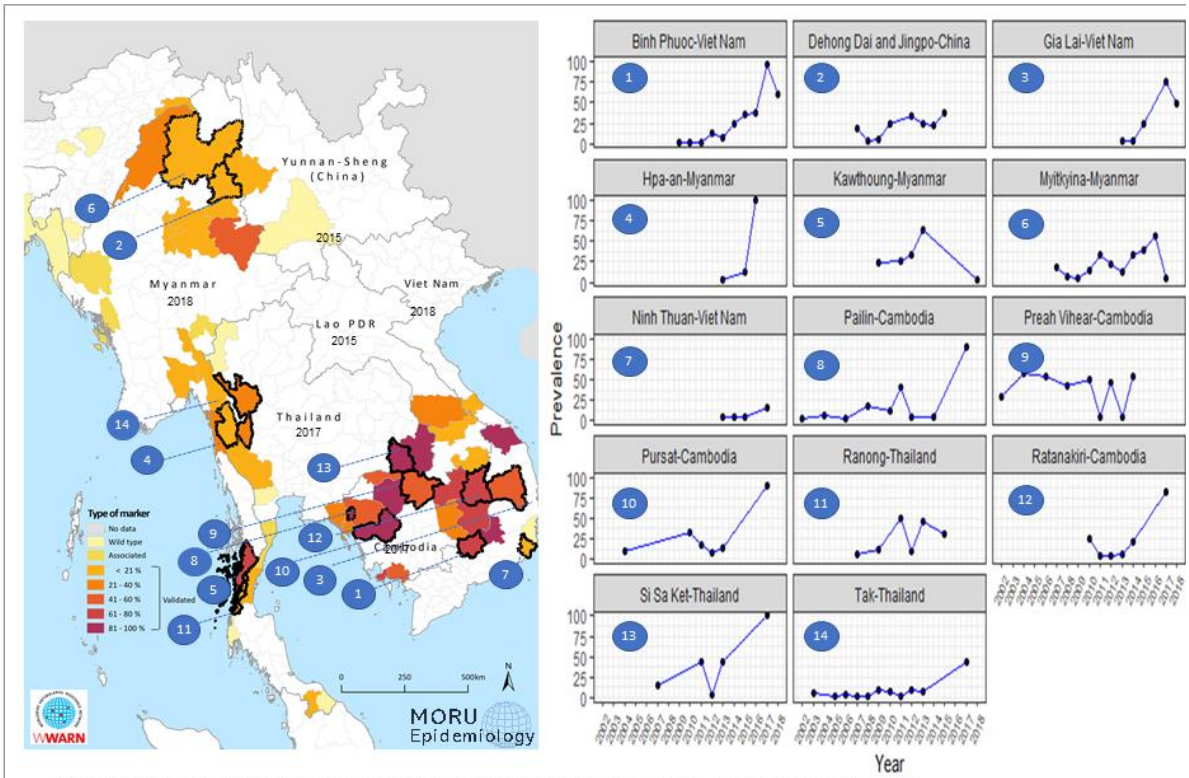
Figure S 5: WHO-validated markers in the GMS



Distribution of the eight different validated SNPs and their prevalence. C580Y had wider distribution throughout the GMS particularly in the east. (Data was aggregated by administrative unit level one (Cambodia, Lao PDR, Thailand and Viet Nam and administrative level two for China, India and Myanmar)

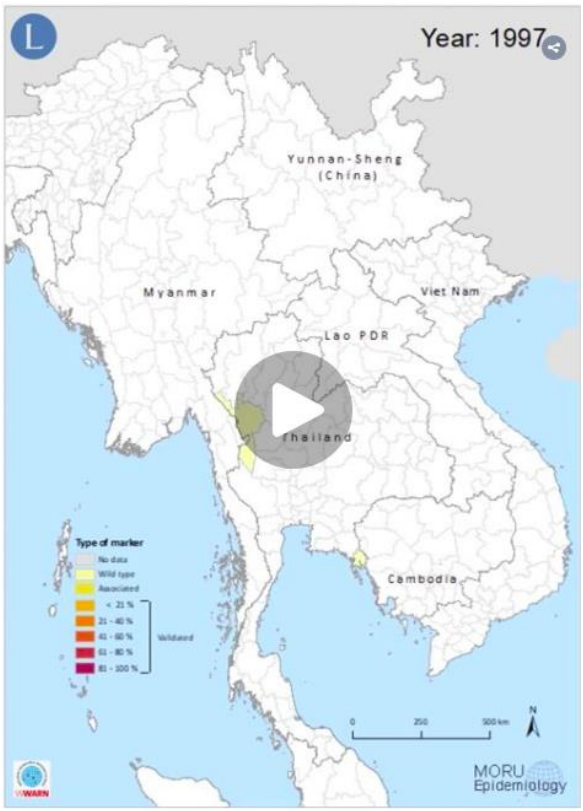
Distribution of K13 markers in the GMS

Figure S 6: Trend of WHO-Validated markers in selected locations in the GMS



Prevalence of WHO-Validated markers over time for selected administrative subunits in the GMS with a minimum of 3 years of data (highlighted by black borders on the map and numbered in blue circles on the plots)

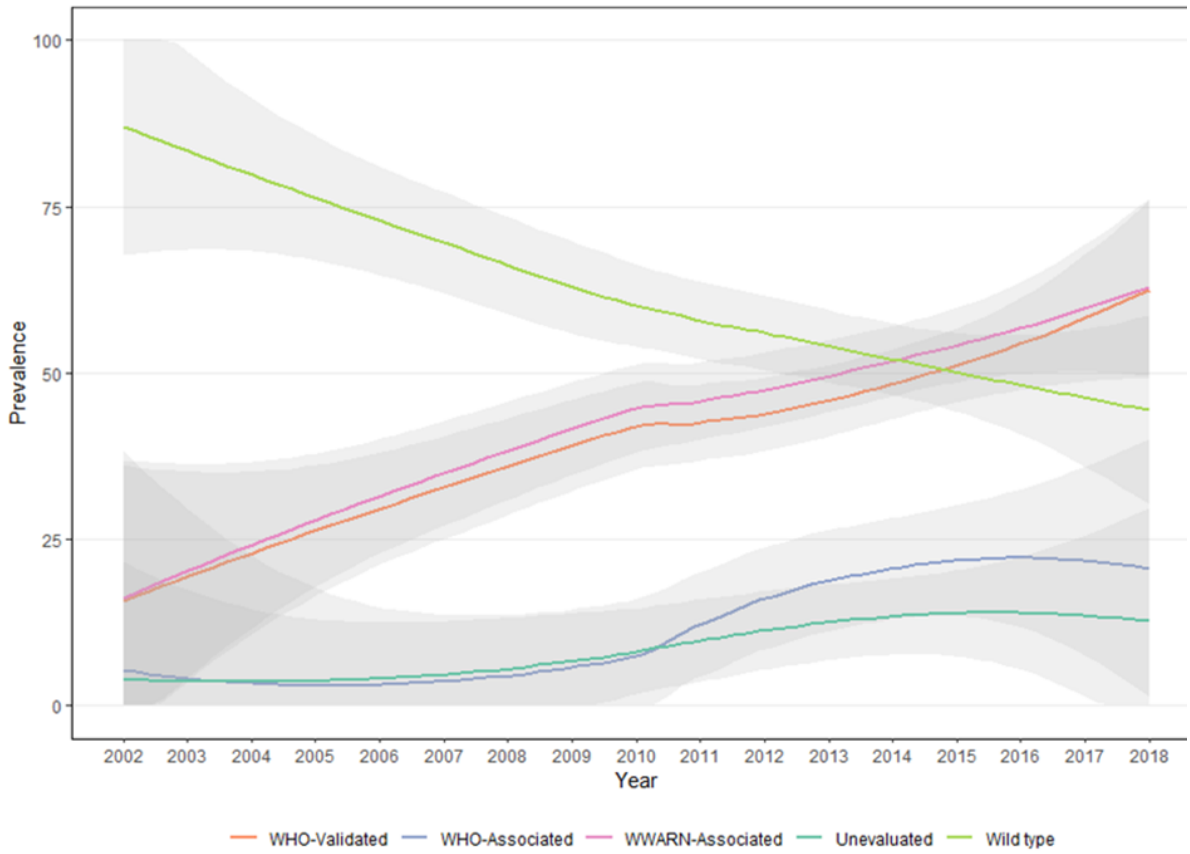
Figure S 7: Temporal and spatial trends of K13 markers in the GMS.



This graphic interchange file displays the change of the overall prevalence of validated molecular markers by administrative unit over time from published K13 studies in the GMS. All countries show an increase in the 'validated' K13 markers.

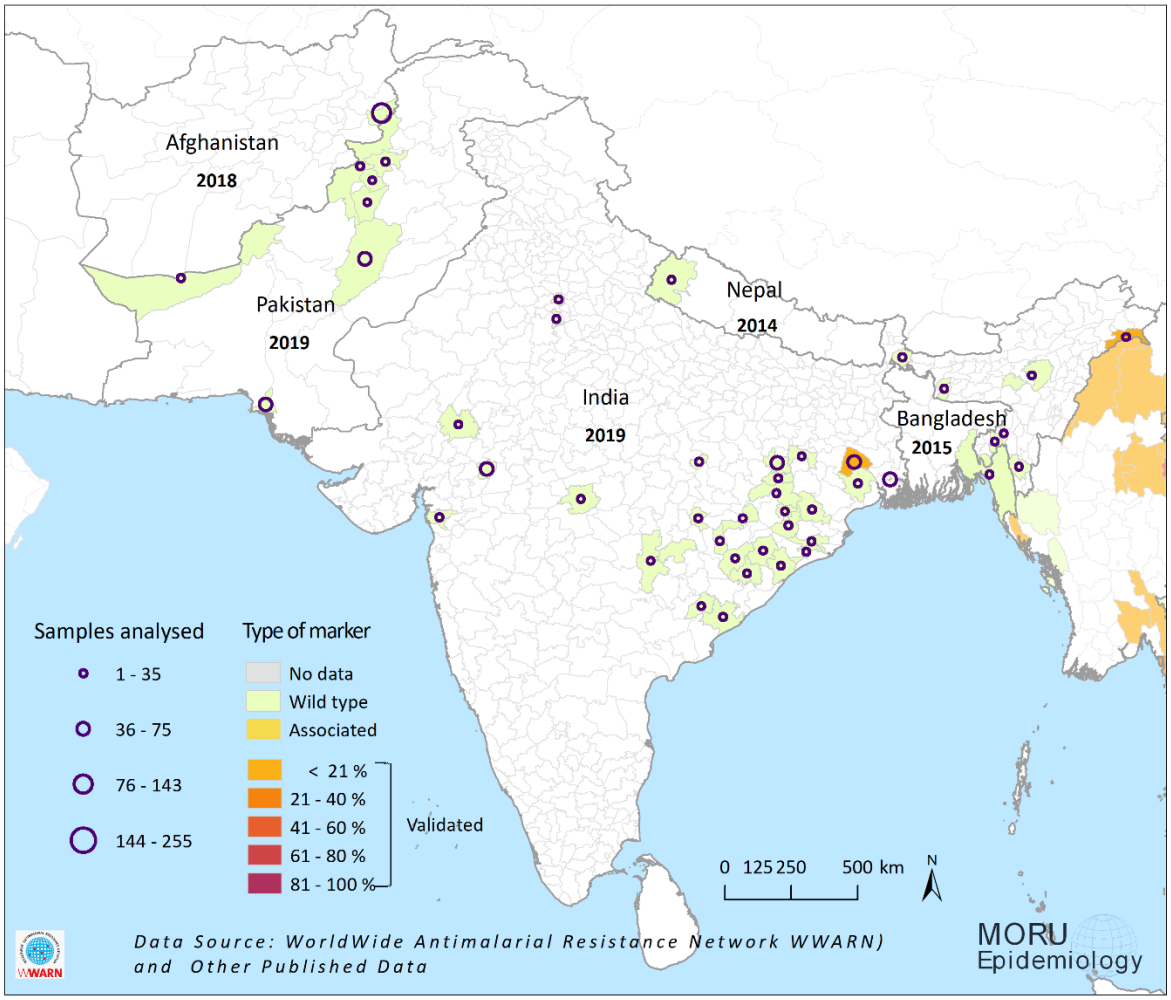
https://www.youtube.com/watch?v=_d0dneGA1Hg&ab_channel=TheLancet

Figure S 8:Temporal trends of K13 marker prevalence in the GMS



Annual prevalence of all K13 markers grouped by category. The lines represent the mean value and bands the 95% confidence intervals. The WHO validated, WHO and WWARN associated markers increased over time from 2002 to 2018 with a decrease in wild type parasites. However, there were wide confidence margins at both ends due to smaller sample sizes.

Figure S 9: Distribution of K13 markers in South Asia



K13 markers in Afghanistan, Bangladesh, India, Nepal and Pakistan. Except for India which had WHO-validated K13 markers (F446I, R539T, R561H/C), other locations had only wild type parasites.

Table S 5: K13 mutations by category.

Categories	SNPs
WHO validated/confirmed	C580Y, F446I, I543T, M476I, N458Y, P553L, P574L, R539T, R561H, Y493H
WHO associated	A481V, A675V, C469F, C469Y/F, E252Q, F673I, G449A, G449A/D, G538V, N537I, P441L, P527H, P574L, R515K, V568G
WWARN associated	A481V, A675V, C580Y, C580C/Y, E252Q, F446I, G449A, G449A/D, G538V, I543T, M476I, N458Y, N537I, P441L, P527H, P553L, P574L, P667T, R515K, R539T, R539R/T, R561H, R561H/C, V568G, Y493H
Unevaluated	A481T, A486V, A504T, A676D, C447Y, C469F, C469Y, C469Y/F, C580F, D281V, D452E, D464E, D512N, D516Y, D584V, E252K, E455K, E556D, E567D, E605G, E605K, E643K, F451I, F483S, F495L, F614L, F673I, G497V, G533A, G533S, G548S, G553A, H366L, H719N, I250T, I437T, I590T, I646L, K189T, K438N, K479I, K503N, L488M, L492S, L618L, M476I , M476V, M562I, M579T, M608K, M608V, N490T, N525D, N554S, N609S, N632D, N657H, N664S, N672R, P443Q, P443S, P553S, P667Q, P667R, P701R, R239Q, R513H, R528G, R528G/T, R529K, R575K, R575K/L, S459L, S485N, S549Y, S621F, S623C, S700L, T474I, T508N, T535A, T535M, T573A, V445G, V454I, V494I, V510G, V510M, V520I, V603E, V637A, V692L, Y493C, Y500C, Y511H, Y541H, Y604H
Not associated	A578S
Wild type	Wild type

A table showing various Single Nucleotide Polymorphisms (SNPs) and their categories as reported in the study.

Tool S 1: The Proposed K13 marker study reporting criteria

To pool data, evaluate trends spatially and temporally, we hereby propose a tool that combines minimal essential information to be included when reporting K13 molecular markers.
This tool consists of four excel sheets which include more information about the tool on sheet (P1-About), the tool to be completed (P2-Tool) horizontally for each sample, variables covered and their meaning (P3-Variables) and their further instructions (P4-Instructions). This tool can also be accessed on the WWARN Malaria Clinical Trials Toolkit (https://www.wwarn.org/tools-resources/kelch-markers-toolkit)

.

Table S 6: K13 markers publications.

S.No	PMID	Title	Authors	Eligible/reason for not-eligible
1	28161569	The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. Lancet Infect Dis. 2017;17(5):491–7.	Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al.	eligible
2	27332904	A Worldwide Map of *Plasmodium falciparum* K13-Propeller Polymorphisms. N Engl J Med. 2016 Jun;374(25):2453–64	Ménard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, et al.	eligible
3	28137815	13 Propeller Mutations in *Plasmodium falciparum* Populations in Regions of Malaria Endemicity in Vietnam from 2009 to 2016. Antimicrob Agents Chemother. 2017 Apr;61(4).	Thuy-Nhien N, Tuyen NK, Tong NT, Vy NT, Thanh NV, Van HT, et al.	eligible
4	25180241	Independent Emergence of Artemisinin Resistance Mutations Among *Plasmodium falciparum* in Southeast Asia. J Infect Dis. 2015 Mar 1;211(5):670–9.	Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al.	eligible
5	25075834	Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2014 Jul;371(5):411–23.	Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al.	eligible
6	25224002	Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine in *Plasmodium falciparum* malaria patients in central Vietnam. Antimicrob Agents Chemother. 2014 Dec;58(12):7049–55.	Thriemer K, Hong N Van, Rosanas-Urgell A, Phuc BQ, Ha DM, Pockele E, et al.	eligible
7	26616851	An outbreak of artemisinin resistant falciparum malaria in Eastern Thailand. Sci Rep. 2015 Nov 30;5:17412.	Imwong M, Jindakhad T, Kunasol C, Sutawong K, Vejakama P, Dondorp AM.	eligible
8	25836766	Selection and Spread of Artemisinin-Resistant Alleles in Thailand Prior to the Global Artemisinin Resistance Containment Campaign. PLoS Pathog. 2015 Apr;11(4):e1004789.	Talundzic E, Okoth SA, Congpuong K, Plucinski MM, Morton L, Goldman IF, et al.	eligible
9	27313266	Declining Efficacy of Artemisinin Combination Therapy Against P. Falciparum Malaria on the Thai-Myanmar Border (2003-2013): The Role of Parasite Genetic Factors. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2016 Sep;63(6):784–91	Phyo AP, Ashley EA, Anderson TJC, Bozdech Z, Carrara VI, Sriprawat K, et al.	eligible

10	20689583	An open-label, randomised study of dihydroartemisinin-piperaquine versus artesunate-mefloquine for falciparum malaria in Asia. PLoS One. 2010 Jul;5(7):e11880.	Valecha N, Phyo AP, Mayxay M, Newton PN, Krudsood S, Keomany S, et al.	eligible
11	25704894	Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. Lancet Infect Dis. 2015 Apr;15(4):415–21.	Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, et al.	eligible
12	27109419	Investigation and control of a *Plasmodium falciparum* malaria outbreak in Shan Special Region II of Myanmar along the China-Myanmar Border from June to December 2014. Infect Dis poverty. 2016 Apr;5:32.	Liu H, Xu J-W, Yang H-L, Li M, Sun C-D, Yin Y-J, et al.	eligible
13	25927592	Prevalence of K13-propeller polymorphisms in *Plasmodium falciparum* from China-Myanmar border in 2007-2012. Malar J. 2015 Apr;14:168.	Wang Z, Shrestha S, Li X, Miao J, Yuan L, Cabrera M, et al.	eligible
14	25537878	Molecular assessment of artemisinin resistance markers, polymorphisms in the k13 propeller, and a multidrug-resistance gene in the eastern and western border areas of Myanmar. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2015 Apr;60(8):1208–15.	Nyunt MH, Hlaing T, Oo HW, Tin-Oo L-LK, Phway HP, Wang B, et al.	eligible
15	27788228	Prevalence of *Plasmodium falciparum* Molecular Markers of Antimalarial Drug Resistance in a Residual Malaria Focus Area in Sabah, Malaysia. PLoS One. 2016;11(10):e0165515.	Norahmad NA, Mohd Abd Razak MR, Abdullah NR, Sastu UR, Imwong M, Muniandy PK, et al.	eligible
16	26688755	No Polymorphism in *Plasmodium falciparum* K13 Propeller Gene in Clinical Isolates from Kolkata, India. J Pathog. 2015;2015:374354.	Chatterjee M, Ganguly S, Saha P, Bankura B, Basu N, Das M, et al	eligible
17	27737665	Therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria from three highly malarious states in India. Malar J. 2016 Oct;15(1):498.	Bharti PK, Shukla MM, Ringwald P, Krishna S, Singh PP, Yadav A, et al.	eligible
18	25691626	Surveillance of artemisinin resistance in *Plasmodium falciparum* in India using the kelch13 molecular marker. Antimicrob Agents Chemother. 2015 May 1;59(5):2548–53.	Mishra N, Prajapati SK, Kaitholia K, Bharti RS, Srivastava B, Phookan S, et al.	eligible
19	25691632	Amplification of pfmdr1, pfcrt, pvmdr1, and K13 propeller polymorphisms associated with *Plasmodium falciparum* and	Feng J, Zhou D, Lin Y, Xiao H, Yan H, Xia Z.	eligible

		Plasmodium vivax isolates from the China-Myanmar border. Antimicrob Agents Chemother. 2015 May 1;59(5):2554–9.		
20	25910630	A Single Mutation in K13 Predominates in Southern China and Is Associated With Delayed Clearance of *Plasmodium falciparum* Following Artemisinin Treatment. J Infect Dis. 2015 Nov 15;212(10):1629–35.	Huang F, Takala-Harrison S, Jacob CG, Liu H, Sun X, Yang H, et al.	eligible
21	26695060	*Plasmodium falciparum* dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays: retrospective and prospective investigations. BMC Med. 2015 Dec;13:305.	Duru V, Khim N, Leang R, Kim S, Domergue A, Kloeung N, et al.	eligible
22	26774243	Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: a multisite prospective cohort study. Lancet Infect Dis. 2016 Mar;16(3):357–65.	Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al.	eligible
23	24352242	A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014 Jan;505(7481):50–5.	Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N, et al.	eligible
24	25288380	Plasmodium prevalence and artemisinin-resistant falciparum malaria in Preah Vihear Province, Cambodia: a cross-sectional population-based study. Malar J. 2014 Oct;13:394.	Bosman P, Stassijns J, Nackers F, Canier L, Kim N, Khim S, et al.	eligible
25	25877962	Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. Lancet Infect Dis. 2015 Jun;15(6):683–91.	Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, et al.	eligible
26	25404021	Mutations in *Plasmodium falciparum* K13 propeller gene from Bangladesh (2009-2013). Malar J. 2014 Nov;13:431.	Mohon AN, Alam MS, Bayih AG, Folefoc A, Shahinas D, Haque R, et al.	eligible
27	26917051	Clinical trials of artesunate plus sulfadoxine-pyrimethamine for *Plasmodium falciparum* malaria in Afghanistan: maintained efficacy a decade after introduction. Malar J. 2016 Feb;15:121.	Awab GR, Imwong M, Pukrittayakamee S, Alim F, Hanpithakpong W, Tarning J, et al.	eligible
28	30535043	Novel pfkelch13 Gene Polymorphism Associates With Artemisinin Resistance in Eastern India. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2019 Sep;69(7):1144–52.	Das S, Manna S, Saha B, Hati AK, Roy S.	eligible

29	29793059	Longitudinal surveillance of drug resistance in *Plasmodium falciparum* isolates from the China-Myanmar border reveals persistent circulation of multidrug resistant parasites. Int J Parasitol Drugs drug Resist. 2018 Aug;8(2):320–8.	Bai Y, Zhang J, Geng J, Xu S, Deng S, Zeng W, et al.	eligible
30	27036739	Parasite clearance rates in Upper Myanmar indicate a distinctive artemisinin resistance phenotype: a therapeutic efficacy study. Malar J. 2016 Mar;15:185.	Tun KM, Jeeyapant A, Imwong M, Thein M, Aung SSM, Hlaing TM, et al.	eligible
31	29345221	Therapeutic Response to Dihydroartemisinin-Piperaquine for P. falciparum and P. vivax Nine Years after Its Introduction in Southern Papua, Indonesia. Am J Trop Med Hyg. 2018 Mar;98(3):677–82.	Poespoprodjo JR, Kenangalem E, Wafom J, Chandrawati F, Puspitasari AM, Ley B, et al.	eligible
32	29813085	Resistance screening and trend analysis of imported falciparum malaria in NSW, Australia (2010 to 2016).	Prosser PC, Meyer MW, Ellis EJ, Lee LR	eligible
33	27301553	*Plasmodium falciparum* parasite population structure and gene flow associated to antimalarial drugs resistance in Cambodia. Malar J. 2016 Jun;15:319.	Dwivedi A, Khim N, Reynes C, Ravel P, Ma L, Tichit M, et al.	eligible
34	27234446	Asymptomatic Plasmodium infections in 18 villages of southern Savannakhet Province, Lao PDR (Laos). Malar J. 2016 May;15(1):296.	Phommasone K, Adhikari B, Henriques G, Pongvongsa T, Phongmany P, von Seidlein L, et al.	eligible
35	28806957	Clinical and molecular surveillance of artemisinin resistant falciparum malaria in Myanmar (2009-2013). Malar J. 2017;16(1):333.	Nyunt MH, Soe MT, Myint HW, Oo HW, Aye MM, Han SS, et al.	eligible
36	30939179	Clinical impact of the two ART resistance markers, K13 gene mutations and DPC3 in Vietnam. PLoS One. 2019;14(4).	Pau MC, Pantaleo A, Tsamesidis I, Hoang H, Tran AT, Nguyen TLH, et al.	eligible
37	28086775	Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin-piperaquine in the south of Vietnam. Malar J. 2017 Jan;16(1):27.	Thanh NV, Thuy-Nhien N, Tuyen NTK, Tong NT, Nha-Ca NT, Dong LT, et al.	eligible
38	31345710	Determinants of dihydroartemisinin-piperaquine treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. Lancet Infect Dis. 2019 Sep;19(9):952–61.	van der Pluijm RW, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, et al.	eligible
39	27585957	Assessing the asymptomatic reservoir and dihydroartemisinin-piperaquine effectiveness in a low	Falq G, Van Den Bergh R, De Smet M,	eligible

		transmission setting threatened by artemisinin resistant *Plasmodium falciparum*. Malar J. 2016 Sep;15(1):446.	Etienne W, Nguon C, Rekol H, et al.	
40	28494763	Artemisinin resistance without pfkelch13 mutations in *Plasmodium falciparum* isolates from Cambodia. Malar J. 2017 Dec 12;16(1):195.	Mukherjee A, Bopp S, Magistrado P, Wong W, Daniels R, Demas A, et al.	eligible
41	29334942	Poor response to artesunate treatment in two patients with severe malaria on the Thai–Myanmar border. Malar J. 2018 Dec 15;17(1):30.	Phyo AP, Win KK, Thu AM, Swe LL, Htike H, Beau C, et al.	eligible
42	27234587	Sustained efficacy of artesunate-sulfadoxine-pyrimethamine against *Plasmodium falciparum* in Yemen and a renewed call for an adjunct single dose primaquine to clear gametocytes. Malar J. 2016 May;15(1):295.	Atroosh WM, Al-Mekhlafi HM, Snounou G, Al-Jasari A, Sady H, Nasr NA, et al.	eligible
43	28388902	Therapeutic efficacy and artemisinin resistance in northern Myanmar: evidence from in vivo and molecular marker studies. Malar J. 2017;16(1):143.	Myint MK, Rasmussen C, Thi A, Bustos D, Ringwald P, Lin K.	eligible
44	27343362	Molecular markers associated with resistance to commonly used antimalarial drugs among *Plasmodium falciparum* isolates from a malaria-endemic area in Taiz governorate-Yemen during the transmission season. Acta Trop. 2016 Oct;162:174–9.	Alareqi LMQ, Mahdy MAK, Lau Y-L, Fong M-Y, Abdul-Ghani R, Mahmud R.	eligible
45	28249583	Molecular surveillance of artemisinin resistance falciparum malaria among migrant goldmine workers in Myanmar. Malar J. 2017 Dec 1;16(1):97.	Nyunt MH, Wang B, Aye KM, Aye KH, Han J-H, Lee S-K, et al.	eligible
46	27084511	Examining *Plasmodium falciparum* and P. vivax clearance subsequent to antimalarial drug treatment in the Myanmar-China border area based on quantitative real-time polymerase chain reaction. BMC Infect Dis. 2016 Apr;16:154.	Lo E, Nguyen J, Oo W, Hemming-Schroeder E, Zhou G, Yang Z, et al.	eligible
47	26548510	Natural selection of K13 mutants of *Plasmodium falciparum* in response to artemisinin combination therapies in Thailand. Clin Microbiol Infect. 2016 Mar;22(3):285.e1-285.e8.	Putaporntip C, Kuamsab N, Kosuwin R, Tantiwattanasub W, Vejakama P, Sueblinvong T, et al.	eligible
48	29996844	Effectiveness and safety of 3 and 5 day courses of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in an area of emerging artemisinin resistance in Myanmar. Malar J. 2018 Jul;17(1):258.	Tun KM, Jeeyapant A, Myint AH, Kyaw ZT, Dhorda M, Mukaka M, et al.	eligible

49	28806961	Molecular analysis demonstrates high prevalence of chloroquine resistance but no evidence of artemisinin resistance in *Plasmodium falciparum* in the Chittagong Hill Tracts of Bangladesh. Malar J. 2017;16(1):335.	Alam MS, Ley B, Nima MK, Johora FT, Hossain ME, Thriemer K, et al.	eligible
50	31009824	In vitro susceptibility of *Plasmodium falciparum* isolates from the China-Myanmar border area to artemisinins and correlation with K13 mutations. Int J Parasitol Drugs drug Resist. 2019 Apr 10;10:20–7.	Zhang J, Li N, Siddiqui FA, Xu S, Geng J, Zhang J, et al.	eligible
51	METFGENRE	METF-SMRU/GenRe	Prof. Francois Nosten and GenRe-Mekong Project	eligible
52	30883571	Artemisinin resistance-associated markers in *Plasmodium falciparum* parasites from the China-Myanmar border: predicted structural stability of K13 propeller variants detected in a low-prevalence area. Carvalho LH, editor. PLoS One. 2019 Mar 18;14(3):e0213686.	He Y, Campino S, Diez Benavente E, Warhurst DC, Beshir KB, Lubis I, et al.	eligible
53	31239407	K13 propeller domain mutations and pfmdr1 amplification in isolates of *Plasmodium falciparum* collected from Thai-Myanmar border area in 2006-2010. Folia Parasitol (Praha). 2019 May;66.	Phompradit P, Chaijaroenkul W, Muhamad P, Na-Bangchang K.	eligible
54	31251812	Pyronaridine-artesunate Efficacy and Safety in Uncomplicated *Plasmodium falciparum* Malaria in Areas of Artemisinin-resistant Falciparum in Viet Nam (2017-2018). Clin Infect Dis an Off Publ Infect Dis Soc Am. 2020 May;70(10):2187–95.	Quang Bui P, Hong Huynh Q, Thanh Tran D, Thanh Le D, Quang Nguyen T, Van Truong H, et al.	eligible
55	28322709	Treatment Failure of Dihydroartemisinin/Piperaquine for *Plasmodium falciparum* Malaria, Vietnam. Emerg Infect Dis. 2017;23(4):715.	Phuc BQ, Rasmussen C, Duong TT, Dong LT, Loi MA, Ménard D, et al.	eligible
56	29110709	The prevalence, incidence and prevention of *Plasmodium falciparum* infections in forest rangers in Bu Gia Map National Park, Binh Phuoc province, Vietnam: a pilot study. Malar J. 2017 Dec 6;16(1):444.	Son DH, Thuy-Nhien N, von Seidlein L, Le Phuc-Nhi T, Phu NT, Tuyen NTK, et al.	eligible
57	29178921	*Plasmodium falciparum* Kelch 13 mutations and treatment response in patients in Hpa-Pun District, Northern Kayin State, Myanmar. Malar J. 2017 Dec 25;16(1):480.	Bonnington CA, Phyo AP, Ashley EA, Imwong M, Sriprawat K, Parker DM, et al.	eligible
58	28549390	Clinical and molecular monitoring of *Plasmodium falciparum*	Mishra S, Bharti PK, Shukla MM, Ali NA,	eligible

		resistance to antimalarial drug (artesunate+sulphadoxine-pyrimethamine) in two highly malarious district of Madhya Pradesh, Central India from 2012-2014. Pathog Glob Health. 2017 Jun;111(4):186–94.	Kashyotia SS, Kumar A, et al.	
59	28903755	Prevalence of K13 mutation and Day-3 positive parasitaemia in artemisinin-resistant malaria endemic area of Cambodia: a cross-sectional study. Malar J. 2017 Sep;16(1):372.	Kheang ST, Sovannaroth S, Ek S, Chy S, Chhun P, Mao S, et al.	eligible
60	31833468	Artemether-lumefantrine and dihydroartemisinin-piperaquine retain high efficacy for treatment of uncomplicated *Plasmodium falciparum* malaria in Myanmar. Am J Trop Med Hyg. 2020;102(3):598–604.	Han KT, Lin K, Myint MK, Thi A, Aye KH, Han ZY, et al.	eligible
61	31791329	Genetic profiling of the *Plasmodium falciparum* parasite population in uncomplicated malaria from India. Malar J. 2019 Dec 2;18(1):385.	Kumar A, Singh SP, Bhatt R, Singh V.	eligible
62	31548652	Characterisation of drug resistance and genetic diversity of *Plasmodium falciparum* parasites from Tripura, Northeast India. Sci Rep. 2019 Dec 1;9(1).	Patgiri SJ, Sarma K, Sarmah N, Bhattacharyya N, Sarma DK, Nirmolia T, et al.	eligible
63	31533403	Molecular surveillance of Pfkelch13 and Pfmdr1 mutations in *Plasmodium falciparum* isolates from southern Thailand. Korean J Parasitol. 2019 Aug 1;57(4):369–77.	Khammanee T, Sawangjaroen N, Buncherd H, Tun AW, Thanapongpichat S.	eligible
64	31416468	Molecular detection of drug resistant malaria in Southern Thailand. Malar J. 2019 Aug;18(1):275.	Noisang C, Prosser C, Meyer W, Chemoh W, Ellis J, Sawangjaroen N, et al.	eligible
65	31069209	Investigation and evaluation of genetic diversity of kelch 13 polymorphisms in *Plasmodium falciparum* from southern China. Front Public Heal. 2019;7(APR).	Feng J, Kong X, Xu D, Yan H, Zhou H, Tu H, et al.	eligible
66	32524960	Efficacy and Safety of Pyronaridine-Artesunate for the Treatment of Uncomplicated *Plasmodium falciparum* and Plasmodium vivax Malaria in Myanmar. Am J Trop Med Hyg. 2020 Sep;103(3):1088–93.	Han KT, Lin K, Han ZY, Myint MK, Aye KH, Thi A, et al.	eligible
67	32258150	Sequence Analysis of the K13-Propeller Gene in Artemisinin Challenging *Plasmodium falciparum* Isolates from Malaria Endemic Areas of Odisha, India: A Molecular Surveillance Study. Biomed Res Int. 2020;2020:8475246.	Rana R, Ranjit M, Bal M, Khuntia HK, Pati S, Krishna S, et al.	eligible

68	32513171	Surveillance of genetic markers associated with *Plasmodium falciparum* resistance to artemisinin-based combination therapy in Pakistan, 2018-2019. Malar J. 2020 Jun 8;19(1):206.	Khan AQ, Khan AQ, Pernaute-Lau L, Pernaute-Lau L, Khattak AA, Luijcx S, et al.	eligible
69	33320907	Emergence of artemisinin-resistant *Plasmodium falciparum* with kelch13 C580Y mutations on the island of New Guinea. PLoS Pathog. 2020 Dec 15;16(12)	Miotto O, Sekihara M, Tachibana SI, Yamauchi M, Pearson RD, Amato R, et al.	eligible
70	33168025	Kelch 13-propeller polymorphisms in *Plasmodium falciparum* from Jazan region, southwest Saudi Arabia. Malar J. 2020 Dec 1;19(1):397.	Dafalla OM, Alzahrani M, Sahli A, Al Helal MA, Alhazmi MM, Noureldin EM, et al.	eligible
71	32854686	Efficacy of artemether-lumefantrine for treating uncomplicated *Plasmodium falciparum* cases and molecular surveillance of drug resistance genes in Western Myanmar. Malar J. 2020 Aug 27;19(1)	Wu Y, Soe MT, Aung PL, Zhao L, Zeng W, Menezes L, et al.	eligible
72	31351071	In vitro synergistic interaction of potent 4-aminoquinolines in combination with dihydroartemisinin against chloroquine-resistant *Plasmodium falciparum*. Acta Trop. 2019 Nov;199:105109.	Agarwal D, Singh S, Gupta RD, Awasthi SK.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
73	31345709	Evolution and expansion of multidrug-resistant malaria in southeast Asia: a genomic epidemiology study. Lancet Infect Dis. 2019 Sep;19(9):943–51.	Hamilton WL, Amato R, van der Pluijm RW, Jacob CG, Quang HH, Thuy-Nhien NT, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-used previously published samples
74	31267499	An Update on Artemisinin Resistance. Methods Mol Biol. 2019; 2013:141–9.	Ariey F, Ménard D.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-review
75	31232939	Intervention of artemisinin in macular edema associated with retinal vein occlusion: A protocol for a systematic review and meta-analysis. Medicine (Baltimore). 2019 Jun;98(25):e16044.	Xu J, Hao X, Lu B, Ming J, Li X, Qi Y, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-no, other-protocol

76	31210357	Artemether for severe malaria. Cochrane database Syst Rev. 2019 Jun;6(6):CD010678.	Esu EB, Effa EE, Opie ON, Meremikwu MM.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-review
77	31185976	An improved nucleic acid extraction method from dried blood spots for amplification of *Plasmodium falciparum* kelch13 for detection of artemisinin resistance. Malar J. 2019 Jun;18(1):192.	Zainabadi K, Nyunt MM, Plowe C V.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-no, other-no
78	31108084	Modulation of in vitro antimalarial responses by polymorphisms in Plasmodium falciparum ABC transporters (pfmdr1 and pfmdr5). Acta Trop. 2019 Aug; 196:126–34.	Gendrot M, Wague Gueye M, Tsombeng Foguim F, Madamet M, Wade KA, Bou Kounta M, et al.	antimalarial-no, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
79	31075171	Antimalarial Immunity to Measures of Parasite Clearance in Therapeutic Efficacy Studies of Artemisinin Derivatives. J Infect Dis. 2019 Aug;220(7):1178–87.	O'Flaherty K, Ataíde R, Zaloumis SG, Ashley EA, Powell R, Feng G, et al.	antimalarial-no, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
80	31015034	Efficacy and resistance of different artemisinin-based combination therapies: a systematic review and network meta-analysis. Parasitol Int. 2020 Feb;74:101919.	Mathenge PG, Low SK, Vuong NL, Mohamed MYF, Faraj HA, Alieldin GI, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-review
81	30995310	Geographic expansion of artemisinin resistance. J Travel Med. 2019 Jun;26(4).	Müller O, Lu GY, von Seidlein L.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
82	30768615	The impact of targeted malaria elimination with mass drug administrations on falciparum malaria in Southeast Asia: A cluster randomised trial. PLoS Med. 2019 Feb;16(2):e1002745.	von Seidlein L, Peto TJ, Landier J, Nguyen T-N, Tripura R, Phommasone K, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-yes, other-no
83	30753425	Targeting malaria parasite invasion of red blood cells as an antimalarial strategy. FEMS	Burns AL, Dans MG, Balbin JM, de Koning-Ward TF, Gilson PR, Beeson JG, et al.	antimalarial-yes, K13 information-no, clinical

		Microbiol Rev. 2019 May;43(3):223–38.		study/invivo study-no, Asia-yes, other-no
84	30717149	Artemisinin Combination Therapies (ACTs): Do Not Forget the Partner Drug! Trop Med Infect Dis. 2019 Feb;4(1).	Nsanzabana C.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-no
85	30701807	Problems of clinical diagnosis and treatment of P. falciparum malaria in Russian Federation. Vol. 90, Terapevticheskii arkhiv. Russia (Federation); 2018. p. 4–8.	Sergiev VP, Baranova AM, Kozhevnikova GM, Tokmalayev AK, Chernyshov D V, Chentsov VB, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-no, other-no
86	30654808	Susceptibility of *Plasmodium falciparum* to artemisinins and Plasmodium vivax to chloroquine in Phuoc Chien Commune, Ninh Thuan Province, south-central Vietnam. Malar J. 2019 Jan;18(1):10.	Phong NC, Chavchich M, Quang HH, San NN, Birrell GW, Chuang I, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
87	30620055	Pyronaridine-artesunate for treating uncomplicated *Plasmodium falciparum* malaria. Cochrane database Syst Rev. 2019 Jan;1(1):CD006404.	Pryce J, Hine P.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-review
88	30607137	Patients' adherence to artemisinin-based combination therapy and healthcare workers' perception and practice in Savannakhet province, Lao PDR. Trop Med Health. 2018;46:44.	Takahashi E, Nonaka D, Iwagami M, Phoutnalong V, Chanthakoumane K, Kobayashi J, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
89	30580023	Overexpression of plasmepsin II and plasmepsin III does not directly cause reduction in *Plasmodium falciparum* sensitivity to artesunate, chloroquine and piperaquine. Int J Parasitol Drugs drug Resist. 2019 Apr;9:16–22.	Loesbanluechai D, Kotanan N, de Cozar C, Kochakarn T, Ansbro MR, Chotivanich K, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-yes, other-no
90	30572592	Artemether and Praziquantel: Origin, Mode of Action, Impact, and Suggested Application for Effective Control of Human Schistosomiasis. Trop Med Infect Dis. 2018 Dec;3(4).	Bergquist R, Elmorshedy H.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-no, other-no

91	30563521	Genetic association between the Pfk13 gene mutation and artemisinin resistance phenotype in *Plasmodium falciparum* isolates from Yunnan Province, China. Malar J. 2018 Dec;17(1):478.	Dong Y, Wang J, Sun A, Deng Y, Chen M, Xu Y, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-secondary data from another study already included
92	30558597	Origins and spread of novel genetic variants of sulfadoxine-pyrimethamine resistance in *Plasmodium falciparum* isolates in Indonesia. Malar J. 2018 Dec;17(1):475.	Basuki S, Fitriah, Risamasu PM, Kasmijati, Ariami P, Riyanto S, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
93	30514877	The origins of malaria artemisinin resistance defined by a genetic and transcriptomic background. Nat Commun. 2018 Dec;9(1):5158.	Zhu L, Tripathi J, Rocamora FM, Miotto O, van der Pluijm R, Voss TS, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-secondary data from another study already included
94	30499404	Antimalarials: Review of Plasmepsins as Drug Targets and HIV Protease Inhibitors Interactions. Curr Top Med Chem. 2019;18(23):2022–8.	Miller Iii WA, Teye J, Achieng AO, Mogire RM, Akala H, Ong'echa JM, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-review
95	30486796	Altered expression of K13 disrupts DNA replication and repair in Plasmodium falciparum. BMC Genomics. 2018 Nov;19(1):849.	Gibbons J, Button-Simons KA, Adapa SR, Li S, Pietsch M, Zhang M, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-transcriptonic lab study
96	30478733	Current scenario and future strategies to fight artemisinin resistance. Parasitol Res. 2019 Jan;118(1):29–42.	Pasupureddy R, Atul, Seshadri S, Pande V, Dixit R, Pandey KC.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review

97	30447701	K13-propeller gene polymorphisms in *Plasmodium falciparum* parasite population: a systematic review protocol of burden and associated factors. Syst Rev. 2018 Nov;7(1):199.	Ocan M, Akena D, Nsobya S, Kamya MR, Senono R, Kinengyere AA, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
98	30390647	The dynamic of asymptomatic *Plasmodium falciparum* infections following mass drug administrations with dihydroarteminisin-piperaquine plus a single low dose of primaquine in Savannakhet Province, Laos. Malar J. 2018 Nov;17(1):405.	Pongvongsa T, Phommasone K, Adhikari B, Henriques G, Chotivanich K, Hanboonkunupakarn B, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-did not asses kelch markers
99	30367653	A single nucleotide polymorphism in the *Plasmodium falciparum* atg18 gene associates with artemisinin resistance and confers enhanced parasite survival under nutrient deprivation. Malar J. 2018 Oct;17(1):391.	Breglio KF, Amato R, Eastman R, Lim P, Sa JM, Guha R, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
100	30269689	High-level artemisinin-resistance with quinine co-resistance emerges in P. falciparum malaria under in vivo artesunate pressure. BMC Med. 2018 Oct;16(1):181.	Tyagi RK, Gleeson PJ, Arnold L, Tahar R, Prieur E, Decosterd L, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-no
101	30154519	Surveillance of Antimalarial Resistance Pfcrt, Pfmdr1, and Pfkelch13 Polymorphisms in African *Plasmodium falciparum* imported to Shandong Province, China. Sci Rep. 2018 Aug 28;8(1):12951.	Xu C, Wei Q, Yin K, Sun H, Li J, Xiao T, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-yes, other-imported cases - travellers from African countries
102	30139985	Expression of key genes affecting artemisinin content in five Artemisia species. Sci Rep. 2018 Aug;8(1):12659.	Salehi M, Karimzadeh G, Naghavi MR, Naghdi Badi H, Rashidi Monfared S.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
103	30135481	Fitness Loss under Amino Acid Starvation in Artemisinin-Resistant Plasmodium falciparum Isolates from Cambodia. Sci Rep. 2018 Aug;8(1):12622.	Bunditvorapoom D, Kochakarn T, Kotanan N, Modchang C, Kümpornsin K,	antimalarial-yes, K13 information-no, clinical study/invivo

			Loesbanluechai D, et al.	study-yes, Asia-yes, other-no
104	30115924	Emerging Southeast Asian PfCRT mutations confer *Plasmodium falciparum* resistance to the first-line antimalarial piperaquine. Nat Commun. 2018 Aug;9(1):3314.	Ross LS, Dhingra SK, Mok S, Yeo T, Wicht KJ, Kümpornsin K, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
105	30071877	Implications of population-level immunity for the emergence of artemisinin-resistant malaria: a mathematical model. Malar J. 2018 Aug;17(1):279.	Scott N, Ataide R, Wilson DP, Hellard M, Price RN, Simpson JA, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-modelling study
106	30016713	Variation in intronic microsatellites and exon 2 of the *Plasmodium falciparum* chloroquine resistance transporter gene during modification of artemisinin combination therapy in Thailand. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2018 Nov;65:35–42.	Seethamchai S, Buppan P, Kuamsab N, Teeranaipong P, Putaporntip C, Jongwutiwes S.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
107	29980936	Dihydroartemisinin-piperaquine treatment failure in uncomplicated Plasmodium falciparum malaria case imported from Ethiopia. Infection. 2018 Dec;46(6):867–70.	Russo G, L'Episcopia M, Menegon M, Souza SS, Dongho BGD, Vullo V, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-no, other-no
108	29976207	Introduction of F446I mutation in the K13 propeller gene leads to increased ring survival rates in *Plasmodium falciparum* isolates. Malar J. 2018 Jul;17(1):248.	Wang J, Huang Y, Zhao Y, Ye R, Zhang D, Pan W.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-transgenic study
109	29973212	Efficacy of two artemisinin-based combinations for the treatment of malaria in pregnancy in India: a randomized controlled trial. Malar J. 2018 Jul;17(1):246.	Anvikar AR, Kuepfer I, Mishra V, Bruce J, Arya T, Mishra DR, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no evaluation of kelch markers
110	29843734	An innovative diagnostic technology for the codon mutation	Imai K, Tarumoto N, Runtuwene LR, Sakai	antimalarial-yes, K13

		C580Y in kelch13 of *Plasmodium falciparum* with MinION nanopore sequencer. Malar J. 2018 May;17(1):217.	J, Hayashida K, Eshita Y, et al.	information-yes, clinical study/invivo study-no, Asia-yes, other-diagnostic study
111	29798745	Therapeutic and Transmission-Blocking Efficacy of Dihydroartemisinin/Piperaquine and Chloroquine against Plasmodium vivax Malaria, Cambodia. Emerg Infect Dis. 2018;24(8):1516–9.	Popovici J, Vantaux A, Primault L, Samreth R, Piv EP, Bin S, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-p.vivax study
112	29798745	Therapeutic and Transmission-Blocking Efficacy of Dihydroartemisinin/Piperaquine and Chloroquine against Plasmodium vivax Malaria, Cambodia. Emerg Infect Dis. 2018;24(8):1516–9.	Popovici J, Vantaux A, Primault L, Samreth R, Piv EP, Bin S, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-p.vivax study
113	29703425	Effect of generalised access to early diagnosis and treatment and targeted mass drug administration on *Plasmodium falciparum* malaria in Eastern Myanmar: an observational study of a regional elimination programme. Lancet. 2018 May 12;391(10133):1916–26.	Landier J, Parker DM, Thu AM, Lwin KM, Delmas G, Nosten FH, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-yes, other-no
114	29690890	A novel field-based molecular assay to detect validated artemisinin-resistant k13 mutants. Malar J. 2018 Apr;17(1):175.	Vachot-Ganée L, Khim N, Iannello A, Legrand E, Kim S, Eam R, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-diagnostic study
115	29676250	*Plasmodium falciparum* resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination. Parasite. 2018;25:24.	Ouji M, Augereau J-M, Paloque L, Benoit-Vical F.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
116	29659945	*Plasmodium falciparum* Falcipain-2a Polymorphisms in Southeast Asia and Their Association With Artemisinin Resistance. J Infect Dis. 2018 Jul;218(3):434–42.	Siddiqui FA, Cabrera M, Wang M, Brashear A, Kemirembe K, Wang Z, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes,

				Asia-yes, other-no
117	29615130	New endoperoxides highly active in vivo and in vitro against artemisinin-resistant *Plasmodium falciparum*. Malar J. 2018 Apr;17(1):145.	Lobo L, Cabral LIL, Sena MI, Guerreiro B, Rodrigues AS, de Andrade-Neto VF, et al. *Plasmodium falciparum*. Malar J. 2018 Apr;17(1):145.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-no, other-new therapeutic study
118	29615130	New endoperoxides highly active in vivo and in vitro against artemisinin-resistant *Plasmodium falciparum*. Malar J. 2018 Apr;17(1):145.	Lobo L, Cabral LIL, Sena MI, Guerreiro B, Rodrigues AS, de Andrade-Neto VF, et al. *Plasmodium falciparum*. Malar J. 2018 Apr;17(1):145.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-no, other-new therapeutic study
119	29566683	Population pharmacokinetic and pharmacodynamic properties of artesunate in patients with artemisinin sensitive and resistant infections in Southern Myanmar. Malar J. 2018 Mar;17(1):126.	Lohy Das JP, Kyaw MP, Nyunt MH, Chit K, Aye KH, Aye MM, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-PKPD study
120	29563721	Suspected Artesunate Resistant Malaria in South India. Vol. 10, Journal of global infectious diseases. 2018. p. 26–7.	Akunuri S, Shraddha P, Palli V, MuraliSantosh B.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-case reports without K13 markers evaluation
121	29538461	Oxidative stress and protein damage responses mediate artemisinin resistance in malaria parasites. PLoS Pathog. 2018 Mar;14(3):e1006930.	Rocamora F, Zhu L, Liong KY, Dondorp A, Miotto O, Mok S, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-no, other-no
122	29535546	Multidrug-resistant malaria and the impact of mass drug administration. Infect Drug Resist. 2018;11:299–306.	Zuber JA, Takala-Harrison S.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no

123	29512604	In vitro susceptibility of Indian *Plasmodium falciparum* isolates to different antimalarial drugs & antibiotics. Indian J Med Res. 2017 Nov;146(5):622–8.	Agarwal P, Anvikar AR, Pillai CR, Srivastava K.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-PKPD study
124	29398391	Origins of the current outbreak of multidrug-resistant malaria in southeast Asia: a retrospective genetic study. Lancet Infect Dis. 2018 Mar;18(3):337–45.	Amato R, Pearson RD, Almagro-Garcia J, Amaratunga C, Lim P, Suon S, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
125	29355852	Drug resistance in Plasmodium. Nat Rev Microbiol. 2018 Mar;16(3):156–70.	Haldar K, Bhattacharjee S, Safeukui I.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-no, other-no
126	29318819	Analysis of spatial distribution of artemisinin in Artemisia annua in China. Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China J Chinese Mater medica. 2017 Nov;42(22):4277–81.	Zhang X-B, Guo L-P, Qiu Z-D, Qu X-B, Wang H, Jing Z-X, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
127	29258508	Functional analysis of *Plasmodium falciparum* subpopulations associated with artemisinin resistance in Cambodia. Malar J. 2017 Dec;16(1):493.	Dwivedi A, Reynes C, Kuehn A, Roche DB, Khim N, Hebrard M, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-previous samples
128	29192183	Prevalence of mutations linked to antimalarial resistance in *Plasmodium falciparum* from Chhattisgarh, Central India: A malaria elimination point of view. Sci Rep. 2017 Nov;7(1):16690.	Patel P, Bharti PK, Bansal D, Ali NA, Raman RK, Mohapatra PK, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
129	29177421	Endoperoxide-based compounds: cross-resistance with artemisinins and selection of a *Plasmodium falciparum* lineage with a K13 non-synonymous polymorphism. J Antimicrob Chemother. 2018 Feb;73(2):395–403.	Paloque L, Witkowski B, Lelièvre J, Ouji M, Ben Haddou T, Ariey F, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-no

130	29150282	Artesunate-quercetin/luteolin dual drug nanofacilitated synergistic treatment for malaria: A plausible approach to overcome artemisinin combination therapy resistance. Med Hypotheses. 2017 Nov;109:176–80.	Puttappa N, Kumar RS, Yamjala K.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
131	29132370	Expanding malaria diagnosis and treatment in Lao PDR: lessons learned from a public-private mix initiative. Malar J. 2017 Nov;16(1):460.	Simmalavong N, Phommixay S, Kongmanivong P, Sichanthongthip O, Hongvangthong B, Gopinath D, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
132	29078767	Correction to: Prevalence of K13 mutation and Day-3 positive parasitaemia in artemisinin-resistant malaria endemic area of Cambodia: a cross-sectional study. Malar J. 2017 Oct;16(1):435.	Kheang ST, Sovannaroth S, Ek S, Chy S, Chhun P, Mao S, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-addendum - original paper already included
133	29062913	Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant falciparum malaria: A pilot trial in four villages of Eastern Myanmar. Wellcome open Res. 2017;2:81.	Landier J, Kajeechiwa L, Thwin MM, Parker DM, Chaumeau V, Wiladphaingern J, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-yes, other-no
134	29020373	Artemether-Lumefantrine Versus Chloroquine for the Treatment of Uncomplicated Plasmodium knowlesi Malaria: An Open-Label Randomized Controlled Trial CAN KNOW. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2018 Jan;66(2):229–36.	Grigg MJ, William T, Barber BE, Rajahram GS, Menon J, Schimann E, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
135	29020247	Lumefantrine Dispersible Tablets in Pediatric Patients With Acute Uncomplicated *Plasmodium falciparum* Malaria: A Phase 3, Rand. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2017 Oct;65(10):1711–20.	Toure OA, Mwapasa V, Sagara I, Gaye O, Thompson R, Maheshwar A V, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
136	28934435	Declining Transmission and Immunity to Malaria and Emerging Artemisinin Resistance in Thailand: A Longitudinal Study. J Infect Dis. 2017 Sep;216(6):723–31.	Ataíde R, Powell R, Moore K, McLean A, Phyo AP, Nair S, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no

				kelch markers reported
137	28927405	The use of respondent-driven sampling to assess malaria knowledge, treatment-seeking behaviours and preventive practices among mobile and migrant populations in a setting of artemisinin resistance in Western Cambodia. Malar J. 2017 Sep;16(1):378.	Ly P, Thwing J, McGinn C, Quintero CE, Top-Samphor N, Habib N, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
138	28895080	Population Pharmacokinetic and Pharmacodynamic Modeling of Artemisinin Resistance in Southeast Asia. AAPS J. 2017 Nov;19(6):1842–54.	Lohy Das J, Dondorp AM, Nosten F, Phyo AP, Hanpithakpong W, Ringwald P, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-modelling study
139	28895080	Population Pharmacokinetic and Pharmacodynamic Modeling of Artemisinin Resistance in Southeast Asia. AAPS J. 2017 Nov;19(6):1842–54.	Lohy Das J, Dondorp AM, Nosten F, Phyo AP, Hanpithakpong W, Ringwald P, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-modelling study
140	28854635	Partner-Drug Resistance and Population Substructuring of Artemisinin-Resistant *Plasmodium falciparum* in Cambodia. Genome Biol Evol. 2017 Jun 1;9(6):1673–86.	Parobek CM, Parr JB, Brazeau NF, Lon C, Chaorattanakawee S, Gosi P, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
141	28797235	Polymorphisms of *Plasmodium falciparum* k13-propeller gene among migrant workers returning to Henan Province, China from Africa. BMC Infect Dis. 2017;17(1):560.	Yang C, Zhang H, Zhou R, Qian D, Liu Y, Zhao Y, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-returning travellers from africa
142	28777791	Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. Nat Med. 2017 Aug;23(8):917–28.	Blasco B, Leroy D, Fidock DA.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
143	28711439	Updates on k13 mutant alleles for artemisinin resistance in	Zaw MT, Emran NA, Lin Z.	antimalarial-yes, K13

		Plasmodium falciparum. J Microbiol Immunol Infect. 2018 Apr;51(2):159–65.		information-yes, clinical study/invivo study-no, Asia-yes, other-review
144	28537265	A tetraoxane-based antimalarial drug candidate that overcomes PfK13-C580Y dependent artemisinin resistance. Nat Commun. 2017 May;8:15159.	O'Neill PM, Amewu RK, Charman SA, Sabbani S, Gnädig NF, Straimer J, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-PKPD
145	28533179	Polymorphisms in pfdhfr and pfdhps genes after five years of artemisinin combination therapy (ACT) implementation from urban Kolkata, India. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2017 Sep;53:155–9.	Chatterjee M, Ganguly S, Saha P, Guha SK, Maji AK.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
146	28473165	Unpacking "Artemisinin Resistance". Trends Pharmacol Sci. 2017 Jun;38(6):506–11.	Wang J, Xu C, Lun Z-R, Meshnick SR.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
147	28454557	Longitudinal genomic surveillance of *Plasmodium falciparum* malaria parasites reveals complex genomic architecture of emerging artemisinin resistance. Genome Biol. 2017 Apr;18(1):78.	Cerqueira GC, Cheeseman IH, Schaffner SF, Nair S, McDew-White M, Phyo AP, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-retrospective samples aready added from other studies
148	28438194	Malaria profiles and challenges in artemisinin resistance containment in Myanmar. Infect Dis poverty. 2017 Apr;6(1):76.	Nwe TW, Oo T, Wai KT, Zhou S, van Griensven J, Chinnakali P, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
149	28438155	The malaria testing and treatment landscape in the southern Lao People's Democratic Republic (PDR). Malar J. 2017 Apr;16(1):169.	Phanalasy S.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no

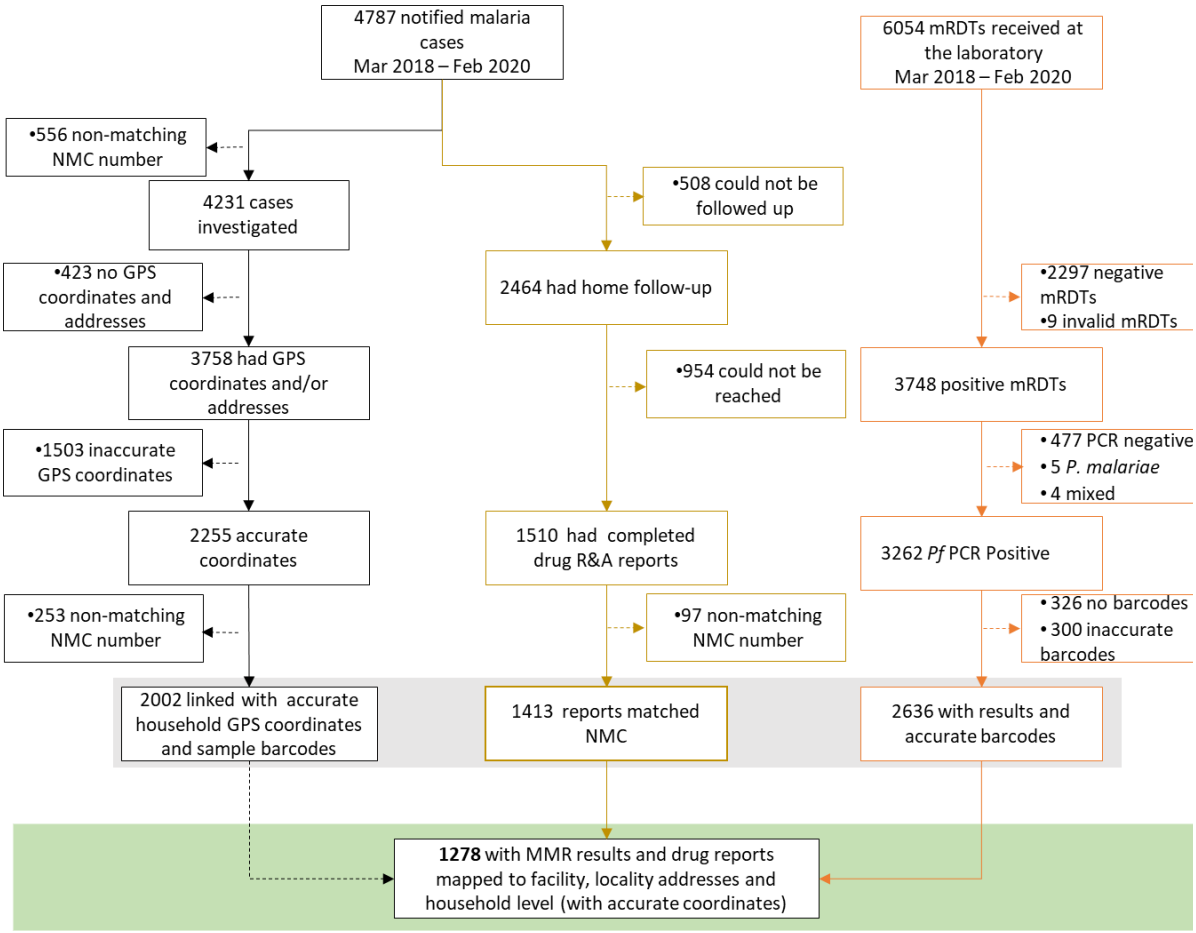
150	28438145	Insights into the availability and distribution of oral artemisinin monotherapy in Myanmar: evidence from a nationally representative outlet survey. Malar J. 2017 Apr;16(1):170.	Thein ST, Khin HSS, Thi A.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
151	28410610	An intricate case of multidrug resistant *Plasmodium falciparum* isolate imported from Cambodia. Malar J. 2017 Apr;16(1):149.	Dell'Acqua R, Fabrizio C, Di Gennaro F, Lo Caputo S, Saracino A, Menegon M, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-no, other-imported case to italy
152	28221121	Molecular Evidence of Drug Resistance in Asymptomatic Malaria Infections, Myanmar, 2015. Emerg Infect Dis. 2017;23(3):517.	Nyunt MH, Shein T, Zaw NN, Han SS, Muh F, Lee S-K, et al.	antimalarial-yes, K13 information-no, clinical study/invivo study-yes, Asia-yes, other-no
153	33319728	Case Report: The First Case of Genotypically Confirmed K13 Propeller Mutation in Sri Lanka and Its Implications on the Elimination Status of Malaria. Am J Trop Med Hyg. 2020 Dec;104(3):964–7.	Fernando D, Weerasekera CJ, Gunasekera WMKT de AW, Hapuarachchi HC, Koo C, Munas M, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-yes, Asia-no, other-imported case
154	32679084	Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study. Lancet Infect Dis. 2020 Jul 14;	Imwong M, Dhorda M, Myo Tun K, Thu AM, Phyo AP, Proux S, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-samples already included from other studies
155	33060063	Triple Artemisinin-Based Combination Therapies for Malaria - A New Paradigm? Trends Parasitol. 2021 Jan;37(1):15–24.	van der Pluijm RW, Amaratunga C, Dhorda M, Dondorp AM.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-review
156	33139275	Transmission of Artemisinin-Resistant Malaria Parasites to Mosquitoes under Antimalarial	Witmer K, Dahalan FA, Delves MJ, Yahiya S, Watson OJ, Straschil U, et al.	antimalarial-yes, K13 information-yes, clinical

		Drug Pressure. Antimicrob Agents Chemother. 2020 Dec;65(1).		study/invivo study-no, Asia-no, other-review
157	33271239	In vitro reduction of *Plasmodium falciparum* gametocytes: Artemisia spp. tea infusions vs. artemisinin. J Ethnopharmacol. 2021 Mar;268:113638.	Snider D, Weathers PJ.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-yes, other-no
158	33370279	Genetic background and PfKelch13 affect artemisinin susceptibility of PfCoronin mutants in *Plasmodium falciparum*. PLoS Genet. 2020 Dec;16(12):e1009266.	Sharma AI, Shin SH, Bopp S, Volkman SK, Hartl DL, Wirth DF.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-no
159	33690638	In vitro growth competition experiments that suggest consequences of the substandard artemisinin epidemic that may be accelerating drug resistance in P. falciparum malaria. PLoS One. 2021;16(3):e0248057.	Hassett MR, Roepe PD.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-no
160	28289248	Antimalarial Drug Resistance: A Threat to Malaria Elimination. Cold Spring Harb Perspect Med. 2017 Jul;7(7).	Menard D, Dondorp A.	antimalarial-no, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
161	28187990	How to Contain Artemisinin- and Multidrug-Resistant Falciparum Malaria. Trends Parasitol. 2017 May;33(5):353–63.	Dondorp AM, Smithuis FM, Woodrow C, Seidlein L von.	antimalarial-no, K13 information-no, clinical study/invivo study-no, Asia-no, other-no
162	33764971	Novel anti-malarial drug strategies to prevent artemisinin partner drug resistance: A model-based analysis. PLoS Comput Biol. 2021 Mar;17(3):e1008850.	Kunkel A, White M, Piola P.	antimalarial-yes, K13 information-no, clinical study/invivo study-no, Asia-no, other-modelling studyy
163	33197753	Evidence for linkage of pfmdr1, pfcrt, and pfk13 polymorphisms to lumefantrine and mefloquine susceptibilities in a *Plasmodium falciparum* cross. Int J Parasitol	Windle ST, Lane KD, Gadalla NB, Liu A, Mu J, Caleon RL, et al.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no,

		Drugs drug Resist. 2020 Dec;14:208–17.		Asia-no, other-genetic cross study
164	31563454	Importance of kelch 13 C580Y mutation in the studies of artemisinin resistance in *Plasmodium falciparum* in Greater Mekong Subregion. J Microbiol Immunol Infect. 2020 Oct;53(5):676–81.	Zaw MT, Lin Z, Emran NA.	antimalarial-yes, K13 information-yes, clinical study/invivo study-no, Asia-yes, other-no

Chapter 3

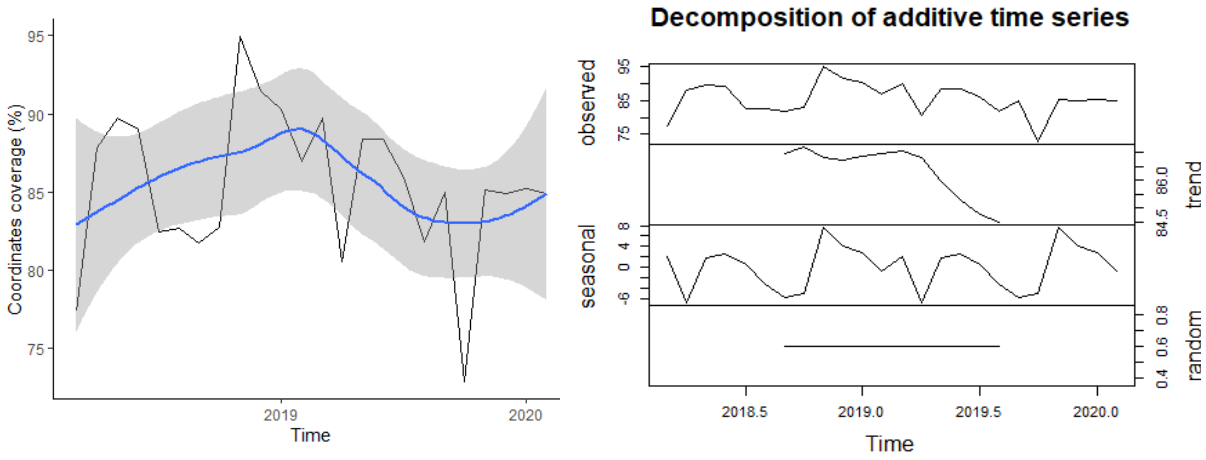
Figure S 10: Data flow and linkage for notified malaria cases and case investigation reports on drug adherence and response.



A chart showing data flow from the DHIS2 (including clinical data of malaria notifications and tested samples captured by NMC and case investigation data captured on case investigation forms) and molecular laboratory data. Case investigation reports on drug adherence and response were only available from January 2019 when home follow-up for adherence and response was initiated. (MMR=Molecular markers of resistance results, mRDT=malaria rapid diagnostic test and P.f = Plasmodium falciparum)

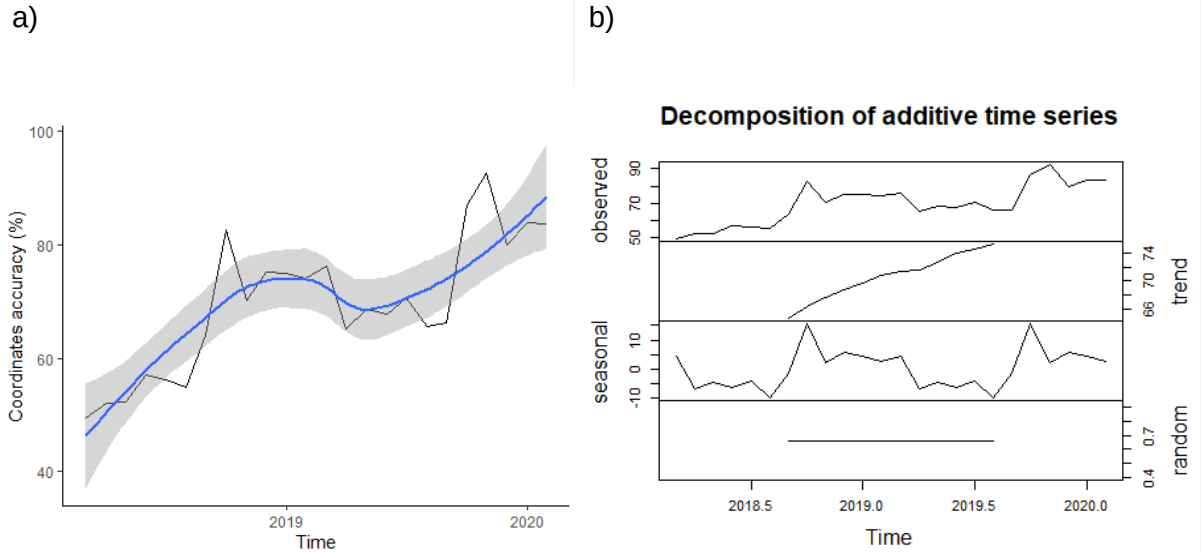
Figure S 11: GPS coordinates' coverage trend over the two-year study period (2018-2020)

a) b)



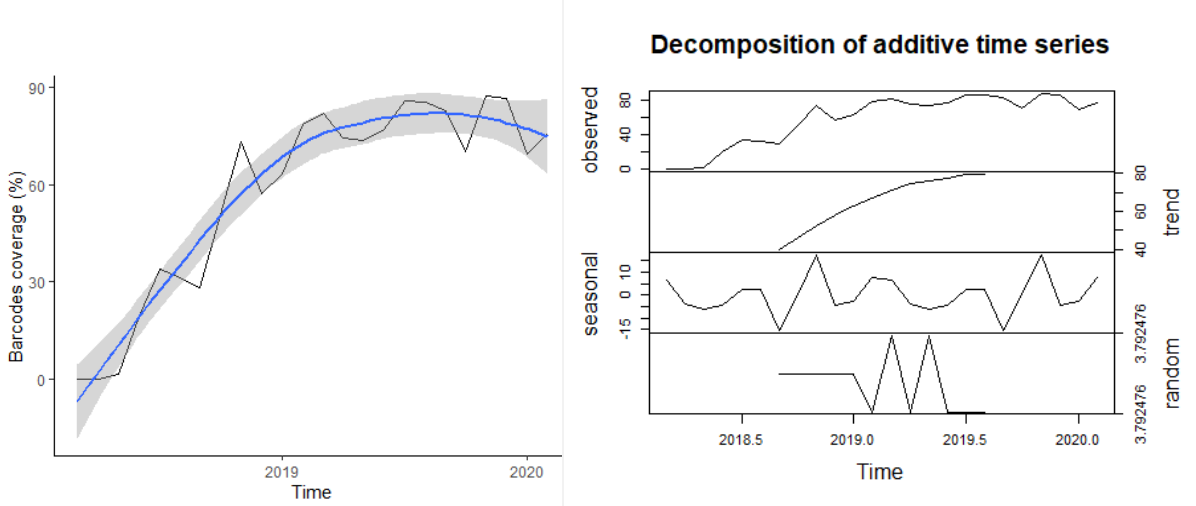
a) a plot of mean coverage of coordinates, with 95% confidence intervals b) A decomposed time series of cases. The series was investigated with residential coordinates recorded over the two-year study period and accounted for the effect of random errors. The coverage had stationarity at 88% from 2018 until the second quarter of 2019, when it dropped to 85%.

Figure S 12: Accuracy trend of malaria case residential coordinates collected over the two-year study period (2018-2020)



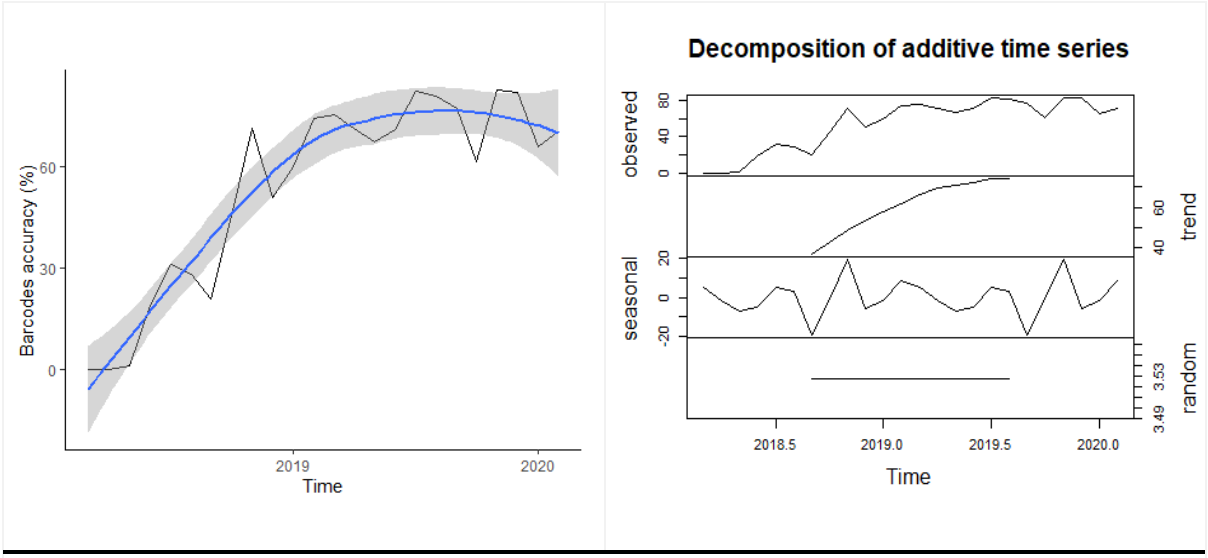
a) Chart showing the trend for coordinate accuracy, with 95% confidence intervals, and b)a decomposed time series over the two-year study period. Accuracy ranged 48% (95%CI: 39 - 56%) at baseline to 89% (95% CI: 80 - 98%) by the first quarter of 2020 (Fig. S3. After accounting for the effect of random errors, there was a 1.4% (95% CI: 0.9 – 1.9 %) average increment in accuracy every month for two years (p≤ 0.001)

Figure S 13: Percentage of mRDTs barcoded over the two-year study period (2018-2020)



a) Chart showing the trend of barcode coverage and its 95% confidence interval, and b) a decomposed time series for two years. After accounting for the effect of random errors in the exploratory analysis, the coverage increased steadily throughout the two years of the study, with an average increment of 4.6% (95% CI: 2.2 – 4. 7%, p < 0.0001) per month.

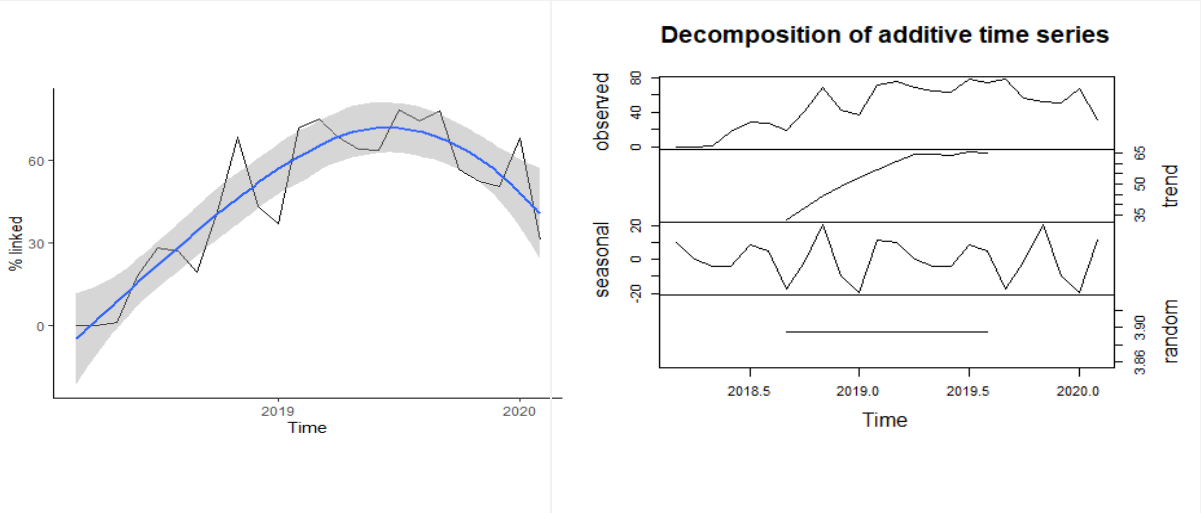
Figure S 14: Barcode accuracy trend over the two-year study period (2018-2020)



Figures showing a) the trend of barcode accuracy and its 95% confidence interval for two years, and b) its decomposed time series. After accounting for the effect of random errors, the accuracy increased steadily throughout with a 4.3% (2.1– 4.5 %) average increment per month ($p < 0.0001$).

Figure S 15: Linkage of the patients' and antimalarial resistance data over the two-year study period (2018-2020)

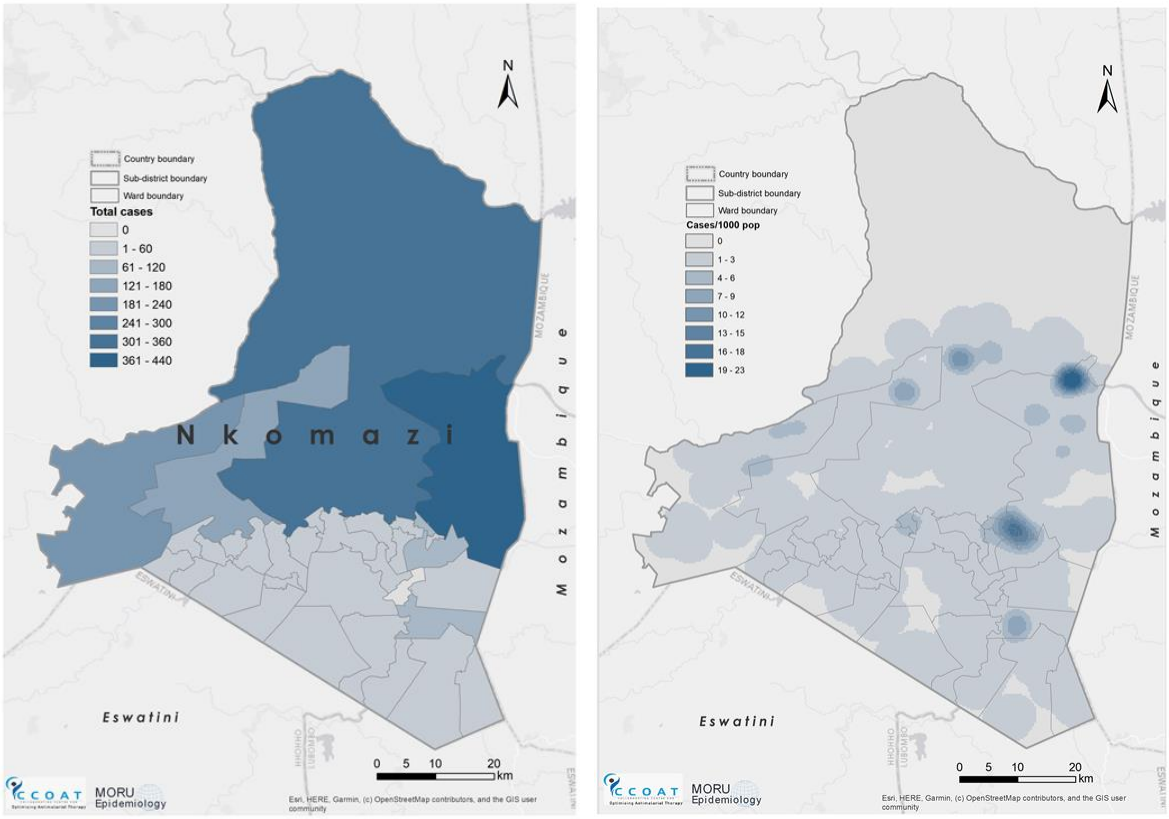
In an exploratory analysis, after accounting for the effect of random errors, the linkage ability increased with a 3.1% (1.2– 4.1 %) average increment in linkage per month (p<0.0001) over the two-year study period until the 1st quarter of 2019 but plateaued after that (Fig. S6).



Figures showing a) the trend of *data linkage, and its 95% confidence interval for two years, and b) its decomposition time series. after accounting for the effect of random errors, the linkage ability increased with a 3.1% (1.2– 4.1 %) average increment in linkage per month (p<0.0001) over the two-year study period until the 1st quarter of 2019 but plateaued after that (Fig. S6)*

Figure S 16: Two different types of shapefiles evaluated for the study area

a) b)



Malaria case distribution maps evaluated for the study area; a) a thematic map of distribution of malaria cases by ward b) a 0.5 X 0.5 density map of cases. The latter was selected as suitable for further mapping malarial cases and drug resistance as a background shapefile.

Tool S 2: GPS tools for training malaria case investigators

1. Tool S2 a: A training guide for trainers

Section 1: Introduction

Strengthening surveillance activities towards accurate and comprehensive data and reporting is central in achieving malaria elimination. Apart from enhancing monitoring and planning activities, improving the quality of malaria surveillance immensely helps in prioritisation of resources. The 2016 – 2030 WHO strategy recommends surveillance to be adopted in the health system as a core intervention. Programmes need to focus resources in training their surveillance teams to be able to keep up with current requirements and the use of new technologies such as tablets and GPS machines. Coupled with the knowledge of the work flow of data through different health information systems such as DHIS2 (District Health Information Systems II) surveillance personnel with supervisory roles are gate-keepers and provide a major link between field data collection and programme management.

This training is therefore aiming at empowering surveillance case investigators in good and well-informed practice to perform case investigation using GPS devices.

This training should be carried out in three parts. A theoretical part should be completed in class first, with the aim of achieving the learning goals below and the second part should be a supervised field practical. The third part is for visualisation of the coordinates collected during the field practical, discussion and feedback sharing. This last part should also address the transcription and copying errors.

Tasks for the trainers/supervisors

This training manual assumes the environment health officers and information officers to be fluent with case investigation, data flow and reporting, therefore be able to train the case investigators regularly to ensure high quality of data.

Learning goals

1. Each case investigator to understand the basic concepts in describing location and direction.
2. Each case investigator to understand the minimum requirements of case/household identifiers to be able to find a case's household.
3. Each case investigator to be fully knowledgeable in understanding how to calibrate a GPS device, obtain coordinates and troubleshoot frequent location queries.
4. Each case investigator to be fluent in the information required, and be able to complete location information, in the case investigation form and the rationale of each input.
5. Each case investigator to be able to independently capture and copy down location correctly and understand how coordinates are visualised.

Materials

Functioning and powered GPS devices and/or tablets, scratch book, pens and papers.

Standard Operating Procedures for GPS and/or tablets.

Flip card or PowerPoint (PPT) presentation (attached) can be used to summarise the important information.

Procedure

Part 1 – Basic concepts in telling and capturing location using GPS devices or tablets (15 – 20 mins)

Identify the level of knowledge:

You can assess the knowledge gap by asking the team to respond to a prepared survey to understand how to customise your training. However, if that is time consuming you can use a consensus approach as below.

Begin by allowing case investigators to explain to you their role and field activities. Ask one or two to fully describe the procedure and ask if there any other alternatives to performing case investigation. Building on their view and addressing the gaps, use the PPT to guide you through the process.

Allow time for a discussion by asking few questions or responding to questions after every 2-3 slides.

Go through the PPT and discuss on the basics of describing location and direction. Use relevant examples to probe participants to describe and give directions of arriving at a place e.g. the training venue, nearby hospital or any famous structure or farm.

The next part will be going through the SOPs, spending between 20 – 30 mins for Garmin eTrex SOP reading, operating the device and discussion. Use the icons listed in each instruction to help guide the participants to arrive at the right setting. In groups of 2 -3 participants, allow 5 – 10 minutes for each group to individually go through the process again and discuss any challenges.

Use similar time for the chosen android application (e.g. *'GPS Essentials')* and follow the same procedure as above.

Part 2A - Writing coordinates (10 mins discussion)

By using the case investigation fields, discuss with case investigators on how the coordinates, direction and address information should be completed.

Part 2B - Field data collection (30 mins exercise)

Ask case investigators to go to the nearby well-known structures and record the addresses and coordinates on a paper.

Write your findings as below	
Name of the building	……
Country it is located	……
City it is located	……
Locality it is located	……
Location	Longitude……Latitude……
Any comments?	……

Part 3 – Coordinates' recording and visualisation (30 mins)

Ask case investigators to swap their copied locations

Create an excel sheet and list the places as below.

Participant/Group number	Structur's name	Country	City	Locality	Longitude	Latitude	Comments
1							
2							
3							
……							

Use Google Maps to visualise these coordinates;

- Open Google Maps on your computer
- Select 'your locations'
- Select 'maps'
- Select 'create maps'
- Select 'add layer'
- Select 'upload file' (here you will upload your excel sheet) and your coordinates will appear on the Google Maps
- Zoom in to show the participants and probe if their locations have been captured correctly

Assessment method

You can send a post training survey (paper- or online based tool) to assess the previous identified knowledge gap and see if the learning goals have been achieved. You can also ask the participants the degree of satisfaction with the training and obtain their feedback for future use.

Alternatively, you can use the consensus approach for a general feedback to see if they have understood. Project the learning objectives slide and see how much they agree by show of hands to each learning goal.

Other supportive information

2. Tool S1B: Standard operating procedures for eTrex 10 GPS device (adapted from Health Geolab Collaborative, 2018) [280].

A. Plan your day:

Turn your GPS machine on and off to make sure there is enough battery for the day.

B. At your facility, identify the cases you will be investigating for the day and plan your site visits

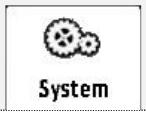
C. When you arrive at the site for obtaining the GPS coordinates, first, identify and write down the name or address (stand/street number/ward/subdistrict), then, follow through the procedures below;

3. Find an **open space** and turn the GPS device on.

4. Click twice on the **menu** button on the left of the device to get to the menu page.

5. Scroll down the menu page using the thumbstick, until the "**Setup**" icon is highlighted. Next, press the thumbstick to access the Setup sub-menu.

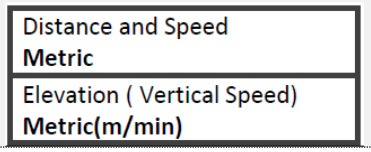
6. Scroll down the menu page using the thumbstick, until the "System" icon is highlighted. Next, press the thumbstick to access the **System** sub-menu.



8. Press the **back** button on the right of the device to return to the Setup sub-menu.

9. Use the thumbstick again to arrive at the "**Units**" icon is highlighted. Next, press the thumbstick to access the Units submenu

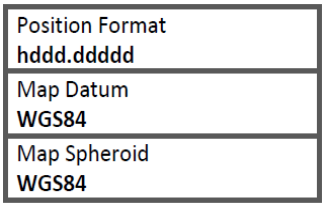
10. Set both the "**Distance and Speed**" as well as "**Elevation**" items to the metric system as shown here:



11. Click on the **back** button on the right of the device to return to the Setup sub-menu.

12. Use the thumbstick again until the "**Position Format**" icon is highlighted. Next, press the thumbstick to access the Units sub-menu.

13. Set the "**Position Format**," "**Map Datum**," and "**Map Spheroid**" as presented here:

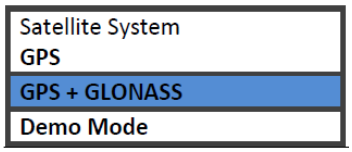


14. Click on the **back** button twice to return to the menu page

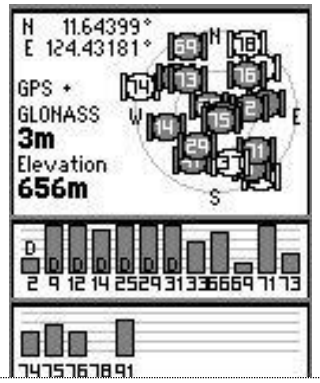
15. Hold the GPS device horizontally in front of you, use the thumbstick to scroll down the menu page until the "**Satellite**" icon is highlighted:

7. Under "Satellite System" highlight the **"GPS+GLONASS"** option and press the thumbstick to save the selection.

This will allow you to use both the GPS and GLONASS constellations of satellites as shown here

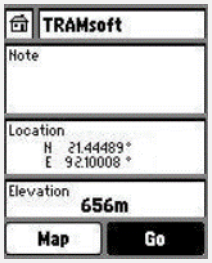


14. Next, press the thumbstick to access the "**Satellite page**" which looks as follows:



17. Once the accuracy value is below 15 metres with at least 4 satellite signals, **temporarily write down** the number of satellite signals and the accuracy measure on a piece of paper.

18. Mark the waypoint by pressing and holding the thumbstick. This will take you to the "**Mark Waypoint**" page as seen here:



19. If this is not the case, this means that the coordinates fall outside the ranges. **Recheck** the units and position format of the device (see steps 7 to 11 above).

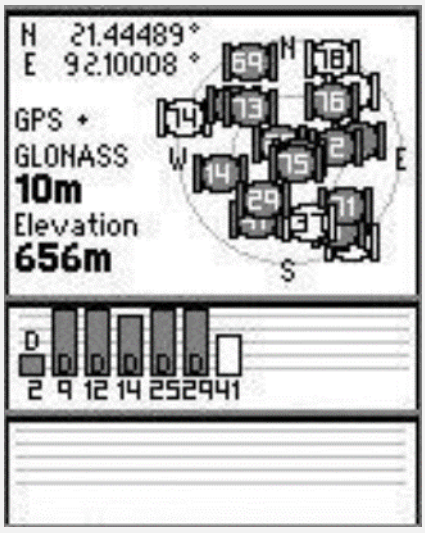
20. **Note** the final number of satellite signals and accuracy to make sure there is a minimum of 4 satellites and the accuracy is within 15m.

21. Record the **coordinates** with 5 decimals in the case investigation form.

e.g. *Latitude (decimal degrees)*
Longitude (decimal degrees)

15. Wait for the accuracy value to become less than **15 metres** with at least **4 satellite signals** received (number of grey bars at the bottom of the page). Stay for at least one minute at the same spot to allow for the best reading possible.

In the example shown here, the accuracy is 10 m with 6 satellite signals received (the transparent bar is not counted).



16. If there is **no fixing** of the satellite signal, make sure you are under open sky and take a few (2 or 3) steps and repeat step 15

- 2 5 . 4 7 9 0 8

0 3 0 . 9 7 2 9 4

22. **Check**. Go back through the form and complete any missing fields.

23. Move to the next household and start again from step A. IMPORTANT: Turn off the GPS device by holding down the '**on/off'** button until you are at the next point to be collected.

Tool S2 c: Standard operating procedures for GPS-Essentials android application

Instructions for downloading, obtaining & recording coordinates using the 'GPS Essentials' App on an Android Mobile Phone or Tablet

Shortened version:

i. Download 'GPS Essentials' App from 'Google Play Store'

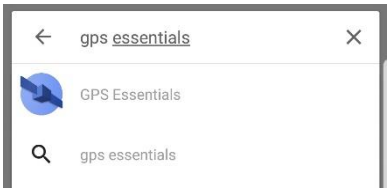
ii. Customise the settings to; "Units" – meters (SI), "Position Datum" – World Geodetic System 1984, "Position format" – Decimal

iii. Open "Satellites", the App will display coordinates, satellites and accuracy.

iv. Select "Dashboard" and add items you want to be displayed every time you are collecting coordinates.

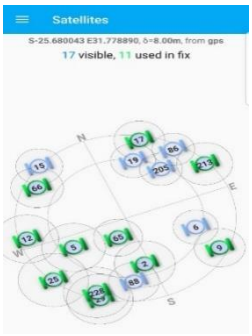
Extended version:

1. To download this App you will need;
   a. An internet connection through WIFI or using data via your mobile subscriber.
   b. A 'Play Store account', you can proceed with steps below if you already have an account. If you do not have it, open 'Google Play Store' and follow instructions on registering an account or proceed with the instructions below if you already have an account to download applications.
2. In the Play Store, click on the search window and type 'GPS Essentials'

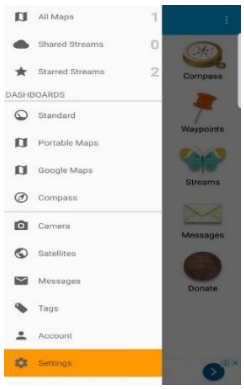


3. Select the App and choose 'INSTALL' for it to be installed in your advice.
4. If you get a warning "Cannot download App from unknown sources", go to settings>>allow downloads from unknown resources>>select to it to allow (on).
5. Wait for a few minutes, the App will download and install automatically. After the installation select 'OPEN' to have the app displayed in your tablet/phone. It will show the App menu as below.

6. Select “Satellites” and coordinates, precision and number of satellites will be displayed as below in two lines



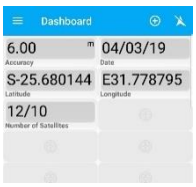
7. Select the 3 lines on the left side corner to open the sliding submenu and scroll down to “Settings”.



8. In settings, scroll down to “**Presentation**” and select;
    a. **Units** and set this to ***Meters (SI)***
    b. **Position Datum** and set it to ***World Geodetic System 1984***
    c. **Position format** and set it to ***Decimal***
9. Click the back arrow to get back to the main Menu and select **“Dashboard”**. Here we will add a dashboard to display our readings as follows: select the plus **‘+’** item on the right upper side of the screen to open the dashboard submenu with the items to select.
    a. Accuracy
    b. Date
    c. Latitude
    d. Longitude
    e. Number of Satellites

Note on the left side the colour highlights on the left side when an item gets selected. Then choose ‘close’ to end the submenu window

10. Your dashboard will be ready with the five items to display always when collecting coordinates.
11. When you arrive at the point for collecting coordinates. Open the ‘GPS Essentials’ and select dashboard to get the reading. Record the coordinates on your paper forms.



Guide S 1: Treatment adherence and response form

Mpumalanga Department of Health
Malaria Elimination Programm

health
MPUMALANGA PROVINCE
REPUBLIC OF SOUTH AFRICA

Malaria Case Investigation Report:
Treatment adherence and response

Patient Name:_____ Patient phone number:_____

Case Number:_____ Notifying Health Facility:_____

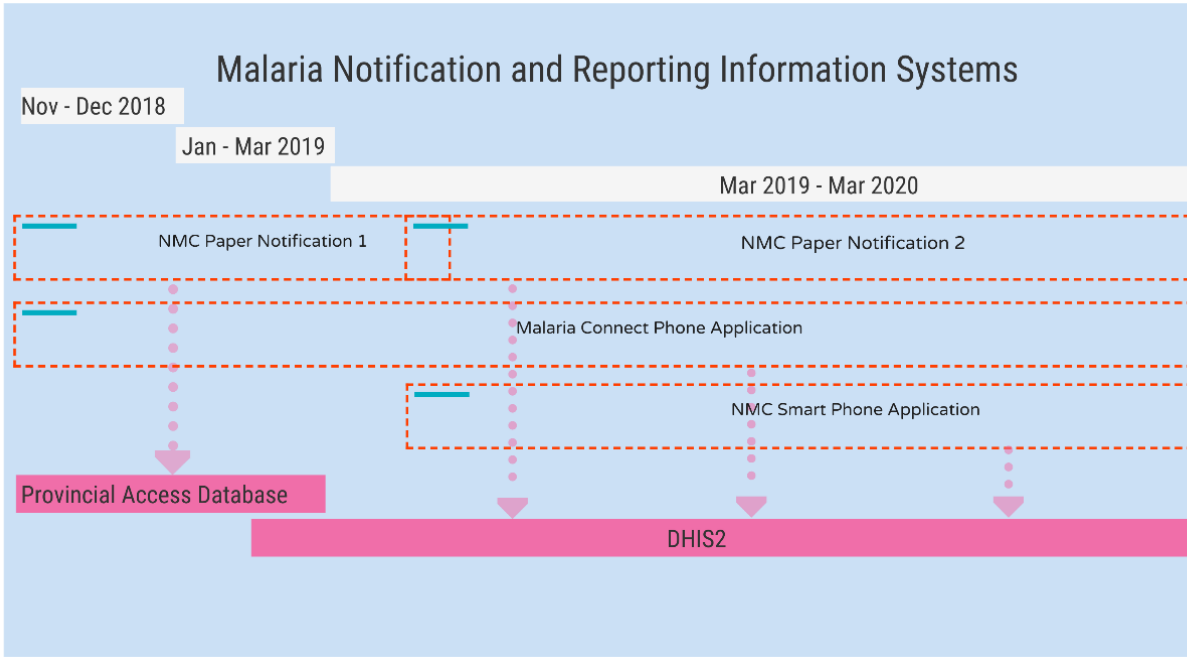
Case detection: Active ☐ Passive ☐ Contact: Home visit ☐ Telephonic ☐ Untraceable ☐

SECTION G: MALARIA TREATMENT QUESTIONS TO PATIENT			
1. Did you (the patient / your child) receive malaria treatment?		Yes ☐	No ☐
If YES: Date treatment started?	20YY/MM/DD		
2. What malaria treatment were you given at the health facility? (Check all treatments given)	Yellow tablets (Coartem®) ☐	A pink tablet (Primaquine) ☐	
	An injection ☐	A drip ☐	
Other treatment (please specify / describe):			
3. How did you (the patient, your child) feel after the malaria treatment?			
Fully Recovered ☐	Still ill? ☐	Died ☐	
If still ill:			
What symptoms?			
Was the patient sent to hospital? Yes ☐ No ☐	*If YES,* Hospital Name:		
4. For each malaria treatment you were given to take at home:			
4a) *For the Coartem® (yellow tablets):* How many tablets were you given? _____ Usually taken with: food ☐/ milk ☐/ water ☐/ nothing ☐ How many tablets do you have left?_____	4b) *For the other malaria treatment:* How many tablets were you given? _____ tablets Usually taken with: food ☐/ milk ☐/ water ☐/ nothing ☐ How many tablets do you have left?_____ tablets		
5. Did you (the patient / your child) experience any problems with the malaria treatment?		Yes ☐	No ☐
If YES, please describe what symptoms / problems were experienced:			
If YES, what was the outcome?			
Fully Recovered ☐	Ongoing ☐	Disability ☐	Died ☐
If ongoing, was the patient sent to hospital? *If YES:*	Hospital Name:		

Please mark applicable areas with an X

Case Investigator Name: _____

Figure S 17: Malaria Notification Systems



An illustration showing the different malaria notification systems involved during the study on integrating molecular markers of resistance into routine malaria notification system.

Data collection tools

Data Collection Instrument 1: SS4ME project survey:
This is a survey tool to collect primary healthcare staff's experience on additional activities introduced during SS4ME on integrating molecular markers of resistance into the routine malaria notification system.

Guide S 2: Health workers' survey (SS4ME Project)

SS4ME project survey			
Clinic name		**Clinic ID (if known)**	
Your job title		**Today's date**	__/_____/___ Dd/month/year

WE APPRECIATE YOUR HELP WITH THIS SURVEY ABOUT THE SS4ME PROJECT

THE QUESTIONS SHOULD TAKE ABOUT 30 MINUTES OF YOUR TIME

THERE ARE NO WRONG ANSWERS!

Please read this background to the survey first

There are parts of the world where malaria parasites have developed resistance to all malaria treatments.

So far, the treatments recommended here work well, but we need to improve our systems to make sure we are able to detect and contain any resistance that occurs.

For the SS4ME project we collected positive and negative malaria RDTs from your clinic/hospital and sent them to a laboratory in Johannesburg (NICD). We also asked staff to collect and send a filter paper sample from any left-over blood instead of wiping it away with a webcol/cotton wool.

The lab is now testing the RDTs and blood spots to see if there was any resistance to Co-Artem®

1	**How was your experience of labelling individual RDTs?**	**Mark ONE of the following 4 answers:**	
		This is not part of my work	☐
		It is easy for me to do	☐
		It is sometimes difficult for me to do	☐
		It is difficult for me to do	☐
Please briefly explain your answer to question 1 here if you would like:			
2	**How was your experience of labelling individual filter paper samples (blood spots)?**	**Mark ONE of the following 4 answers:**	
		This is not part of my work	☐
		It is easy for me to do	☐
		It is sometimes difficult for me to do	☐

		It is difficult for me to do	☐
Please briefly explain your answer to question 2 here if you would like:			
3	**How was your experience of putting positive RDTs into individual packets for collection by the malaria programme and negatives RDTs in a different bag?**	**Mark ONE of the following 4 answers:**	
		This is not part of my work	☐
		It is easy for me to do	☐
		It is sometimes difficult for me to do	☐
		It is difficult for me to do	☐
Please briefly explain your answer to question 3 here if you would like:			
4	**How was your experience of putting filter paper blood spots into packets for collection by the malaria programme?**	**Mark ONE of the following 4 answers:**	
		This is not part of my work	☐
		It is easy for me to do	☐
		It is sometimes difficult for me to do	☐
		It is difficult for me to do	☐
Please briefly explain your answer to question 4 here if you would like:			
5	**Which tools for malaria case notification have you used in the last 6 months?**	**Check all that apply:**	
		Malaria	☐
		Malaria Connect	☐
		NMC Phone app	☐
		Other (please name): _____	☐
6	**How was your experience of using the paper NMC form to notify malaria cases?**	**Mark ONE of the following 4 answers:**	
		This is not part of my work	☐
		It is easy for me to use	☐
		It is sometimes difficult for me to use	☐
		It is difficult for me to use	☐
Please briefly explain your answer to question 5 here if you would like:			
7	**What was the effect of SS4ME on your work?**	**Mark ONE of the following 3 answers:**	
		It had no effect	☐
			☐

		It affected my work in a negative way (i.e. caused me problems with my work) It affected my work in a positive way	☐
Please briefly explain your answer to question 7 here if you would like:			
8	**Do you think the SS4ME project is useful?**	**Mark ONE of the following 3 answers:**	
		Yes No Don't know	☐ ☐ ☐
Please briefly explain your answer to question 8 here if you would like:			
9	**What should we do differently if we wanted to ask a new clinic to start SS4ME?**	**Mark ONE of the following 2 answers:**	
		Keep things as they are Change things	☐ ☐
Please briefly explain your answer to question 9 here if you would like:			
10	**The department of health introduced a single low dose of primaquine to clinics in the last year. Please let us know how this has affected your work**	**Mark ONE of the following 3 answers:**	
		It had no effect It affected my work in a negative way (i.e. caused me problems with my work) It affected my work in a positive way	☐ ☐ ☐
Please briefly explain your answer to question 10 here if you would like:			
Finally	**Please write any other comments or suggestions that you can think of for improving malaria notifications in this area if you would like**		
THANK YOU FOR YOUR HELP			

Data Collection Instrument 2:

Guide S 3: A focus group discussion guide (SS4ME Project)

This is a semi-structured interview guide for the focus group discussions to collect experience on integrating molecular markers of resistance into routine malaria notification system.

FGD IDNO |__|__|__|__| **Facilitator Initials** |__|__|__| **Note-taker Initials** |__|__|__|
Audio file: |__|__|__| **Date** |__|__/__|__/__|__|
Introduction
I am _____ from _____ (facilitator)
I am _____ from _____ (note-taker)

- ✓ Ask group to introduce themselves using first names
- ✓ Capture demographic details – using first name for discussion
- ✓ Explain general purpose of the FGDs:

  To understand the experiences of participants with malaria diagnosis, treatment and notification and their ideas about a new system
- ✓ Aims of the discussion and expected duration (1 hour)
- ✓ Who is involved in the process (other participants)
- ✓ Why the participants' cooperation is important
- ✓ What will happen with the collected information and how the participant/target group will benefit
- ✓ Ask group to define their own ground rules, for example:
  - Only one person talks at a time.
  - It is important for us to hear everyone's ideas and opinions. There are no right or wrong answers to questions – just ideas, experiences and opinions, which are all valuable.
  - It is important for us to hear all sides of an issue – the positive and the negative.
  - Confidentiality is assured. "What is shared in the room stays in the room."
- ✓ Any questions?
- ✓ Check position and functioning of tape recorder
- ✓ Check for everyone's consent to participate and be recorded
- ✓ Refreshments will be served after the discussion

Domain	Topic and probes
Roles	As a reminder, briefly, what does your job involve? Why is this important?
Adding resistance data to notifications	There are parts of the world where malaria parasites have developed resistance to all treatments available. So far the treatments recommended here still seem to work very well, but we need to improve our surveillance systems to make sure we detect and contain any drug resistance that may occur here. The SS4ME project involved linking malaria notifications with molecular markers of antimalarial drug resistance obtained from RDT samples and blood spots sent to the NICD. We then can put the results on maps with

	GIS coordinates of where the RDTs were taken, where people live and sometimes where people had travelled from (if different). In time this will allow us to predict areas of drug resistant parasites which may guide where more work is needed to contain resistance and, for instance, change treatment policy. Let's talk about your experience of this project. (Probe: were you aware of the project? What role did you play?) We would first like your advice on whether we need to refine the practical processes • For instance, we asked [you][clinic staff] to label used RDTs and blood spots on filter paper to dab blood remaining after finger prick. These were collected and stored at clinics for despatch to the NICD - What was your experience with this, if at all? Probe: labelling, bagging, labelling bags, logs, transfer to NICD (what will happen if the project coordinator is not available to help with some aspects of this, e.g. logs?) • We then linked the malaria notifications for each patient with his or her positive RDT resistance data results from the NICD by using the Notifiable Medical Conditioner Identifier (NMC ID) - What do you think of this? Probe: are there other identifiers that could be used instead or as well? • What other systems have been in place for malaria notifications in the past 6 months in your facility/area? Probe: MalariaConnect®, NMC App. How was your work affected by these systems? Duplication, missing data? During the project in the same area (Nkomazi sub-district), the department of health introduced a single low dose of a new (unregistered) drug called primaquine to reduce the transmission of malaria within the community and therefore increase the chance of malaria elimination. Clinics were asked to dose patients with uncomplicated malaria (with a few restrictions) with PQ and add a record of this dosing to the notification forms [and for case investigations an additional page 3 to assess treatment response] • How did this impact you in your work? Probe: malaria notifications/ case investigations
Maps (MEP staff only – show maps)	Parasite resistance to malaria treatment has spread in eight countries in Asia and in the past resistance has previously transferred from Asia to Africa. • Do you think we should be concerned? Why? Probe: if no, what level of resistance would give rise to concern? As part of monitoring and early warning systems, maps of antimalarial resistance are being used to monitor spread. • How useful are maps for monitoring drug resistance? • What type of maps, and information in them, would you prefer? Probe use of different colours for cases, resistance etc. Here is a set of drug resistance maps produced from the Nkomazi linked data; • Which of the formats do you find easier to understand? Why? • Would you like any changes on this format to improve it? Probe: change of colours, symbols, titles, captions, or administrative units/localities

	Here is another set of maps produced from another area in the world • If this was South Africa, what is your impression of the data? Probe: what steps might be needed on seeing these data?
Overall utility and impact	• What do you think are the benefits of SS4ME? And the challenges? • What do you think are the benefits of introducing SLD PQ? And the challenges? • Is this project of linking notifications and drug resistance data worthwhile to your work? Probe: can you see any value for your patients? [MEP programme - value for identifying foci for investigation] • How best can this model be scaled up? Probe: if we were to introduce this to Bushbuckridge or Mbombela, what would you advise? • MEP staff only: do you think the SS4ME project is the right one to generate information that will help you and others to do your role?
Closing We are now approaching the end of our discussion. Is there anything else anyone would like to add? ✓ Summarise ✓ Thank participants Collect participant demographic details	

Guide S 4: An in-depth interview guide for key informants (SS4ME Project)

A semi-structured guide for the key informant interviews to collect experience on integrating molecular markers of resistance into routine malaria notification system.

IDI IDNO |__|__|__|__| **Facilitator Initials** |__|__|__| **Note-taker Initials** |__|__|__|
Audio file: |__|__|__| **Date** |__|__/__|__/__|__|

Introduction
I am _____ from _____

- ✓ General purpose of the study
- ✓ Aims of the interview and expected duration
- ✓ Who is involved in the process (other participants)
- ✓ Why the participant's cooperation is important
- ✓ What will happen with the collected information and how the participant/target group will benefit
- ✓ Any questions?
- ✓ Consent

Warm up [demographic & work history]
Can I ask some details about you and your job?
Job title _____
Years worked in this role |__|__|yrs|__|__|mths
Highest educational grade attained ___ __ Year of graduation_____
Are you originally from this area? □ Yes □ No

Domain	Topic and probes
Role	As a reminder, briefly, what does your job involve? Why is this important?
Linking resistance data to notifications	There are parts of the world where malaria parasites have developed resistance to all treatments currently available. So far treatments recommended here still seem to work very well, but we need to improve our surveillance systems to make sure we can promptly detect and contain any drug resistance that may occur here. The SS4ME project involved linking data from malaria notifications with molecular markers of antimalarial drug resistance from RDT samples and blood spots by the NICD. Data were then mapped according to GIS coordinates of sampling, residence (and suspected location of infection, if different). This feeds into a geo-statistical model, yielding a predictive map of estimated prevalence of drug resistant parasites showing levels of uncertainty, to inform drug resistance containment efforts and – if needed – changes in malaria treatment policy. Let's talk about your experience of this project. (Probe: were you aware of the project? What role did you play?) We would first like your advice on whether we need to refine the practical processes • For instance, we asked clinic staff to label RDTs and blood spots on filter paper to dab blood remaining after finger prick which were collected and stored at clinics for despatch to the NICD - What was your experience with this, if at all? Probe: labelling, bagging, labelling bags, logs, transfer to NICD (what will happen if the project coordinator is not available to help with some aspects of this, such as the logs?)

	• We then linked the notification data for each patient with his or her positive RDT using the Notifiable Medical Conditioner Identifier (NMC ID) - What do you think of this? Probe: are there other identifiers that could be used instead or as well? • What other systems are in place for malaria notifications? Probe: MalariaConnect®, NMC App. How was the project affected by these systems and vice versa? Duplication, missing data? • During the project in the same area (Nkomazi sub-district), the department of health introduced a single low dose of a new (unregistered) drug called primaquine to reduce the transmission of malaria within the community and therefore increase the chance of malaria elimination. This involved reporting details of patients treated with PQ (notification forms) and for case investigations an additional page 3 to assess treatment response. - How do you think this impacted the project and the routine malaria case management/case investigation system? Probes: how did introducing PQ affect notifications? How did introducing PQ affect case investigations?
Maps – show maps	Resistance to artemisinins has spread in eight countries in Asia. Drug resistance has previously transferred from Asia to Africa, and malaria partner drugs (like lumefantrine) have already started showing tolerance in Africa. • Do you think we should be concerned? Why? Probe: if no, what level of resistance would give rise to concern? As part of monitoring and early warning systems, maps of antimalarial resistance showing the distribution of drug resistance are being used to monitor geographical spread of artemisinin and lumefantrine resistance. • How useful are maps for monitoring drug resistance? • What type of maps, and information in them, would you prefer? • [general discussion on colours for cases, resistance etc.] Here is a set of drug resistance maps produced from the Nkomazi linked data; • Which of the formats do you find easier to understand? Why? • Would you like any changes on this format to improve it? Probe: change of colours, symbols, titles, captions, or administrative units/localities Here is another set of maps produced from another area in the world • If this was South Africa, what is your impression of the data? Probe: what steps might be needed on seeing these data?
Overall utility and impact	Overall, do you think the SS4ME project is fit-for-purpose to generate information that will help you and others to do your role? • What were the benefits of SS4ME? And the challenges? • What were the benefits of introducing SLD PQ? And the challenges? • Are the outputs worthwhile? Probe: opportunity costs, for identifying foci for investigation • How sustainable is this project? Probe: how best can this model be scaled up • Overall, what is the utility of this model for supporting changes to malaria treatment policy
Closing	

We are now approaching the end of our discussion. Is there anything else you would like to add?

- ✓ Summarise
- ✓ Thank participant

Tool S 3: A Process-oriented logic model for assessing the integration of early warning interventions in existing surveillance system

Below are table structured summaries displaying the process-oriented logic framework, including processes, inputs, outputs, and outcomes for evaluating the integration of molecular markers of resistance into a routine malaria notification system.

Tool S3 A: Illustration of the process-oriented logic framework without data.

		Logic						
		Inputs/Activities	***Outputs***		***Outcomes***			
			Intended	Unintended	Unintended	Short-term	Long-term	Quotes/example
Processes	Process I, II, III …	Activity 1, 2, 3 …						Quote 1, 2, 3
	Process I, II, III …	Activity 1, 2, 3 …						Quote 1, 2, 3
	Process I, II, III …	Activity 1, 2, 3 …						Quote 1, 2, 3

Tool S3 B: The process-oriented logic framework with processes, inputs, outputs and selected quotes.

		Processes					
		Detection, notification and reporting	***Sample collection and reporting***	***Case investigation***	***Data capturing***	***Analysis and reporting***	***Others***
Inputs/Activities		Filling forms (additional contact information and address details)	Sample collection (dried blood spot collection and labelling) Assigning barcodes stickers	New location (GPS) collection SoPs	Barcode scanner Barcode scanning	Monthly downloads of data and analysis Monthly data reporting	Training Usage of devices Sample collection Meaning of MMR & surveillance in general
Outputs	*Intended*	completed demographics and patient tracking information	barcoded filter papers of blood samples for molecular marking	Increased GPS accuracy	Automatic capture of barcodes avoidance of transcription errors	Data quality assessment	trained staff on GPS devices, DBS sample collection and filling the notification forms informed staff on the meaning and importance of MMR and surveillance

	Unintended	Daunting start Increased workload Multiple reporting systems	increased workload poor packaging and filing of the sample details Lack of barcode assignment	Extra training sessions Sense of ownership of the project that triggered the agency Threatened working relationships Requirement for airtime	Inconsistent barcode assignment Manual input of barcodes Increased workload	increased workload due to the merging of the different three forms	Increased awareness of resistance Added confidence to staff Positive perception of the added tasks
Outcomes	*Short-term*	Resistance leads to a slow start Incomplete tracking information	Inadequate quality samples and data shipped to the central lab for molecular analysis	Increased accuracy of patients' location Motivated teams	Non-barcoded-samples Transcription errors Slow data-capturing process	quarterly reports of cases' notification, investigation and linkage Feedback of areas of improvement and activities for areas of improvement	Increased dedication to working with correct processes Contradictions (knowing vs not knowing staff, willing vs not-willing staff)
	Long-term	Acceptance and adapting Increased programmatic support and monitoring		Failure to track all patients	Increased linked cases at the household level Overall increase in willingness and uptake of the programme	Improvement of data quality, timeliness in reporting and willingness to carry out MMR-RMSS Increased linkage	Raised knowledge about malaria and drug resistance Raised quality of data and samples. Increased linkability

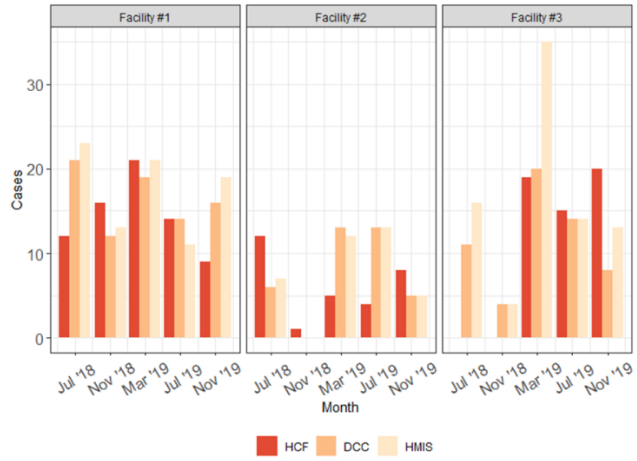
Quotes	'We do the paper first, the notification book first, the remaining depends with their time or workload'. [Nurse, IDI06]. 'But the volume you can look at the CHCs [Community Health Centres] and eight hour, there are more people coming in because day and night we are working.' [Nurse FGD03]. 'Basically at first it was quite a daunting task because now [I] remember we had facilities that recently introduced the new NMC form and also Malaria Connect and all of that. So when we went to train the health facilities they were resistant because they feel that it is extra work on their behalf. … Then even though they complied, we were getting a hint that they were not completely filling incorrectly the samples that we needed them to.' [Environmental Health Officer, FGD01].	'Yes, there is not enough (blood from one prick). There is a little but it is not enough. So most of the time they have to prick maybe twice or three times sometimes.' And added by another staff member 'I think the experience has taught us that mostly when the people are working in the farms to draw blood, sometimes they prick harder. So it is either they have to prick maybe two times or three times.' [Case investigator, FGD01]. 'These activities have helped us to know what we are doing and to help the patients, if they ask me now why we are taking another blood sample, it is for monitoring drug resistance and make sure you are cured. So it's a very good thing' [Nurse, FGD04]. 'remember we went with the supervisor to a couple of clinics when we	'We drive almost 40 kms or more to find a case, then the number is wrong and there is only one line for address. Just looking for one case you take the whole day while other notifications are waiting.' [Case investigator, FGD01] 'Sometimes I call a clinic before leaving home to know if there are cases near where I leave. And..travelling to Clinic easy closer to where I leave than the office, so I don't spend too much time on the road going back and forth' [Case investigation officer, FGD05]. 'Sometimes I call a clinic before leaving home to know if there are cases near where I leave. And..travelling to Clinic easy closer to where I	'When it is the rainy days and the cases increase, sometimes it's only two of us capturing data. Therefore, we would be late to capture… Late for a few days to one or two weeks.' [Data clerk, FGD01]. 'Typing is easier than using a barcode scanner'. [Data clerk, FGD01]. 'Yes, that are missing, maybe the facility code or anything like that then maybe three or four times we have experience that the RDT came alone like just the RDT without the filter paper, without a barcode. So most of the times we just discard because there is nothing we can do or go back and find this person and do the thing again' [Surveillance	'…what I do is to, I need to merge the NMC form together with the page three case investigation form and to take it outside the computer and merge even if it is excel it is prone to changing formats and that could introduce issues that could lead to un-matching within the DHIS2 framework.' [Information officer, KII03]. 'Once notifications were submitted and paper forms were captured in the HMIS system, ideally, all cases were merged and duplicates removed. In our analysis and feedback some cases had duplicates that required further cleaning.' [Information officer, KII03]. 'Ja I think another thing that is good our case investigation has also improved because I remember when we started our base line was thirty five percent. For forty eight hours and seventy two hours[time between case reporting and investigation] it was around forty eight percent. So now on third quarter we reported above sixty five which was even above the target we set for this financial year. I think with them making the follow ups, having to go to	'The ownership thing you know when you understand exactly what it is you are capturing and how important it is then it makes all the difference in terms of making sure it is on file because, how these things usually get presented it is a study and there is a lot of studies that we come across and it is always just another study that we do not know how it is going to end up, who it is going to benefit. So I think also that approach of you [referring to the study team] is excellent as we know what this is.. and what this is for. This is how important it is, it is ours only. [Information officer, FG02]

		identified that problem (sample lacking respective DBS or with insufficient DBS) and then we went back and told them that now we no longer just take the RDT. We also need to take the dry blood spots, so we need enough blood for the dry blood because sometimes we find that the dry blood is just a tiny bit of the blood and it really cannot be used. So we had to make sure that we go back to them and tell and train them that when you prick make sure you prick enough just in case it is a positive you can also get more blood. They are currently a lot better' [Nurse, FGD03].	leave than the office, so I don't spend too much time on the road going back and forth' [Case investigation officer, FGD05]. 'Yes, they do so (call) because in the notification book we also write our phone numbers when we notify a patient, if there is something missing they can call. Even if we are not on duty, yah, threatening our working relationship.' [Nurse, FGD03] 'The ownership thing you know when you understand exactly what it is you are capturing and how important it is then it makes all the difference in terms of making sure it is on file because, how these things usually get presented it is a study and there is a lot of studies that we come across	supervisor, FGD02]	the facilities to check on the stocks and everything has made them to also be involved in going there and investigating the cases because the thing was XXXX and XXXX [mentioning MMR-RMSS project staff] would be analysing and supporting with feedback e.g., saying no but the Primaquine the facility is this much and then we are not having the cases recorded. So ja I think our system improved with the Primaquine and the smart surveillance it has pushed them to go and investigate the cases within the required time.' [Supervisory staff, KII02]	

			and it is always just another study that we do not know how it is going to end up, who it is going to benefit. So I think also that approach of you [referring to the study team] is excellent as we know what this is.. and what this is for. This is how important it is, it is ours only. [Information officer, FG02]			

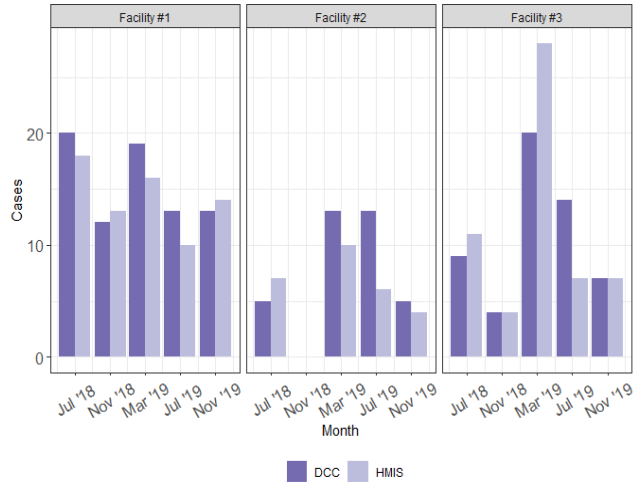
Figure S 18: Comparison of data from source (Health Care Facilities – HCF), Data Collection Centre and DHIS2

(a) NMC1 cases notified



This figures shows data from three HCFs. The case notification report counts matched in 40% (6/15) of the evaluations. Four of these six matching evaluations occurred in the latest two quarters (July and November 2019), which correlated with a period of training and supportive supervisory visits. At no quarter all three levels had similar counts. The two secondary levels had less data variability than their primary source health facility.

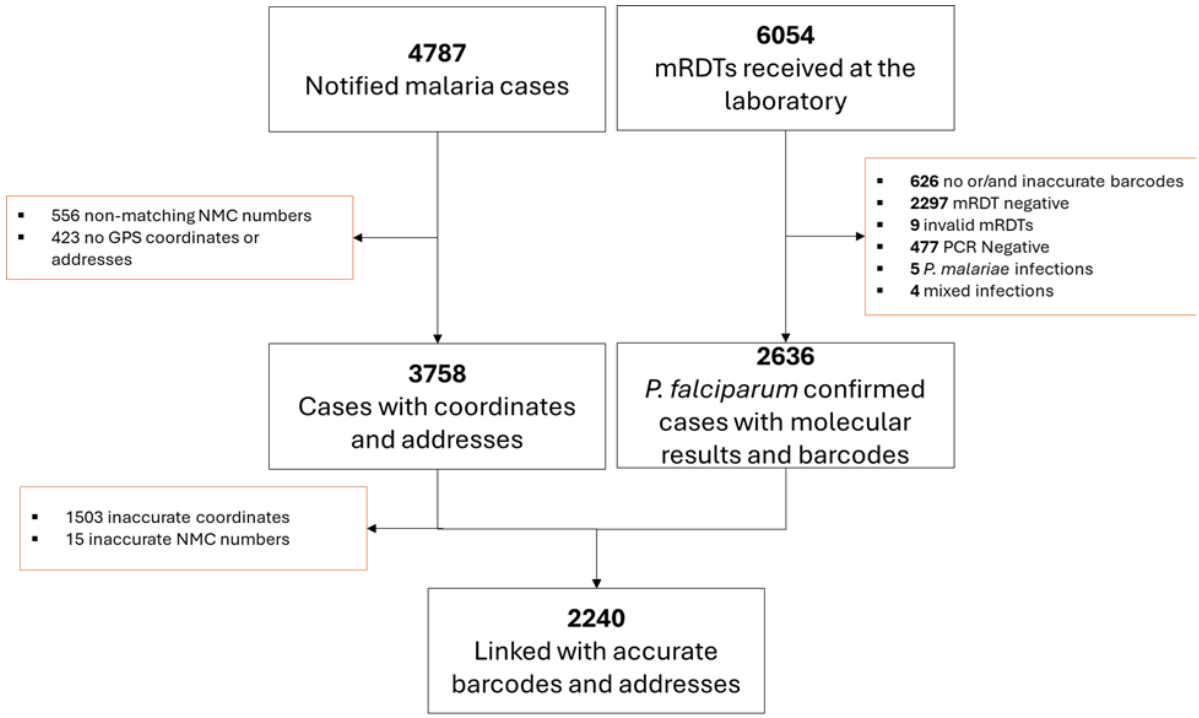
(b) Cases investigated



Case investigation report counts matched across two levels in three evaluations (n=15) in September – November 2018 & 2019 and April – July 2019. The data between the DCC and DHIS2 differed in most evaluations (86.7%; 13/16), with the third level (HIMS) having more data than the second level in the remaining 3 evaluations.

The comparison of data from source (Health Care Facilities – HCF), DCC and DHIS2 using three HCFs as the primary source. Malaria notifications were aggregated and compared in the different levels for every first month of the five quarters in Nkomazi, Mpumalanga South Africa.

Figure S 19: Flow chart of the notified malaria cases and laboratory data linkage from March 2018 to February 2020.

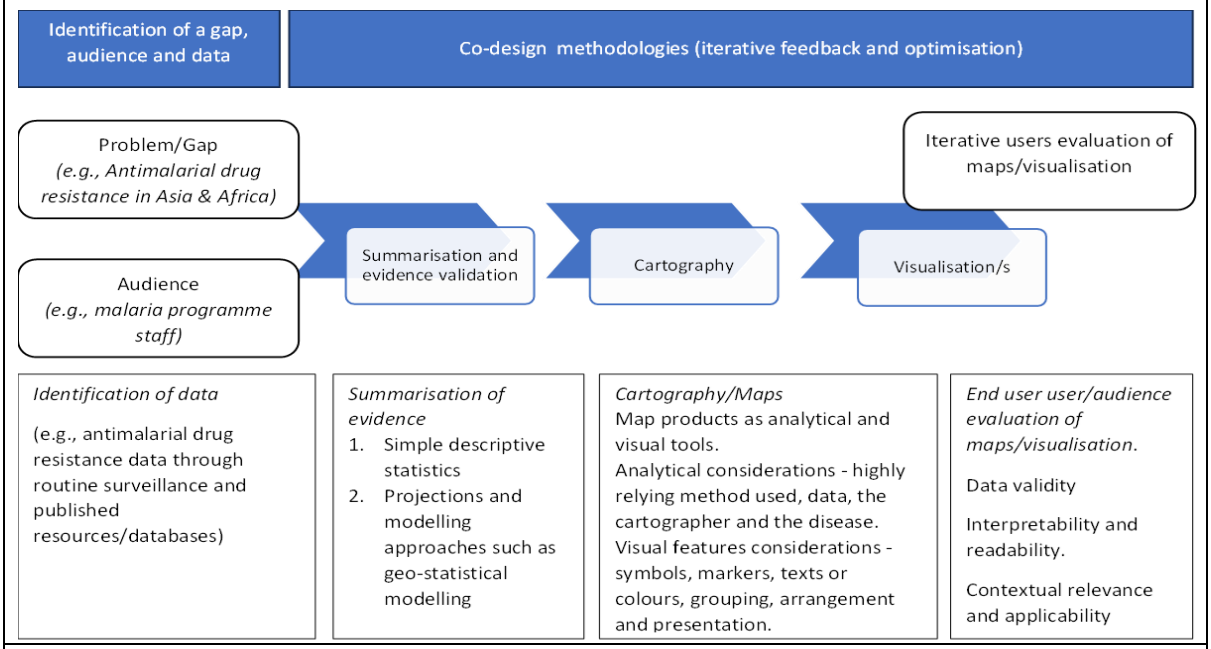


Malaria cases detected and laboratory data linkage data flow from March 2018 to February 2020 in Nkomazi sub-district, Mpumalanga. The notifiable medical condition (NMC) forms captured the data on malaria cases and later fed into the District Health Information System II (DHIS2). Laboratory samples included malaria rapid diagnostic tests (mRDTs) with their respective filter paper-dried blood spots, which were further analysed using PCR for confirmation and species detection.

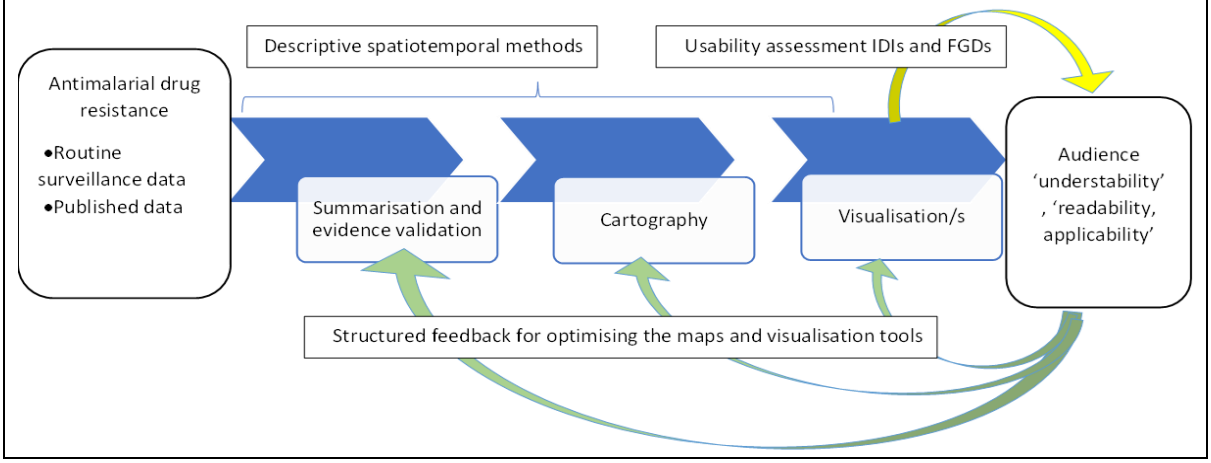
Chapter 5

Tool S 4: A framework for developing audience-specific maps and associated visualisations

a) Overall tool



b) Optimised tool for antimalarial drug resistance maps and associated visualisations



Ethics approvals

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone (021) 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

10 February 2020

HREC REF:030/2020

Prof K Barnes
Division of Clinical Pharmacology
K-Floor, OMB

Dear Prof Barnes

PROJECT TITLE: DEVELOPMENT AND OPTIMISATION OF PLASMODIUM FALCIPARUM ARTEMISININ AND PARTNER DRUGS RESISTANCE MAPS FOR COMMUNICATION TO POLICY MAKERS IN SOUTHERN AFRICA AND SOUTH-EAST ASIA (SUB-STUDY 519/2017) (PHD DEGREE - DR FRANK M KAGORO)

Thank you for your response letter dated 30 January 2020, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Franko Kagoro will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 030/2020sa

NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is n compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G 50 Old Main Building
Groote Schuur Hospital
Observatory 7925
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

15 January 2020

HREC REF: 698/2019

Prof Karen Barnes
Division of Pharmacology
K-Floor OMB GSH

Dear Prof Barnes

PROJECT TITLE: IMPROVEMENT OF ROUTINE DATA FOR DEVELOPING MAPS OF PLASMODIUM FALCIPARUM ARTEMISININ AND PARTNER DRUG RESISTANCE FOR MALARIA PROGRAMMES AND POLICYMAKERS (MAKING DATA MAP-WORTHY) (SUB-STUDY 519/2017) (PHD DEGREE - DR FRANK M. KAGORO)

Thank you for submitting your response to the concerns raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study subject.

Approval is granted for one year until the 30 January 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period. (Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledges that the student: Dr Frank M. Kagoro will also be involved in this study.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 698/2019
OL

NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, University Offices
Wellington Square, Oxford OX1 2JD
Tel: +44 (0)1865 (2)82106
E-mail: oxtrec@admin.ox.ac.uk

Dr. Frank Kagoro
3/F Santasiri Sornmani Bldg
Faculty of Tropical Medicine, Mahidol University
420/6 Ratchawithi Rd, Phaya Thai
Ratchathewi, Bangkok 10400

31 October 2018

Dear Dr. Kagoro

Full Title of Study: Optimisation of artemisinin resistance maps for communication to policymakers

OxTREC Reference: 553-18

Thank you for your email of the 25 October 2018, and for your minimal risk application form.

I am pleased to confirm that approval has now been granted for this study. This is valid for the first five years and is subject to receiving the local ethical approval (if this approval has not yet been received).

The documents approved for this study are as follows:

Documents:	Version:	Date:
Minimal risk application form		
ICF	V2.0	24/10/18
Questionnaire	V2.0	24/10/18

Any subsequent changes to the application must be submitted to the Committee as an Amendment. This should include a letter to give the reasons for the proposed modifications and all revised documents with changes tracked.

Please ensure that you submit a completed Annual Report form on every anniversary of this approval and a final End of Study Report. The relevant forms can be found on the OxTREC website: https://researchsupport.admin.ox.ac.uk/governance/ethics/applytoctrec

Finally, please note the following **important information**:

Data safety—all studies
It is the responsibility of the PI to ensure that all data collected during the course of the study is stored and transferred safely and securely. Further guidance and advice is available from the Research Data Team.

Studies that will involve storing human tissue samples in Oxford
As you are planning to import the samples into England, you will need to make arrangements

Tel: +44 (0)1865 (2)82106
Email: oxtrec@admin.ox.ac.uk
Web: https://researchsupport.admin.ox.ac.uk/governance/ethics

before the samples are transferred to store them under the governance of a Human Tissue Authority (HTA) licence. It is a legal requirement that any tissue or fluid made up of or containing human cells to be used for the purpose of research is stored on premises licensed by the HTA unless covered by an exemption. OxTREC approval is not a recognised exemption. Further information may be found on the University's human tissue governance web pages: https://researchsupport.admin.ox.ac.uk/governance/human-tissue.

Yours sincerely

Signed by candidate

Dr Rebecca Bryant
Research Ethics Manager, OxTREC

7 References

[1] Pongtavornpinyo W, Yeung S, Hastings IM, et al. Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. *Malar J* 2008; 7: 229.

[2] World Health Organization. *World malaria report 2023*. Geneva, https://cdn.who.int/media/docs/default-source/malaria/world-malaria-reports/world-malaria-report-2023-spreadview.pdf (2023).

[3] Delacollette C, D'Souza C, Christophel E, et al. Malaria trends and challenges in the Greater Mekong Subregion. *Southeast Asian J Trop Med Public Health* 2009; 40: 674–91.

[4] World Health Organization. *Eliminating malaria in the Greater Mekong Subregion: United to end a deadly disease*. Geneva, http://www.who.int/about/licensing/copyright_form/ (2016, accessed 27 February 2017).

[5] Manzoni G, Try R, Guintran JO, et al. Progress towards malaria elimination in the Greater Mekong Subregion: perspectives from the World Health Organization. *Malar J* 2024; 23: 1–16.

[6] Patrick Mansoni. Tropical Diseases. In: *A Manual of the Diseases of Warm Climates*. London: Cassell and Company, Limited, pp. 1–21.

[7] Snow RW, Amratia P, Kabaria CW, et al. The Changing Limits and Incidence of Malaria in Africa: 1939– 2009. In: *Advances in Parasitology*, pp. 169–262.

[8] World Health Organization. *Global technical strategy and targets for malaria 2016-2030*, http://apps.who.int/gb/ebwha/pdf_files/WHA68/A68_R2-en.pdf?ua=1 (2016, accessed 20 May 2019).

[9] Roll Back Malaria. *The Global Malarial Action Plan for A Malaria Free World*. Geneva, https://www.afro.who.int/sites/default/files/2017-06/Gmapfull.pdf (2008, accessed 7 January 2017).

[10] World Health Organization. *World Malaria Report 2009*. Geneva, https://www.who.int/publications-detail/world-malaria-report-2009 (2019, accessed 6 December 2019).

[11] World Health Organization. *Report on antimalarial drug efficacy, resistance and response 10 years of surveillance (2010 - 2019)*. Geneva, https://www.who.int/publications/i/item/9789240012813 (2020).

[12] Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 2014; 505: 50–55.

[13] Menard D, Ariey F. Towards real-time monitoring of artemisinin resistance. *The Lancet Infectious Diseases* 2015; 15: 367–368.

[14] Vachot-Ganée L, Khim N, Iannello A, et al. A novel field-based molecular assay to detect validated artemisinin-resistant k13 mutants. *Malar J* 2018; 17: 175.

[15] WWARN. Molecular Surveyor K13 Methodology. *Worldwide Antimalarial Resistance Network*, http://www.wwarn.org/tracking-resistance/molecular-surveyor-k13/molecular-surveyor-k13-methodology (2017, accessed 7 August 2017).

[16] Menard D, Ariey F. Towards real-time monitoring of artemisinin resistance. *The Lancet Infectious Diseases* 2015; 15: 367–368.

[17] Hay SI, Snow RW. The Malaria Atlas Project: Developing Global Maps of Malaria Risk. *PLoS Med* 2006; 3: e473.

[18] Omumbo JA, Noor AM, Fall IS, et al. How Well Are Malaria Maps Used to Design and Finance Malaria Control in Africa? *PLoS One* 2013; 8: e53198.

[19] Maude RJ, Mercado CEG, Rowley J, et al. Estimating malaria disease burden in the Asia-Pacific. *Wellcome Open Res* 2019; 4: 59.

[20] Barr-Walker J. Evidence-based information needs of public health workers: a systematized review. *J Med Libr Assoc* 2017; 105: 69–79.

[21] Silal SP, Little F, Barnes KI, et al. Towards malaria elimination in Mpumalanga, South Africa: a population-level mathematical modelling approach. *Malar J* 2014; 13: 297.

[22] Kraemer MUG, Hay SI, Pigott DM, et al. Progress and Challenges in Infectious Disease Cartography. *Trends Parasitol* 2016; 32: 19–29.

[23] Couclelis H, Gottsegen J. What maps mean to people: Denotation, connotation, and geographic visualization in land-use debates. pp. 151–162.

[24] Yu Q. Understanding the Impact of Culture on Interpretation: A Relevance Theoretic Perspective, http://web.uri.edu/iaics/files/Qiufen-YU.pdf (2014, accessed 11 August 2017).

[25] Ory J, Christophe S, Fabrikant SI, et al. How Do Map Readers Recognize a Topographic Mapping Style? *Cartogr J* 2015; 52: 193–203.

[26] Dondorp AM, Nosten F, Yi P, et al. Artemisinin Resistance in *Plasmodium falciparum* Malaria. *New England Journal of Medicine* 2009; 361: 455–467.

[27] Das S, Manna S, Saha B, et al. Novel pfkelch13 Gene Polymorphism Associates With Artemisinin Resistance in Eastern India. *Clin Infect Dis* 2019; 69: 1144–1152.

[28] Ménard D, Khim N, Beghain J, et al. A Worldwide Map of *Plasmodium falciparum* K13-Propeller Polymorphisms. *N Engl J Med* 2016; 374: 2453–2464.

[29] Rathore D, McCutchan TF, Sullivan M, et al. Antimalarial drugs: current status and new developments. *Expert Opin Investig Drugs* 2005; 14: 871–883.

[30] World Health Organization. *Emergency response to artemisinin resistance in the Greater Mekong subregion: regional framework for action 2013-2015*. Geneva, www.who.int (2013, accessed 22 May 2019).

[31] Ndwiga L, Kimenyi KM, Wamae K, et al. A review of the frequencies of *Plasmodium falciparum* Kelch 13 artemisinin resistance mutations in Africa. *Int J Parasitol Drugs Drug Resist* 2021; 16: 155–161.

[32] Nzoumbou-Boko R, Gildas Panté-Wockama C-B, Ngoagoni C, et al. Molecular assessment of kelch13 non-synonymous mutations in *Plasmodium falciparum* isolates from Central African Republic. *Malar J* 2020; 19: 191.

[33] Bwire GM, Ngasala B, Mikomangwa WP, et al. Detection of mutations associated with artemisinin resistance at k13-propeller gene and a near complete return of chloroquine susceptible falciparum malaria in Southeast of tanzania. *Sci Rep*; 10. Epub ahead of print 2020. DOI: 10.1038/s41598-020-60549-7.

[34] Uwimana A, Umulisa N, Venkatesan M, et al. Association of *Plasmodium falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. *Lancet Infect Dis* 2021; 21: 1120–1128.

[35] Anderson TJC, Nair S, McDew-White M, et al. Population Parameters Underlying an Ongoing Soft Sweep in Southeast Asian Malaria Parasites. *Mol Biol Evol* 2017; 34: 131–144.

[36] Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2014; 371: 411–423.

[37] Conrad MD, Asua V, Garg S, et al. Evolution of Partial Resistance to Artemisinins in Malaria Parasites in Uganda. *New England Journal of Medicine* 2023; 389: 722–732.

[38] Statistics South Africa. *South African census 2011, statistical release (Revised)*. Pretoria, www.statssa.gov.za (2012, accessed 15 July 2019).

[39] Sharp BL, le Sueur D. Malaria in South Africa--the past, the present and selected implications for the future. *S Afr Med J* 1996; 86: 83–9.

[40] Maharaj R, Morris N, Seocharan I, et al. The feasibility of malaria elimination in South Africa. *Malar J* 2012; 11: 423.

[41] South African National Department of Health. *National malaria statistics*. Pretoria, 2013.

[42] South Africa Government News. SA records over 7 400 malaria cases | SAnews. *News*, https://www.sanews.gov.za/south-africa/sa-records-over-7-400-malaria-cases (2023, accessed 5 April 2024).

[43] Maharaj R, Raman J, Morris N, et al. Epidemiology of malaria in South Africa: From control to elimination. *South African Medical Journal* 2013; 103: 779–783.

[44] Raman J, Morris N, Frean J, et al. Reviewing South Africa's malaria elimination strategy (2012–2018): progress, challenges and priorities. *Malar J* 2016; 15: 438.

[45] Balawanth R, Ba I, Qwabe B, et al. Assessing Kwa-Zulu-Natal's progress towards malaria elimination and its readiness for sub-national verification. *Malar J* 2019; 18: 108.

[46] National Department of Health South Africa. *Malaria Elimination Strategic Plan for South Africa 2019 - 2023*. Pretoria, https://www.nicd.ac.za/wp-content/uploads/2019/10/MALARIA-ELIMINATION-STRATEGIC-PLAN-FOR-SOUTH-AFRICA-2019-2023-MALARIA-ELIMINATION-STRATEGIC-PLAN-2019-2023.pdf (2019, accessed 18 February 2022).

[47] National Institute for Communicable Diseases (NICD). *National guidelines for the treatment of malaria, South Africa 2018 (Updated January 2019)*. Pretoria, https://www.nicd.ac.za/wp-content/uploads/2017/09/National_Guidelinesfor_the_Treatment_of_Malaria_update_June_2018.pdf (2019, accessed 20 October 2021).

[48] World Health Organization. *World malaria report 2020: 20 years of global progress and challenges*. Geneva, https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020 (2020, accessed 7 December 2020).

[49] Shretta R, Silal SP, Celhay OJ, et al. Malaria elimination transmission and costing in the Asia-Pacific: Developing an investment case. *Wellcome Open Res* 2019; 4: 60.

[50] Adjuik M, Babiker A, Garner P, et al. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 2004; 363: 9–17.

[51] Tran TH, Dolecek C, Pham PM, et al. Dihydroartemisinin-piperaquine against multidrug-resistant *Plasmodium falciparum* malaria in Vietnam: randomised clinical trial. *Lancet* 2004; 363: 18–22.

[52] World Health Organization. *Global Plan for Artemisinin Resistance Containment (GPARC)*. Geneva, http://apps.who.int/medicinedocs/en/m/abstract/Js18636en (2011, accessed 16 January 2020).

[53] Bai Y, Zhang J, Geng J, et al. Longitudinal surveillance of drug resistance in *Plasmodium falciparum* isolates from the China-Myanmar border reveals persistent circulation of multidrug resistant parasites. *Int J Parasitol Drugs Drug Resist* 2018; 8: 320–328.

[54] Prosser C, Meyer W, Ellis J, et al. Resistance screening and trend analysis of imported falciparum malaria in NSW, Australia (2010 to 2016). *PLoS One* 2018; 13: e0197369.

[55] Imwong M, Dhorda M, Myo Tun K, et al. Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study. *Lancet Infect Dis* 2020; 20: 1470–1480.

[56] Xu C, Wei Q, Yin K, et al. Surveillance of Antimalarial Resistance Pfcrt, Pfmdr1, and Pfkelch13 Polymorphisms in African *Plasmodium falciparum* imported to Shandong Province, China. *Sci Rep* 2018; 8: 12951.

[57] Imwong M, Suwannasin K, Kunasol C, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis* 2017; 17: 491–497.

[58] World Health Organization. *Artemisinin resistance and artemisinin-based combination therapy efficacy (Status report)*. Geneva, 2018.

[59] Worldwide Antimalarial Resistance Network (WWARN). Artemisinin Molecular Surveyor. *Surveyor*, http://www.wwarn.org/molecular/surveyor/k13/index.html?t=201608031200#0 (2021, accessed 21 August 2021).

[60] World Health Organization (WHO). Malaria Threat Map. *Maps*, https://apps.who.int/malaria/maps/threats/ (2019, accessed 26 August 2020).

[61] Worldwide Antimalarial Resistance Network (WWARN). Molecular Surveyor K13 Methodology. *Surveyor*, http://www.wwarn.org/tracking-resistance/molecular-surveyor-k13/molecular-surveyor-k13-methodology (2017, accessed 7 August 2017).

[62] O'Mara-Eves A, Thomas J, McNaught J, et al. Using text mining for study identification in systematic reviews: a systematic review of current approaches. *Syst Rev* 2015; 4: 5.

[63] Global Administrative Areas (GADM). Maps and data. *GADM Data*, https://gadm.org/index.html (2018, accessed 11 February 2019).

[64] WWARN K13 Genotype-Phenotype Study Group. Association of mutations in the *Plasmodium falciparum* Kelch13 gene (Pf3D7_1343700) with parasite clearance rates after artemisinin-based treatments-a WWARN individual patient data meta-analysis. *BMC Med*; 17. Epub ahead of print 2019. DOI: 10.1186/s12916-018-1207-3.

[65] Prabhakaran S. Loess Regression and Smoothing With R. *R Tutorial*, http://r-statistics.co/Loess-Regression-With-R.html (2016, accessed 13 July 2020).

[66] Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 2014; 505: 50–55.

[67] Noedl H, Se Y, Sriwichai S, et al. Artemisinin resistance in Cambodia: a clinical trial designed to address an emerging problem in Southeast Asia. *Clin Infect Dis*; 51. Epub ahead of print 1 December 2010. DOI: 10.1086/657120.

[68] Malaria Atlas Project (MAP). Trends in Global Malaria Burden, https://malariaatlas.org/trends/region/MAP/GLOBAL (2017, accessed 26 August 2020).

[69] Hamilton WL, Amato R, van der Pluijm RW, et al. Evolution and expansion of multidrug-resistant malaria in southeast Asia: a genomic epidemiology study. *Lancet Infect Dis* 2019; 19: 943–951.

[70] Imwong M, Hien TT, Thuy-Nhien NT, et al. Spread of a single multidrug resistant malaria parasite lineage (PfPailin) to Vietnam. *Lancet Infect Dis* 2017; 17: 1022–1023.

[71] Landier J, Parker DM, Thu AM, et al. Effect of generalised access to early diagnosis and treatment and targeted mass drug administration on *Plasmodium falciparum*

malaria in Eastern Myanmar: an observational study of a regional elimination programme. *Lancet* 2018; 391: 1916–1926.

[72] Spring MD, Lin JT, Manning JE, et al. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. *Lancet Infect Dis* 2015; 15: 683–691.

[73] Jacob CG, Thuy-Nhien N, Mayxay M, et al. Genetic surveillance in the Greater Mekong subregion and South Asia to support malaria control and elimination. *Elife*; 10. Epub ahead of print 2021. DOI: 10.7554/ELIFE.62997.

[74] World Health Organization. *World Malaria Report 2018*. Geneva, www.who.int/malaria (2018, accessed 8 May 2019).

[75] World Health Organization. *Global report on antimalarial efficacy and drug resistance: 2000-2010*. Geneva, https://www.who.int/publications/i/item/9789241500470 (2010, accessed 16 April 2024).

[76] World Health Organization. Guidelines for the treatment of Malaria - 3rd edition, https://iris.who.int/bitstream/handle/10665/162441/9789241549127_eng.pdf;jsessionid=6A8BD95D41D35B441DB453E032AE1905?sequence=1 (2015, accessed 16 April 2024).

[77] Baker L, Barnes K. New antimalarial treatment for KwaZulu-Natal. *South African Medical Journal* 2001; 91: 358–9.

[78] Ukpe IS, Moonasar D, Raman J, et al. Case management of malaria: treatment and chemoprophylaxis. *S Afr Med J* 2013; 103: 793–798.

[79] Djimdé AA, Fofana B, Sagara I, et al. Efficacy, Safety, and Selection of Molecular Markers of Drug Resistance by Two ACTs in Mali. *Am J Trop Med Hyg* 2008; 78: 455–461.

[80] Raman J, Little F, Roper C, et al. Five Years of Large-Scale dhfr and dhps Mutation Surveillance Following the Phased Implementation of Artesunate Plus Sulfadoxine-Pyrimethamine in Maputo Province, Southern Mozambique. *Am J Trop Med Hyg* 2010; 82: 788.

[81] Dondorp AM, Nosten F, Yi P, et al. Artemisinin Resistance in *Plasmodium falciparum* Malaria . *New England Journal of Medicine* 2009; 361: 455–467.

[82] Tun KM, Imwong M, Lwin KM, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet Infect Dis* 2015; 15: 415.

[83] Das S, Saha B, Hati AK, et al. Evidence of Artemisinin-Resistant *Plasmodium falciparum* Malaria in Eastern India . *New England Journal of Medicine* 2018; 379: 1962–1964.

[84] Lu F, Culleton R, Zhang M, et al. Emergence of Indigenous Artemisinin-Resistant *Plasmodium falciparum* in Africa. *New England Journal of Medicine* 2017; 376: 991–993.

[85] Sutherland CJ, Lansdell P, Sanders M, et al. pfk13-Independent Treatment Failure in Four Imported Cases of *Plasmodium falciparum* Malaria Treated with Artemether-Lumefantrine in the United Kingdom. *Antimicrob Agents Chemother*; 61. Epub ahead of print 1 March 2017. DOI: 10.1128/AAC.02382-16.

[86] Ikeda M, Kaneko M, Tachibana SI, et al. Artemisinin-Resistant *Plasmodium falciparum* with High Survival Rates, Uganda, 2014-2016. *Emerg Infect Dis* 2018; 24: 718–726.

[87] Taylor SM, Parobek CM, De Conti DK, et al. Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in Sub-Saharan Africa: a molecular epidemiologic study. *J Infect Dis* 2015; 211: 680–688.

[88] Southern African Development Community (SADC). *SADC Malaria Report 2017*. Gaborone, www.sadc.int (2017, accessed 17 April 2024).

[89] Craig MH, Kleinschmidt I, Nawn JB, et al. Exploring 30 years of malaria case data in KwaZulu-Natal, South Africa: part I. The impact of climatic factors. *Trop Med Int Health* 2004; 9: 1247–1257.

[90] World Health Organization. *Methods for surveillance of antimalarial drug efficacy*. Geneva, https://apps.who.int/iris/bitstream/handle/10665/44048/9789241597531_eng.pdf?sequence=1%0Ahttp://www.who.int/malaria/publications/atoz/9789241597531/en/ (2009, accessed 17 April 2024).

[91] Raman J, Mauff K, Muianga P, et al. Five Years of Antimalarial Resistance Marker Surveillance in Gaza Province, Mozambique, Following Artemisinin-Based Combination Therapy Roll Out. *PLoS One* 2011; 6: e25992.

[92] Méndez F, Muñoz Á, Carrasquilla G, et al. Determinants of treatment response to sulfadoxine-pyrimethamine and subsequent transmission potential in falciparum malaria. *Am J Epidemiol* 2002; 156: 230–238.

[93] Happi CT, Gbotosho GO, Folarin OA, et al. Polymorphisms in *Plasmodium falciparum* dhfr and dhps genes and age related in vivo sulfadoxine-pyrimethamine resistance in malaria-infected patients from Nigeria. *Acta Trop* 2005; 95: 183–193.

[94] Warhurst DC. A molecular marker for chloroquine-resistant falciparum malaria. *N Engl J Med* 2001; 344: 299–302.

[95] Department of Health SA. *Malaria Elimination Strategic Plan 2012-2108*, http://www.ccoat.uct.ac.za/sites/default/files/image_tool/images/229/SA Malaria Elimination Strategic Plan 2012.pdf (2012, accessed 15 July 2019).

[96] Brooke B, Koekemoer L, Kruger P, et al. Malaria vector control in South Africa. *S Afr Med J* 2013; 103: 784–788.

[97] Ngomane L, De Jager C. Changes in malaria morbidity and mortality in Mpumalanga Province, South Africa (2001-2009): a retrospective study. *Malar J*; 11. Epub ahead of print 2012. DOI: 10.1186/1475-2875-11-19.

[98] Silal SP, Little F, Barnes KI, et al. Towards malaria elimination in Mpumalanga, South Africa: a population-level mathematical modelling approach. *Malar J* 2014; 13: 297.

[99] Govere JM, La Grange JJ, Durrheim DN, et al. Sulfadoxine-pyrimethamine effectiveness against *Plasmodium falciparum* malaria in Mpumalanga Province, South Africa. *Trans R Soc Trop Med Hyg* 1999; 93: 644.

[100] Deacon HE, Freese JA, Sharp BL. Drug-resistant *Plasmodium falciparum* malaria in the eastern Transvaal. *S Afr Med J* 1994; 84: 394–395.

[101] Mabuza A, Govere J, Grange K la, et al. Therapeutic efficacy of sulfadoxine-pyrimethamine for *Plasmodium falciparum* malaria - PubMed. *The South African Medical Journal* 2005; 95: 346–9.

[102] Barnes KI, Durrheim DN, Little F, et al. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLoS Med* 2005; 2: e330.

[103] Wooden J, Kyes S, Sibley CH. PCR and strain identification in *Plasmodium falciparum*. *Parasitology Today* 1993; 9: 303–305.

[104] Mangold KA, Manson RU, Koay ESC, et al. Real-Time PCR for Detection and Identification of Plasmodium spp. *J Clin Microbiol* 2005; 43: 2435.

[105] Padley D, Moody AH, Chiodini PL, et al. Use of a rapid, single-round, multiplex PCR to detect malarial parasites and identify the species present. *Ann Trop Med Parasitol* 2003; 97: 131–7.

[106] Plowe C V., Cortese JF, Djimde A, et al. Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. *J Infect Dis* 1997; 176: 1590–1596.

[107] Sutherland CJ, Haustein T, Gadalla N, et al. Chloroquine-resistant *Plasmodium falciparum* infections among UK travellers returning with malaria after chloroquine prophylaxis. *J Antimicrob Chemother* 2007; 59: 1197–1199.

[108] Price RN, Uhlemann A-C, Brockman A, et al. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. *Lancet* 2004; 364: 438–447.

[109] Talundzic E, Chenet SM, Goldman IF, et al. Genetic Analysis and Species Specific Amplification of the Artemisinin Resistance-Associated Kelch Propeller Domain in P. falciparum and P. vivax. *PLoS One*; 10. Epub ahead of print 20 August 2015. DOI: 10.1371/JOURNAL.PONE.0136099.

[110] Global Administrative Areas (GADM). GADM Maps and Data. *Data*, https://gadm.org/index.html (2018, accessed 11 February 2019).

[111] Brewer CA, Pickle L. Evaluation of Methods for Classifying Epidemiological Data on Choropleth Maps in Series. *Annals of the Association of American Geographers* 2002; 92: 662–681.

[112] Harrower M, Brewer CA. ColorBrewer.org: An Online Tool for Selecting Colour Schemes for Maps. *Cartogr J* 2003; 40: 27–37.

[113] Okell LC, Reiter LM, Ebbe LS, et al. Emerging implications of policies on malaria treatment: genetic changes in the Pfmdr-1 gene affecting susceptibility to artemether–lumefantrine and artesunate–amodiaquine in Africa. *BMJ Glob Health* 2018; 3: e000999.

[114] Venkatesan M, Gadalla NB, Stepniewska K, et al. Polymorphisms in *Plasmodium falciparum* Chloroquine Resistance Transporter and Multidrug Resistance 1 Genes: Parasite Risk Factors That Affect Treatment Outcomes for P. falciparum Malaria After Artemether-Lumefantrine and Artesunate-Amodiaquine. *Am J Trop Med Hyg* 2014; 91: 833–843.

[115] Malmberg M, Ferreira PE, Tarning J, et al. *Plasmodium falciparum* Drug Resistance Phenotype as Assessed by Patient Antimalarial Drug Levels and Its Association With pfmdr1 Polymorphisms. *J Infect Dis* 2013; 207: 842.

[116] Kublin JG, Cortese JF, Njunju EM, et al. Reemergence of Chloroquine-Sensitive *Plasmodium falciparum* Malaria after Cessation of Chloroquine Use in Malawi. *J Infect Dis* 2003; 187: 1870–1875.

[117] Davlantes E, Dimbu PR, Ferreira CM, et al. Efficacy and safety of artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in three provinces in Angola, 2017. *Malar J*; 17. Epub ahead of print 3 April 2018. DOI: 10.1186/S12936-018-2290-9.

[118] Plucinski MM, Dimbu PR, Macaia AP, et al. Efficacy of artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisinin-piperaquine for treatment of uncomplicated *Plasmodium falciparum* malaria in Angola, 2015. *Malar J*; 16. Epub ahead of print 2 February 2017. DOI: 10.1186/S12936-017-1712-4.

[119] Raman J, Allen E, Workman L, et al. Safety and tolerability of single low-dose primaquine in a low-intensity transmission area in South Africa: an open-label, randomized controlled trial. *Malar J*; 18. Epub ahead of print 24 June 2019. DOI: 10.1186/S12936-019-2841-8.

[120] Vaughan-Williams CH, Raman J, Raswiswi E, et al. Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal: An observational cohort study. *Malar J* 2012; 11: 1–9.

[121] Artimovich E, Kapito-Tembo A, Pensulo P, et al. The effect of local variation in malaria transmission on the prevalence of sulfadoxine-pyrimethamine resistant haplotypes and selective sweep characteristics in Malawi. *Malar J* 2015; 14: 1–7.

[122] White NJ. Antimalarial drug resistance. *J Clin Invest* 2004; 113: 1084–1092.

[123] Ishengoma DS, Lwitiho S, Madebe RA, et al. Using rapid diagnostic tests as source of malaria parasite DNA for molecular analyses in the era of declining malaria prevalence. *Malar J* 2011; 10: 6.

[124] Papa Mze N, Ndiaye YD, Diedhiou CK, et al. RDTs as a source of DNA to study *Plasmodium falciparum* drug resistance in isolates from Senegal and the Comoros Islands. *Malar J* 2015; 14: 1–8.

[125] Ndiaye M, Sow D, Nag S, et al. Country-Wide Surveillance of Molecular Markers of Antimalarial Drug Resistance in Senegal by Use of Positive Malaria Rapid Diagnostic Tests. *Am J Trop Med Hyg* 2017; 97: 1593–1596.

[126] World Health Organization. *Malaria surveillance, monitoring and evaluation: a reference manual*. Geneva, https://iris.who.int/bitstream/handle/10665/272284/9789241565578-eng.pdf?ua=1 (2018, accessed 27 June 2019).

[127] World Health Organization. *World malaria report 2020: 20 years of global progress and challenges*. Geneva, 2020.

[128] World Health Organization. *World Malaria Report 2018*. Geneva, 2018.

[129] World Health Organization. *World malaria report 2021*. Geneva, 2021. Epub ahead of print 2021. DOI: Licence: CC BY-NC-SA 3.0 IGO.

[130] Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of Artemisinin Resistance in *Plasmodium falciparum* Malaria. *N Engl J Med* 2014; 371: 411–23.

[131] Tun KM, Imwong M, Lwin KM, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet Infect Dis* 2015; 15: 415–421.

[132] Feng J, Kong X, Xu D, et al. Investigation and Evaluation of Genetic Diversity of *Plasmodium falciparum* Kelch 13 Polymorphisms Imported From Southeast Asia and Africa in Southern China. *Front Public Health* 2019; 7: 95.

[133] Das S, Manna S, Saha B, et al. Novel pfkelch13 Gene Polymorphism Associates With Artemisinin Resistance in Eastern India. *Clin Infect Dis* 2019; 69: 1144–1152.

[134] Maharaj R, Raman J, Morris N, et al. Epidemiology of malaria in South Africa: From control to elimination. *South African Medical Journal* 2013; 103: 779–783.

[135] SADC Elimination 8. *SADC Malaria Elimination Eight Regional Surveillance Quarterly bulletin: Malaria incidence along E8 border districts*. Windhoek, Namibia, 2020.

[136] Ménard D, Khim N, Beghain J, et al. A Worldwide Map of *Plasmodium falciparum* K13-Propeller Polymorphisms. *New England Journal of Medicine* 2016; 374: 2453–2464.

[137] Bwire GM, Ngasala B, Mikomangwa WP, et al. Detection of mutations associated with artemisinin resistance at k13-propeller gene and a near complete return of chloroquine susceptible falciparum malaria in Southeast of tanzania. *Sci Rep*; 10. Epub ahead of print 2020. DOI: 10.1038/s41598-020-60549-7.

[138] Uwimana A, Legrand E, Stokes BH, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat Med* 2020; 26: 1602–1608.

[139] Pacheco MA, Kadakia ER, Chaudhary Z, et al. Evolution and Genetic Diversity of the k13 Gene Associated with Artemisinin Delayed Parasite Clearance in *Plasmodium falciparum*. *Antimicrob Agents Chemother*; 63. Epub ahead of print 2019. DOI: 10.1128/AAC.02550-18.

[140] Tola M, Ajibola O, Idowu ET, et al. Molecular detection of drug resistant polymorphisms in *Plasmodium falciparum* isolates from Southwest, Nigeria. *BMC Res Notes*; 13. Epub ahead of print 1 December 2020. DOI: 10.1186/s13104-020-05334-5.

[141] Uwimana A, Umulisa N, Venkatesan M, et al. Association of *Plasmodium falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. *Lancet Infect Dis* 2021; 21: 1120–1128.

[142] Balikagala B, Fukuda N, Ikeda M, et al. Evidence of Artemisinin-Resistant Malaria in Africa. *https://doi.org/101056/NEJMoa2101746* 2021; 385: 1163–1171.

[143] Global Malaria Programe. *WHO Malaria Policy Advisory Group (MPAG) meeting*. Geneva, 2021.

[144] Straimer J, Gnädig NF, Witkowski B, et al. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. *Science (1979)*; 347.

[145] Chenet SM, Akinyi Okoth S, Huber CS, et al. Independent Emergence of the *Plasmodium falciparum* Kelch Propeller Domain Mutant Allele C580Y in Guyana. *Journal of Infectious Diseases* 2016; 213: 1472–1475.

[146] Mathieu LC, Cox H, Early AM, et al. Local emergence in Amazonia of *Plasmodium falciparum* k13 C580Y mutants associated with in vitro artemisinin resistance. *Elife* 2020; 9: 1–21.

[147] Olivera MJ, Guerra AP, Cortes LJ, et al. Artemether–Lumefantrine Efficacy for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Choco, Colombia after 8 Years as First-Line Treatment. *Am J Trop Med Hyg* 2020; 102: 1056.

[148] Ndwiga L, Kimenyi KM, Wamae K, et al. A review of the frequencies of *Plasmodium falciparum* Kelch 13 artemisinin resistance mutations in Africa. *Int J Parasitol Drugs Drug Resist* 2021; 16: 155–161.

[149] World Health Organization. *A framework for malaria elimination*. Geneva, 2017.

[150] Huang F, Liu H, Yan H, et al. Antimalarial Drug Resistance Surveillance in China, 2016–2020. *China CDC Wkly* 2021; 3: 366.

[151] Sudathip P, Saejeng A, Khantikul N, et al. Progress and challenges of integrated drug efficacy surveillance for uncomplicated malaria in Thailand. *Malaria Journal 2021 20:1* 2021; 20: 1–16.

[152] World Health Organization. *Malaria surveillance, monitoring & evaluation: a reference manual*. Geneva, 2018.

[153] Kagoro FM, Barnes KI, Marsh K, et al. Mapping genetic markers of artemisinin resistance in *Plasmodium falciparum* malaria in Asia: a systematic review and spatiotemporal analysis. *Lancet Microbe*. Epub ahead of print January 2022. DOI: 10.1016/S2666-5247(21)00249-4.

[154] Raman J, Kagoro FM, Mabuza A, et al. Absence of kelch13 artemisinin resistance markers but strong selection for lumefantrine-tolerance molecular markers following 18 years of artemisinin-based combination therapy use in Mpumalanga Province, South Africa (2001-2018). *Malar J*; 18. Epub ahead of print 22 August 2019. DOI: 10.1186/s12936-019-2911-y.

[155] Ndiaye M, Sow D, Nag S, et al. Country-Wide Surveillance of Molecular Markers of Antimalarial Drug Resistance in Senegal by Use of Positive Malaria Rapid Diagnostic Tests. *Am J Trop Med Hyg* 2017; 97: 1593–1596.

[156] Jacob CG, Thuy-Nhien N, Mayxay M, et al. Genetic surveillance in the Greater Mekong subregion and South Asia to support malaria control and elimination. *Elife*; 10. Epub ahead of print 10 August 2021. DOI: 10.7554/ELIFE.62997.

[157] Snow RW, Marsh K, Le Sueur D. The Need for Maps of Transmission Intensity to Guide Malaria Control in Africa. *Parasitology Today*; 12.

[158] Mera maritza, Gonzalez Calorina LMD. Towards an Intelligent Decision Support System for Public Health Surveillance - A Qualitative Analysis of Information Needs. In: *Integrating Information Technology and Management for Quality of Care*. 2005, pp. 44–47.

[159] Carroll LN, Au AP, Todd Detwiler L, et al. Visualization and analytics tools for infectious disease epidemiology: A systematic review. *J Biomed Inform* 2014; 51: 287–298.

[160] NICD. *National guidelines for the treatment of malaria, South Africa 2018 (Updated January 2019)*. Pretoria, 2019.

[161] National Department of Health South Africa. *Malaria Elimination Strategic Plan for South Africa 2019 - 2023*. Pretoria, 2019.

[162] Libiseller C, Grimvall A. Performance of partial Mann–Kendall tests for trend detection in the presence of covariates. *Environmetrics* 2002; 13: 71–84.

[163] Silverman BW. Density estimation for statistics and data analysis. In: *Monographs on Statistics and Applied Probability*. Chapman and Hall, 1986, pp. 1–22.

[164] Statistics South Africa. *Census 2011, Statistical release (Revised)*. 2011.

[165] Sharp BL, le Sueur D. Malaria in South Africa--the past, the present and selected implications for the future. *S Afr Med J* 1996; 86: 83–9.

[166] Maharaj R, Morris N, Seocharan I, et al. The feasibility of malaria elimination in South Africa. *Malar J* 2012; 11: 423.

[167] Stepniewska K, Humphreys GS, Gonçalves BP, et al. Efficacy of single dose primaquine with artemisinin combination therapy on P. falciparum gametocytes

and transmission: A WWARN individual patient meta-analysis. *J Infect Dis*. Epub ahead of print 11 August 2020. DOI: 10.1093/INFDIS/JIAA498.

[168] Taylor WR, Naw HK, Maitland K, et al. Single low-dose primaquine for blocking transmission of *Plasmodium falciparum* malaria – a proposed model-derived age-based regimen for sub-Saharan Africa. *BMC Med*; 16. Epub ahead of print 18 January 2018. DOI: 10.1186/S12916-017-0990-6.

[169] Raman J, Allen E, Workman L, et al. Safety and tolerability of single low-dose primaquine in a low-intensity transmission area in South Africa: an open-label, randomized controlled trial. *Malar J*; 18. Epub ahead of print 24 June 2019. DOI: 10.1186/S12936-019-2841-8.

[170] Barnes K, Mabuza A, Frean PJ, et al. *Smart surveillance towards malaria elimination in Mpumalanga , South Africa (SS4ME): novel approaches for mapping antimalarial resistance (Protocol)*. 2017.

[171] Centre for Humanitarian Data. Humanitarian Data Exchange, https://data.humdata.org/ (2021, accessed 12 May 2021).

[172] Padley D, Moody AH, Chiodini PL, et al. Use of a rapid, single-round, multiplex PCR to detect malarial parasites and identify the species present. *Ann Trop Med Parasitol* 2003; 97: 131–7.

[173] Sutherland CJ, Haustein T, Gadalla N, et al. Chloroquine-resistant *Plasmodium falciparum* infections among UK travellers returning with malaria after chloroquine prophylaxis. *J Antimicrob Chemother* 2007; 59: 1197–1199.

[174] Price RN, Uhlemann A-C, Brockman A, et al. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. *Lancet* 2004; 364: 438–447.

[175] Talundzic E, Chenet SM, Goldman IF, et al. Genetic Analysis and Species Specific Amplification of the Artemisinin Resistance-Associated Kelch Propeller Domain in P. falciparum and P. vivax. *PLoS One*; 10. Epub ahead of print 20 August 2015. DOI: 10.1371/JOURNAL.PONE.0136099.

[176] Talundzic E, Okoth SA, Congpuong K, et al. Selection and Spread of Artemisinin-Resistant Alleles in Thailand Prior to the Global Artemisinin Resistance Containment Campaign. *PLoS Pathog* 2015; 11: e1004789.

[177] WWARN K13 Genotype-Phenotype Study Group. Association of mutations in the *Plasmodium falciparum* Kelch13 gene (Pf3D7_1343700) with parasite clearance rates after artemisinin-based treatments-a WWARN individual patient data meta-analysis. DOI: 10.1186/s12916-018-1207-3.

[178] Team R. stats-package: The R Stats Package.

[179] Pohlert T. Non-Parametric Trend Tests and Change-Point Detection [R package trend version 1.1.4].

[180] Shretta R, Liu J, Cotter C, et al. *Malaria Elimination and Eradication*. The International Bank for Reconstruction and Development / The World Bank, 2017. Epub ahead of print 3 November 2017. DOI: 10.1596/978-1-4648-0524-0/CH12.

[181] World Health Organization. *Report on antimalarial drug efficacy, resistance and response 10 years of surveillance (2010 - 2019)*. Geneva, 2020.

[182] Nsanzabana C, Djalle D, Guérin PJ, et al. Tools for surveillance of anti-malarial drug resistance: an assessment of the current landscape. *Malar J* 2018; 17: 75.

[183] Chen H, Hailey D, Wang N, et al. A Review of Data Quality Assessment Methods for Public Health Information Systems. *OPEN ACCESS Int J Environ Res Public Health* 2014; 11: 11.

[184] World Health Organization. Data Quality Review (DQR) Toolkit, https://www.who.int/healthinfo/tools_data_analysis/dqr_modules/en/ (accessed 14 January 2020).

[185] Nguyen TT, Nzigou Mombo B, Lalremruata A, et al. DNA recovery from archived RDTs for genetic characterization of *Plasmodium falciparum* in a routine setting in Lambaréné, Gabon. *Malar J*; 18. Epub ahead of print 2 October 2019. DOI: 10.1186/S12936-019-2972-Y.

[186] Morris U, Aydin-Schmidt B, Shakely D, et al. Rapid diagnostic tests for molecular surveillance of *Plasmodium falciparum* malaria -assessment of DNA extraction methods and field applicability. *Malar J* 2013; 12: 106.

[187] Ishengoma DS, Lwitiho S, Madebe RA, et al. Using rapid diagnostic tests as source of malaria parasite DNA for molecular analyses in the era of declining malaria prevalence. *Malar J* 2011; 10: 6.

[188] Cnops L, Boderie M, Gillet P, et al. Rapid diagnostic tests as a source of DNA for Plasmodium species-specific real-time PCR. *Malar J*; 10. Epub ahead of print 2011. DOI: 10.1186/1475-2875-10-67.

[189] Tessema SK, Raman J, Duffy CW, et al. Applying next-generation sequencing to track falciparum malaria in sub-Saharan Africa. *Malar J* 2019; 18: 1–9.

[190] Taylor SM, Parobek CM, Aragam N, et al. Pooled Deep Sequencing of *Plasmodium falciparum* Isolates: An Efficient and Scalable Tool to Quantify Prevailing Malaria Drug-Resistance Genotypes. *J Infect Dis* 2013; 208: 1998–2006.

[191] Morris U, Aydin-Schmidt B, Shakely D, et al. Rapid diagnostic tests for molecular surveillance of *Plasmodium falciparum* malaria -assessment of DNA extraction methods and field applicability. *Malar J* 2013; 12: 106.

[192] Diakité SAS, Traoré K, Sanogo I, et al. A comprehensive analysis of drug resistance molecular markers and *Plasmodium falciparum* genetic diversity in two malaria endemic sites in Mali. *Malar J* 2019; 18: 361.

[193] World Health Organization. Malaria Threat Map, https://apps.who.int/malaria/maps/threats/ (2019, accessed 26 August 2020).

[194] Baraka V, Mavoko HM, Nabasumba C, et al. Impact of treatment and re-treatment with artemether-lumefantrine and artesunate-amodiaquine on selection of *Plasmodium falciparum* multidrug resistance gene-1 polymorphisms in the Democratic Republic of Congo and Uganda. *PLoS One* 2018; 13: e0191922.

[195] Venkatesan M, Gadalla NB, Stepniewska K, et al. Polymorphisms in *Plasmodium falciparum* Chloroquine Resistance Transporter and Multidrug Resistance 1 Genes: Parasite Risk Factors That Affect Treatment Outcomes for P. falciparum Malaria After Artemether-Lumefantrine and Artesunate-Amodiaquine. *Am J Trop Med Hyg* 2014; 91: 833–843.

[196] Ljolje D, Dimbu PR, Kelley J, et al. Prevalence of molecular markers of artemisinin and lumefantrine resistance among patients with uncomplicated *Plasmodium falciparum* malaria in three provinces in Angola, 2015. *Malar J*; 17. Epub ahead of print 20 February 2018. DOI: 10.1186/S12936-018-2233-5.

[197] Davlantes E, Dimbu PR, Ferreira CM, et al. Efficacy and safety of artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in three provinces in Angola, 2017. *Malar J*; 17. Epub ahead of print 3 April 2018. DOI: 10.1186/S12936-018-2290-9.

[198] Gansané A, Moriarty LF, Ménard D, et al. Anti-malarial efficacy and resistance monitoring of artemether-lumefantrine and dihydroartemisinin-piperaquine shows inadequate efficacy in children in Burkina Faso, 2017–2018. *Malar J* 2021; 20: 48.

[199] Wu Y, Soe MT, Aung PL, et al. Efficacy of artemether-lumefantrine for treating uncomplicated *Plasmodium falciparum* cases and molecular surveillance of drug resistance genes in Western Myanmar. *Malar J*; 19. Epub ahead of print 27 August 2020. DOI: 10.1186/s12936-020-03376-5.

[200] Bretscher MT, Dahal P, Griffin J, et al. The duration of chemoprophylaxis against malaria after treatment with artesunate-amodiaquine and artemether-lumefantrine and the effects of pfmdr1 86Y and pfcrt 76T: a meta-analysis of individual patient data. *BMC Med*; 18. Epub ahead of print 25 February 2020. DOI: 10.1186/S12916-020-1494-3.

[201] Dimbu PR, Horth R, Cândido ALM, et al. Continued low efficacy of artemether-lumefantrine in Angola in 2019. *Antimicrob Agents Chemother*; 65. Epub ahead of print 1 February 2021. DOI: 10.1128/AAC.01949-20.

[202] Conrad MD, Rosenthal PJ. Antimalarial drug resistance in Africa: the calm before the storm? *Lancet Infect Dis* 2019; 19: e338–e351.

[203] Davlantes E, Dimbu PR, Ferreira CM, et al. Efficacy and safety of artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in three provinces in Angola, 2017. *Malar J*; 17. Epub ahead of print 3 April 2018. DOI: 10.1186/s12936-018-2290-9.

[204] Baloyi RE, Shandukani MB, Graffy R, et al. Evaluating a 24-h mobile reporting system for malaria notifications in comparison with a paper-based system in South Africa, 2015. *Malar J*; 17. Epub ahead of print 23 August 2018. DOI: 10.1186/S12936-018-2451-X.

[205] Githinji S, Oyando R, Malinga J, et al. Completeness of malaria indicator data reporting via the District Health Information Software 2 in Kenya, 2011-2015. *Malar J*; 16. Epub ahead of print 17 August 2017. DOI: 10.1186/s12936-017-1973-y.

[206] Gesicho MB, Were MC, Babic A. Data cleaning process for HIV-indicator data extracted from DHIS2 national reporting system: a case study of Kenya. *BMC Med Inform Decis Mak*; 20. Epub ahead of print 1 December 2020. DOI: 10.1186/s12911-020-01315-7.

[207] Bhattacharya AA, Umar N, Audu A, et al. Quality of routine facility data for monitoring priority maternal and newborn indicators in DHIS2: A case study from Gombe State, Nigeria. *PLoS One*; 14. Epub ahead of print 1 January 2019. DOI: 10.1371/journal.pone.0211265.

[208] World Health Organization. *World Malaria Report*. Geneva, 2016.

[209] Hotchkiss DR, Diana ML, Foreit KGF. How can routine health information systems improve health systems functioning in lowand middle-income countries? assessing the evidence base. *Adv Health Care Manag* 2012; 12: 25–58.

[210] World Health Organization. *World malaria report 2019*. Geneva, 2019.

[211] Hyde E, Bonds MH, Ihantamalala FA, et al. Estimating the local spatio-temporal distribution of malaria from routine health information systems in areas of low health care access and reporting. *Int J Health Geogr*; 20. Epub ahead of print 1 December 2021. DOI: 10.1186/s12942-021-00262-4.

[212] Hamre KES, Pierre B, Namuyinga R, et al. Establishing a National Molecular Surveillance Program for the Detection of *Plasmodium falciparum* Markers of Resistance to Antimalarial Drugs in Haiti. *Am J Trop Med Hyg* 2020; 103: 2217.

[213] Fontecha GA, Sanchez AL, Mendoza M, et al. A four-year surveillance program for detection of *Plasmodium falciparum* chloroquine resistance in Honduras. *Mem Inst Oswaldo Cruz* 2014; 109: 492.

[214] GenRe-Mekong. Genomic Epidemiology Network (MalariaGen), https://www.malariagen.net/parasite/genre-mekong (2022, accessed 17 February 2022).

[215] Casanova D, Baptista V, Costa M, et al. Artemisinin resistance-associated gene mutations in *Plasmodium falciparum*: A case study of severe malaria from Mozambique. *Travel Med Infect Dis* 2023; 57: 102684.

[216] Mesia Kahunu G, Wellmann Thomsen S, Wellmann Thomsen L, et al. Identification of the PfK13 mutations R561H and P441L in the Democratic Republic of Congo. *Int J Infect Dis* 2023; 139: 41–49.

[217] Fola AA, Feleke SM, Mohammed H, et al. *Plasmodium falciparum* resistant to artemisinin and diagnostics have emerged in Ethiopia. *Nat Microbiol* 2023; 8: 1911.

[218] Uwimana A, Legrand E, Stokes BH, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat Med* 2020; 26: 1602–1608.

[219] Balikagala B, Fukuda N, Ikeda M, et al. Evidence of Artemisinin-Resistant Malaria in Africa. *https://doi.org/101056/NEJMoa2101746* 2021; 385: 1163–1171.

[220] World Health Organization. *Malaria surveillance, monitoring & evaluation: a reference manual*. Geneva, http://apps.who.int/iris/bitstream/handle/10665/272284/9789241565578-eng.pdf (2018, accessed 20 October 2021).

[221] World Health Organization. *Strategy to respond to antimalarial drug resistance in Africa*. Geneva, https://www.who.int/news/item/18-11-2022-tackling-emerging-antimalarial-drug-resistance-in-africa (2022, accessed 23 February 2023).

[222] Rosenthal PJ. The interplay between drug resistance and fitness in malaria parasites. *Mol Microbiol* 2013; 89: 1025.

[223] Kagoro FM, Allen E, Mabuza A, et al. Making data map-worthy—enhancing routine malaria data to support surveillance and mapping of *Plasmodium falciparum* anti-malarial resistance in a pre-elimination sub-Saharan African setting: a molecular and spatiotemporal epidemiology study. *Malar J* 2022; 21: 1–19.

[224] Christoffels A, Mboowa G, van Heusden P, et al. A pan-African pathogen genomics data sharing platform to support disease outbreaks. *Nat Med* 2023; 29: 1052–1055.

[225] Tanui CK, Tessema SK, Tegegne MA, et al. Unlocking the power of molecular and genomics tools to enhance cholera surveillance in Africa. *Nature Medicine 2023 29:10* 2023; 29: 2387–2388.

[226] Barnes K, Mabuza A, Frean PJ, et al. *Smart surveillance towards malaria elimination in Mpumalanga , South Africa (SS4ME): novel approaches for mapping antimalarial resistance (Protocol)*. 2017.

[227] Craig P, Dieppe P, Macintyre S, et al. Developing and evaluating complex interventions: the new Medical Research Council guidance. *The BMJ* 2008; 337: 979–983.

[228] Rehfuess EA, Booth A, Brereton L, et al. Towards a taxonomy of logic models in systematic reviews and health technology assessments: A priori, staged, and iterative approaches. *Res Synth Methods* 2018; 9: 13–24.

[229] World Health Organization. Module 1 Framework and metrics DATA QUALITY REVIEW, http://apps.who.int/bookorders. (2017, accessed 20 August 2019).

[230] Worldwide Antimalarial Resistance Network (WWARN). Training materials for obtaining accurate malaria case GPS coordinates |, https://www.wwarn.org/tools-resources/training-materials-obtaining-accurate-malaria-case-gps-coordinates (accessed 15 March 2022).

[231] Odendaal WA, Anstey Watkins J, Leon N, et al. Health workers' perceptions and experiences of using mHealth technologies to deliver primary healthcare services: a qualitative evidence synthesis. *Cochrane Database Syst Rev*; 2020. Epub ahead of print 26 March 2020. DOI: 10.1002/14651858.CD011942.PUB2.

[232] Kijsanayotin B, Pannarunothai S, Speedie SM. Factors influencing health information technology adoption in Thailand's community health centers: applying the UTAUT model. *Int J Med Inform* 2009; 78: 404–416.

[233] Kim S, Lee K-H, Hwang H, et al. Analysis of the factors influencing healthcare professionals' adoption of mobile electronic medical record (EMR) using the unified theory of acceptance and use of technology (UTAUT) in a tertiary hospital. *BMC Med Inform Decis Mak*; 16. Epub ahead of print December 2016. DOI: 10.1186/S12911-016-0249-8.

[234] Dehnavieh R, Haghdoost A, Khosravi A, et al. The District Health Information System (DHIS2): A literature review and meta-synthesis of its strengths and operational challenges based on the experiences of 11 countries. *Health Information Management Journal* 2019; 48: 62–75.

[235] Bhattacharya AA, Umar N, Audu A, et al. Quality of routine facility data for monitoring priority maternal and newborn indicators in DHIS2: A case study from Gombe State, Nigeria. *PLoS One* 2019; 14: e0211265.

[236] Githinji S, Oyando R, Malinga J, et al. Completeness of malaria indicator data reporting via the District Health Information Software 2 in Kenya, 2011-2015. *Malar J* 2017; 16: 1–11.

[237] Dehnavieh R, Haghdoost AA, Khosravi A, et al. The District Health Information System (DHIS2): A literature review and meta-synthesis of its strengths and operational challenges based on the experiences of 11 countries. *Health Information Management Journal* 2019; 48: 62–75.

[238] Kiberu VM, Matovu JK, Makumbi F, et al. Strengthening district-based health reporting through the district health management information software system: the Ugandan experience. *BMC Med Inform Decis Mak* 2014; 14: 40.

[239] Diallo CO, Schiøler KL, Drabo KM, et al. Stakeholder's perception of the health information system performance in Burkina Faso. *Pan Afr Med J* 2023; 44: 155.

[240] Garrib A, Stoops N, Mckenzie A, et al. An evaluation of the District Health Information System in rural South Africa. *South African Medical Journal*; 98.

[241] Kiberu VM, Matovu JK, Makumbi F, et al. Strengthening district-based health reporting through the district health management information software system: the Ugandan experience. *BMC Med Inform Decis Making* 2014; 14: 40.

[242] Skivington K, Matthews L, Simpson SA, et al. A new framework for developing and evaluating complex interventions: update of Medical Research Council guidance. *BMJ*; 374. Epub ahead of print 30 September 2021. DOI: 10.1136/BMJ.N2061.

[243] Gil JP, Krishna S. pfmdr1 (*Plasmodium falciparum* multidrug drug resistance gene 1): a pivotal factor in malaria resistance to artemisinin combination therapies. *Expert Rev Anti Infect Ther* 2017; 15: 527–543.

[244] Baraka V, Mavoko HM, Nabasumba C, et al. Impact of treatment and re-treatment with artemether-lumefantrine and artesunate-amodiaquine on selection of

Plasmodium falciparum multidrug resistance gene-1 polymorphisms in the Democratic Republic of Congo and Uganda. *PLoS One* 2018; 13: e0191922.

[245] Tumwebaze PK, Conrad MD, Okitwi M, et al. Decreased susceptibility of *Plasmodium falciparum* to both dihydroartemisinin and lumefantrine in northern Uganda. *Nat Commun*; 13. Epub ahead of print 1 December 2022. DOI: 10.1038/S41467-022-33873-X.

[246] Marwa K, Kapesa A, Baraka V, et al. Therapeutic efficacy of artemether-lumefantrine, artesunate-amodiaquine and dihydroartemisinin-piperaquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in Sub-Saharan Africa: A systematic review and meta-analysis. *PLoS One* 2022; 17: e0264339.

[247] Mesia Kahunu G, Wellmann Thomsen S, Wellmann Thomsen L, et al. Identification of the PfK13 mutations R561H and P441L in the Democratic Republic of Congo. *International Journal of Infectious Diseases* 2024; 139: 41–49.

[248] Mihreteab S, Platon L, Berhane A, et al. Increasing Prevalence of Artemisinin-Resistant HRP2-Negative Malaria in Eritrea. *N Engl J Med* 2023; 389: 1191–1202.

[249] la Fuente IM De, Sagrado Benito MJ, Ousley J, et al. Screening for K13-Propeller Mutations Associated with Artemisinin Resistance in *Plasmodium falciparum* in Yambio County (Western Equatoria State, South Sudan). *Am J Trop Med Hyg* 2023; 109: 1072–1076.

[250] Rosenthal PJ, Asua V, Conrad MD. Nature Reviews Microbiology nature reviews microbiology Emergence, transmission dynamics and mechanisms of artemisinin partial resistance in malaria parasites in Africa. *Nat Rev Microbiol*. Epub ahead of print 2024. DOI: 10.1038/s41579-024-01008-2.

[251] Ngasala B, Chiduo MG, Bushukatale S, et al. Efficacy and safety of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in mainland Tanzania, 2018. *Malar J* 2024; 23: 95.

[252] Wang LT, Bwambale R, Keeler C, et al. Private sector drug shops frequently dispense parenteral anti-malarials in a rural region of Western Uganda. *Malar J* 2018; 17: 1–9.

[253] Sharma AI, Shin SH, Bopp S, et al. Genetic background and PfKelch13 affect artemisinin susceptibility of PfCoronin mutants in *Plasmodium falciparum*. *PLoS Genet*; 16. Epub ahead of print 28 December 2020. DOI: 10.1371/JOURNAL.PGEN.1009266.

[254] Grossman T, Vainer J, Paran Y, et al. Emergence of artemisinin-based combination treatment failure in patients returning from sub-Saharan Africa with P. falciparum malaria. *J Travel Med*; 30. Epub ahead of print 28 December 2023. DOI: 10.1093/JTM/TAAD114.

[255] Delandre O, Daffe SM, Gendrot M, et al. Absence of association between polymorphisms in the pfcoronin and pfk13 genes and the presence of *Plasmodium falciparum* parasites after treatment with artemisinin derivatives in Senegal. *Int J Antimicrob Agents* 2020; 56: 106190.

[256] Grossman T, Vainer J, Paran Y, et al. Emergence of artemisinin-based combination treatment failure in patients returning from sub-Saharan Africa with P. falciparum malaria. *J Travel Med* 2023; 30: 1–9.

[257] Novartis. Novartis and Medicines for Malaria Venture announce decision to move to Phase 3 study for novel ganaplacide/lumefantrine-SDF combination in adults and children with malaria. *News*, https://www.novartis.com/news/media-releases/novartis-and-medicines-malaria-venture-announce-decision-move-phase-3-study-novel-ganaplacidelumefantrine-sdf-combination-adults-and-children-malaria (2023, accessed 11 April 2024).

[258] van der Pluijm RW, Amaratunga C, Dhorda M, et al. Triple Artemisinin-Based Combination Therapies for Malaria - A New Paradigm? *Trends Parasitol* 2021; 37: 15–24.

[259] Zupko RJ, Nguyen TD, Ngabonziza JCS, et al. Modeling policy interventions for slowing the spread of artemisinin-resistant pfkelch R561H mutations in Rwanda. *Nature Medicine 2023 29:11* 2023; 29: 2775–2784.

[260] World Health Organization. *The use of artesunate-pyronaridine for the treatment of uncomplicated malaria*. Geneva, https://www.who.int/medicines/regulation/medicines-safety/publications/ (2019, accessed 23 February 2024).

[261] Ogutu B, Yeka A, Kusemererwa S, et al. Ganaplacide (KAF156) plus lumefantrine solid dispersion formulation combination for uncomplicated *Plasmodium falciparum* malaria: an open-label, multicentre, parallel-group, randomised, controlled, phase 2 trial. *Lancet Infect Dis* 2023; 23: 1051–1061.

[262] Chen I, Ishengoma DS. Ganaplacide plus lumefantrine solid dispersion formulation: considerations for development and rollout. *Lancet Infect Dis* 2023; 23: 990–992.

[263] World Health Organization. WHO recommends R21/Matrix-M vaccine for malaria prevention in updated advice on immunization. *News release*, https://www.who.int/news/item/02-10-2023-who-recommends-r21-matrix-m-vaccine-for-malaria-prevention-in-updated-advice-on-immunization (2023, accessed 23 February 2024).

[264] Hamilton A, Haghpanah F, Hasso-Agopsowicz M, et al. Modeling of malaria vaccine effectiveness on disease burden and drug resistance in 42 African countries. *Communications Medicine 2023 3:1* 2023; 3: 1–10.

[265] Idoga PE, Toycan M, Nadiri H, et al. Assessing factors militating against the acceptance and successful implementation of a cloud based health center from the healthcare professionals' perspective: a survey of hospitals in Benue state, northcentral Nigeria. *BMC Med Inform Decis Mak*; 19. Epub ahead of print 19 February 2019. DOI: 10.1186/S12911-019-0751-X.

[266] Kang SY, Battle KE, Gibson HS, et al. Spatio-temporal mapping of Madagascar's Malaria Indicator Survey results to assess *Plasmodium falciparum* endemicity trends between 2011 and 2016. *BMC Med* 2018; 16: 71.

[267] Gething PW, Patil AP, Smith DL, et al. A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar J* 2011; 10: 378.

[268] Assefa A, Fola AA, Tasew G. Emergence of *Plasmodium falciparum* strains with artemisinin partial resistance in East Africa and the Horn of Africa: is there a need to panic? *Malar J* 2024; 23: 34.

[269] Robinson AC, Chen J, Lengerich EJ, et al. Combining Usability Techniques to Design Geovisualization Tools for Epidemiology. *Cartogr Geogr Inf Sci* 2005; 32: 243–255.

[270] Quasdorf T, Clack L, Laporte Uribe F, et al. Theoretical approaches to process evaluations of complex interventions in health care: a systematic scoping review protocol. *Syst Rev* 2021; 10: 1–7.

[271] World Health Organization. International Pathogen Surveillance Network (IPSN). *Initiatives*, https://www.who.int/initiatives/international-pathogen-surveillance-network (2023, accessed 27 February 2024).

[272] Lyimo BM, Popkin-Hall ZR, Giesbrecht DJ, et al. Potential Opportunities and Challenges of Deploying Next Generation Sequencing and CRISPR-Cas Systems to Support Diagnostics and Surveillance Towards Malaria Control and Elimination in Africa. *Front Cell Infect Microbiol*; 12. Epub ahead of print 13 July 2022. DOI: 10.3389/fcimb.2022.757844.

[273] Onywera H, Ondoa P, Nfii F, et al. Boosting pathogen genomics and bioinformatics workforce in Africa. *Lancet Infect Dis*; 24. Epub ahead of print 1 February 2024. DOI: 10.1016/S1473-3099(23)00394-8.

[274] Inzaule SC, Tessema SK, Kebede Y, et al. Genomic-informed pathogen surveillance in Africa: opportunities and challenges. *Lancet Infect Dis* 2021; 21: e281–e289.

[275] Tessema SK, Raman J, Duffy CW, et al. Applying next-generation sequencing to track falciparum malaria in sub-Saharan Africa. *Malar J* 2019; 18: 1–9.

[276] McIntyre SA, Francis JJ, Gould NJ, et al. The use of theory in process evaluations conducted alongside randomized trials of implementation interventions: A systematic review. *Transl Behav Med* 2020; 10: 168–178.

[277] Nilsen P. Making sense of implementation theories, models and frameworks. *Implement Sci*; 10. Epub ahead of print 21 April 2015. DOI: 10.1186/S13012-015-0242-0.

[278] Kagoro FM, Barnes KI, Marsh K, et al. Mapping genetic markers of artemisinin resistance in *Plasmodium falciparum* malaria in Asia: a systematic review and spatiotemporal analysis. *Lancet Microbe*. Epub ahead of print January 2022. DOI: 10.1016/S2666-5247(21)00249-4.

[279] WHO. *Artemisinin resistance and artemisinin-based combination therapy efficacy (Status report -- August 2018)*. Geneva, 2018.

[280] Ebener S, Maude RJ, Gault P. Guidance for the management and use of geospatial data and technologies in health.

https://www.healthgeolab.net/DOCUMENTS/Guide_HGLC_Part2_4_2.pdf (2022, Accessed 18 February 2022)