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

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Research Article

Keywords: Artemisinin resistance, Kelch-13, lumefantrine, Plasmodium falciparum, Africa, Spatial-temporal model, malaria

Posted Date: February 4th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1320822/v1>

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Abstract

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

Methods: From March 2018 to February 2020, we conducted a sequential mixed-methods study to evaluate the feasibility of the near-real-time linkage of individual patient antimalarial 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.

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 *mdr86*ASN and *crt76*LYS alleles, potentially associated with decreased lumefantrine susceptibility.

Conclusion: Routine near-real-time mapping of molecular markers associated with antimalarial 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.

Background

Malaria has been declining globally, with a 50% reduction in malaria cases and an 84% reduction in malaria deaths from 2000 to 2015.¹ Unfortunately, there has been no significant progress in reducing the global malaria burden since 2015.² The emergence of the SARS-CoV-2 threatens to reverse any such progress, with the World Health Organization (WHO) estimating an additional 47 000 deaths in 2020 linked to pandemic-related disruptions, with the WHO African region accounting for the majority of these cases.³ The emergence and spread of antimalarial drug resistance threaten malaria control and elimination efforts, especially in Southeast Asia (SEA), where parasites resistant to artemisinin-based combination therapies (ACTs) have been confirmed in at least five countries, with resistance markers also reported in China-Myanmar border and eastern India.^{4–7} 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.^{8,9} Mutations in the *Plasmodium falciparum* *kelch* 13 gene (K13) associated with artemisinin resistance have been identified in Central (Central African Republic), Eastern (Rwanda and Tanzania) and Western Africa (Ghana, Nigeria and Senegal), with phenotypic evidence of artemisinin resistance (delayed parasite clearance) recently confirmed in Rwanda and Uganda.^{10–16} More importantly, the K13 561HIS mutation in Rwanda and the 469TYR and 675VAL mutations in Uganda have been documented in up to 20% of infected individuals. These three mutations have been associated with reduced efficacy to artemisinin both *in-vitro* and *in-vivo*.^{12,15,17} Recent studies have demonstrated the independent emergence of artemisinin resistance molecular markers in Guyana, and the presence of novel K13 mutations in Manaus, Brazil and Choco, Colombia.^{18–21} 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, this genomic surveillance was rarely linked to a public health surveillance system.²²

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 be achieved readily, low and very low transmission settings face recruitment challenges due to low malaria case numbers 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 antimalarial drug efficacy into overall malaria case-based surveillance.²³ However, resource constraints limit follow up of all malaria cases and this is seldom feasible for mobile and migrant populations, so many low transmission countries fail to monitor antimalarial efficacy adequately. As far as we are aware, only China and Thailand have published iDES results since its initial recommendation in 2018.^{24–26} As countries move towards malaria elimination, many of those with low to very low case numbers need alternative methods for antimalarial drug resistance surveillance. Surveillance of molecular markers associated with drug resistance collected in different clinical trials and observational research studies has proved useful. However, these are generally short-term, are conducted in a few sites where they are not repeated regularly enough to track resistance trends over longer time periods.²⁷ 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 an early warning of emerging resistance.^{28–30} Such molecular marker surveillance using routine data could then trigger and target confirmatory therapeutic efficacy studies needed to inform antimalarial treatment policies to ensure effective treatment and accelerate local, regional and global elimination goals.

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.^{31–33} While the need for maps showing the distribution of parasites (malaria cases), vectors and vector breeding sites and prevalence of insecticide and antimalarial resistance was realised 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.³⁴ '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.³⁶ Analysing trends in routine data and reviewing results 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 (mRDT) or microscopy before treatment is administered.³⁷ The Malaria Elimination Programme (MEP) in Mpumalanga has stratified Nkomazi sub-district as being in the pre-elimination phase, and has piloted two interventions to enhance monitoring of antimalarial efficacy and advance malaria elimination. The first was Smart Surveillance for Malaria Elimination (SS4ME), started in February 2018, and comprised the collection of mRDTs (and wherever possible capillary blood filter paper samples) for tracking molecular markers of antimalarial resistance. The second was the programmatic roll-out of single low dose primaquine for malaria transmission blocking, in addition to routine treatment of uncomplicated malaria with the artemisinin-based combination therapy, artemether-lumefantrine (AL); this included WHO recommended follow up of treatment adherence and response during malaria case investigations (from January 2019).^{37,38}

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 effective use by policymakers in local, provincial and national malaria programmes in South(ern) Africa.

Methods

Design

We used a sequential explanatory mixed-methods approach with iterative quantitative and qualitative methods 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 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 to define spatial and temporal data patterns for data linkage and mapping.^{39–41} 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 project team worked iteratively to improve data architecture, system and maps produced.

The quantitative component included data aggregations, curation, and analysis to generate data visualisations of the obtained data. These visualisations and summaries of the analysis 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 (Fig. 1). Data were grouped monthly and quarterly to estimate the 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 improvement of GPS coordinate data over time.

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

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.^{33,42} Most malaria cases in South Africa are imported, with some local transmission occurring in the low-altitude 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).

This study was conducted in Nkomazi Sub-District, a pre-elimination area in Mpumalanga province, South Africa. All patients meeting the diagnostic criteria were tested for malaria using a falciparum-specific HRP2-based mRDT.^{38,43} Those who tested positive with uncomplicated malaria are treated with the WHO recommended weight-based 3-day artemether-lumefantrine (Coartem®) regimen as per the South African malaria treatment guidelines.³⁷ Artemether-lumefantrine has been used in the study area since 2007.^{27,37,38} 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 the WHO-recommended transmission-blocking antimalarial primaquine (0.25 mg base/kg (15 mg base adult maximal dose)^{44–46}. 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 mRDT finger prick site, labelled and barcoded, then sent to the malaria laboratory at the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa, together with its respective positive mRDT cassette, for antimalarial resistance marking.⁴⁷ An additional 10% of the negative mRDTs were also collected and sent to the NICD for quality assurance assessment.

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 case investigator from the Malaria Elimination Programme (MEP) 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 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, the case investigation form completed, and information on malaria treatment adherence and response (including any side effects) captured. Once completed these forms are submitted to the sub-district malaria office for quality checking and electronic capture into the DHIS2.

Data

Malaria case data

Malaria case data consisted of notifiable medical condition form (NMC), case investigation and treatment adherence and response forms downloaded as individual case records from the DHIS2.

Geospatial data

The geospatial data consisted of four types. Firstly, each patient's residential address and GPS coordinates were sourced from a) android tablets/handheld GPS devices and b) 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 (GADM) website for South Africa. 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).⁴⁸

Laboratory data

Parasite DNA was extracted from the mRDTs and DBSs using the Qiagen DNA mini extraction kit (Qiagen, Germany), according to the manufacturer's instructions. The extracted DNA was subjected to multiplex PCR to confirm *Plasmodium* species.⁴⁹ To assess possible decreases in lumefantrine susceptibility, samples containing only *P. falciparum* parasites were then subjected to molecular analysis to detect polymorphisms in the chloroquine resistance transporter (*crt*) and multi-drug resistance 1 (*mdr1*) genes.^{49–51} 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.⁵¹ 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.⁵² The amplified products were sent to Inqaba Biotechnologies (South Africa) for Sanger sequencing. Sequences were then aligned against a reference *Pf* K13 gene (XM_001350122.1) using a BLAST search and BioEdit Software to detect polymorphisms in 27 codons associated with delayed parasite clearance in South East Asia.^{20,53} 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/or response in the event of any significant resistance-associated mutations.

Definition of metrics

Coverage

Four measures of coverage were used: 1) percentages of malaria cases with blood samples taken (mRDT/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 the health facility, ward, locality, and household levels.

Study procedures

Using monthly timelines, we evaluated the accuracy, coverage and ability to link the malaria notifications to the case investigation, laboratory data and drug report data. 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 the different GPS devices, 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.

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.

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) ⁵⁴. Loess regression was used to obtain the optimum distribution and the 95% confidence margins of the trend (Fig. S2(a), S3(a) & S4(a)). This time series was further decomposed to evaluate trend, seasonality and random errors using Sen's slope and Mann-Kendall test.^{39,41} Seasonality was further explored using box plots.

Molecular markers analysis

The classification of the 27 *K13* mutations after codon 400 assessed in this study was guided by the WHO and the Worldwide Antimalarial Resistance Network (WWARN).^{20,55} We used the 2020 WHO categories of 'validated', 'associated/candidate', 'not associated' or 'wild type'.⁵⁵ We renamed the 'wild type' parasite 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 *mdr86*ASN and *crt76*LYS alleles but no increase in *mdr1* copy number were categorised as less susceptible (or tolerant) to lumefantrine.

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. 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 with their ward boundaries. Twenty-four case investigators with experience ranging between one and 24 years 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 evaluation of the shapefiles.

Thematic maps of the distribution of malaria cases by ward were produced by using a spatial join tool of 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:

- $i = 1, \dots, n$ 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.⁴⁰

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 optimise 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 calculated by a Poisson process using the known malaria incidence and population settlement data to obtain likelihood ratios and Chi-square test were analysed. To assess the sparsity of malaria case residential data, we used average nearest neighbour analysis to explore for precision in the GPS coordinate dataset.

Results

Malaria notification, case investigation, drug adherence and response reports

From the 1st of March 2018 to the 28th of February 2020, 4787 malaria cases were notified in Nkomazi sub-district. All cases were definitively diagnosed using mRDTs, 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 symptoms onset. As from March 2018, 78.5% (n=3758) of cases were investigated and GPS coordinates captured (Fig. 2). Treatment adherence and response reports were to be captured from January 2019 (Fig. S1), where after, 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).

We performed data linkage 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 mRDT barcodes (n=2527), allowing 89% (n=2002) of accurately investigated cases to be linked to their molecular markers of resistance results. The second group included treatment adherence and response reports, introduced in January 2019, where only 50% (n=1413) of case investigations 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 could be linked to their individual molecular markers of resistance results (Fig. 2).

Of the 6054 mRDTs received by the national laboratory, 61.6% (n=3748) were reported as *falciparum* positive by the Mpumalanga MEP; the remainder were negative mRDTs sent for quality control purposes. Parasite DNA was extracted, and PCR amplified from these positive mRDTs 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.

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 the first quarter of 2020 (Fig. S6).

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 (Fig 3 and 4).

Temporal trends of the selected surveillance metrics

As shown in Fig. 2 (overall) and Fig. 5 (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 mRDT samples received at the NICD laboratory, those barcoded increased from 19% to 85%. The household GPS coordinate accuracy increased from 48% to 76% over the study period.

GPS Coordinate coverage and accuracy

Although the proportion of households with GPS coordinates ("coverage") remained high throughout the study, we noted spikes in coverage that corresponded with the months after on-site training 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 (Fig. 3 and S3)

Barcode coverage and accuracy

Over the course of the study, there was a steady increase in the percentage of accurately barcoded samples. We observed an increase in coverage and accuracy from an average of 5% in the first quarter to

80% in the last quarter and peaks in the months after on-site visits and training, except for the third training (Fig. 4 and S4).

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 (Fig. S5).

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 Fig. 6. We assessed 28 GPS collection devices being used by case investigators in the Mpumalanga MEP. 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°) with the remaining four in the format of degrees, minutes and seconds (DDD° MM' SS.S"). Standard operating procedures (supplemental Tool S1) 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.

Overall, we observed improvement in accuracy and precision of coordinates 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 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 (Fig. S7(a)), 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 (Fig. S7(b)) included malaria cases clustering only in certain areas within the wards (other areas are largely unoccupied farmlands or nature reserves), so 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 (n=5/38); duplicate labelling (n=7/38); and misplaced labels with no consensus (12/38).

Molecular markers of drug resistance

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

The propeller domain of the *K13* gene was successfully amplified and sequenced from 73% (2 385/3262) of the *PCR positive* falciparum samples (Table 1). All sequenced samples were wildtype at the 27 *K13*

single nucleotide polymorphisms (SNPs) known to be associated with artemisinin resistance (delayed parasite clearance).

Table 1. Summary of molecular markers of artemisinin and lumefantrine “resistance”

	Artemisinin	Lumefantrine	
Marker name	<i>K13</i>	<i>Pf mdr186</i>	<i>Pf crtK76T</i>
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%)

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.

Almost all the samples in which the *mdr186* and crt76 SNPs could be assessed carried wild type *mdr186*ASN (99.7%, 2803/2812), and *crt76*LYS (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 Fig. 7.

Discussion

Over the course of this study, we curated data from 4787 notified malaria cases and were able to link 75% of these cases to their individual antimalarial 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’. To our knowledge, this is the first study utilising routine malaria surveillance data individually linked to molecular surveillance data to create near-real-time maps of antimalarial drug resistance. It is also the first study to use routine surveillance data stored in DHIS2 for the spatio-temporal mapping of antimalarial drug resistance data. The evidence generated by this pilot exemplifies the WHO recommendation to transform surveillance into a core intervention.²³ While most evaluations of molecular markers of artemisinin and partner drug resistance are from clinical trials,^{22,56} routine surveillance has the potential to facilitate the early detection of antimalarial 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. Although the WHO recommends iDES for low malaria transmission settings, there is little evidence of how feasible this is for resource-constrained malaria programmes with thousands of malaria cases, particularly if most 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 are needed.⁵⁵

We collected and assessed routine malaria surveillance data over two years to map any spatiotemporal changes in molecular markers of antimalarial drug resistance. A user-centred feedback approach helped to assess data quality and incorporate improvement activities into the 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.³⁵ We observed improvements in the surveillance metrics studied that were sustained at a moderately high level of quality for seven months after the last on-site supervision. Since there are no proposed analysis frameworks for evaluating routine location data from cohorts of infectious diseases patients, we adapted the metrics from the latest systematic review and the WHO's Data Quality Review (DQR) framework as this expert proposed framework was only designed for the assessment of facility-based data. Malaria surveillance data include off-facility activities such as case investigation home visits to assess treatment adherence and response, while seeking any mosquito vector breeding sites.^{57,58}

We found that a 0.5 x 0.5 km malaria density map provided a more accurate representation of the distribution of malaria. This challenges the most prevalent malaria map designs, 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 park with that lack 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. Our study only 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 we were able to link only 55.1% of all reported malaria cases, our iterative analysis, training and feedback sharing 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 with accurate GPS location linked to their individual molecular marker of resistance results, it would be possible to target the correct geolocation of that case for further investigation and prompt response within that community, should any molecular marker of concern be identified.

Almost 45% of individual malaria cases and molecular data could not be linked in our 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 antimalarial drug resistance. To avoid stressing the already stretched health system, further development of assay methods is needed to obtain an adequate yield from mRDTs alone, as filter paper DBS requirement could potentially limit the scalability of this approach.

Although we identified and corrected numerous human and system errors, 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. Despite most of the migrant cases presenting 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.

Although the proportion of filter paper DBS submitted with positive mRDTs 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 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.⁵⁹ More intensive in-person training would be required to improve and sustain progress.

A small proportion (2.2%) of negative mRDTs were malaria positive by PCR. This finding could be due to patients with infections that have parasites loads below the detection limit of the mRDT (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 mRDT and/or DBS or the incorrect use of the mRDT (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 deletions in histidine-rich protein 2 (*hrp2*); available evidence suggests *hrp2* mutations are rare within the southern African region⁶⁰. However, ongoing rigorous testing is required to exclude *hrp2* deletions in this region.

Fortunately, we did not find any 'validated' or 'associated/candidate' *K13* mutations associated with artemisinin resistance in this study.^{20,55} However, the strong selection for the *mdr86*ASN and *crt76*LYS wildtype alleles may indicate some lumefantrine tolerance. A study in Uganda found an increase in relative risk of treatment failure (PCR-adjusted) associated with the presence of the *mdr86*ASN allele. Venkatesan et al. 2014 found a slight increase in recrudescence and reinfection for parasites carrying *pfcr76* and *mdr86*ASN alleles following AL treatment.^{61,62} However, no other African studies have demonstrated an increased therapeutic failure rate when these alleles are present.^{63–65} In Asia, clinical failure rates have been linked to the increase in *mdr1* copy number as compared to the *mdr86*ASN

alleles.⁶⁶ The increase in *mdr1* copy number has rarely been reported in Africa, and our study did not observe any such increase.^{51,67}

Our study highlights the need for continued rigorous surveillance, particularly in the light of multiple reports of the independent emergence of *K13* resistance markers in Eastern, Central and Western African countries.^{12–15,22} 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) is of some concern,^{55,68} although consecutive studies in 2017 and 2019 showed adequate parasite clearance rates.^{69–71} The extreme AL drug pressure in southern African and decreased AL efficacy in Rwanda and Uganda calls for strengthening of resistance surveillance across Africa, to inform efficient targeting of further investigation of parasite clearance rates and ACT efficacy and prompt response to failing treatment.

Our study had some of the challenges and limitations often seen with the use of routine surveillance data, 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 harmonisation 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, the malarial short messaging service and NMC mobile applications.⁷² Ideally, these two systems should feed into the DHIS2 system and remove duplication; however, we could not access the two mobile-phone-based databases for confirmation. Insufficient staff and resources may also hamper data quality. During the two years of our 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.^{73–75} Such challenges limit the usage of routine data in decision-support systems especially in low- and middle-income countries. 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, 3,76–78} We did not explore 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, given the focus of our study.^{33,79}

Routine near-real-time mapping of molecular markers of antimalarial drug resistance data to the healthcare facility, locality and patient household levels offers malaria programmes rapid and efficient monitoring of spatio-temporal changes in antimalarial 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, hence allocating their resources strategically. Pragmatic and innovative approaches such as co-designing can enable precision mapping, contextualisation of analyses and meeting the malaria MEP needs. While enhancing the quality of routine data can be a daunting task, identifying, monitoring and improving important surveillance metrics and indicators by MEPs and researchers working collaboratively can be critical to both evaluating progress and achieving malaria elimination targets.

Conclusions

In low malaria transmission settings in sub-Saharan Africa, near-real-time fine-scale mapping of molecular markers of antimalarial drug resistance can assist in rapidly and efficiently monitoring antimalarial drug resistance and identifying areas requiring further interventions. However, the sustainability of such a strategy requires continuous training, close supervision and strong programmatic support. The methods piloted, and lessons learnt 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. FMK and JR summarised 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, as well as the Clinton Health Access Initiative, Humana People to People (HPP) and all healthcare facility staff who collaborated in this study for all their support.

References

1. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. Geneva; 2020.
2. World Health Organization. World Malaria Report 2018. Geneva; 2018.
3. World Health Organization. World malaria report 2021. Geneva; 2021.
4. Feng J, Kong X, Xu D, Yan H, Zhou H, et al. Investigation and evaluation of genetic diversity of kelch 13 polymorphisms in plasmodium falciparum from southern China. *Front Public Heal*. 2019;7(APR).
5. He Y, Campino S, Diez Benavente E, Warhurst DC, Beshir KB, et al. 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. *PLoS One*. 2019;14(3):e0213686.
6. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, et al. Spread of Artemisinin Resistance in Plasmodium falciparum Malaria. *N Engl J Med*. 2014;371:411–23.
7. Das S, Manna S, Saha B, Hati AK, Roy S. 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.

8. Maharaj R, Raman J, Morris N, Moonasar D, Durrheim DN, et al. Epidemiology of malaria in South Africa: From control to elimination. *South African Med J*. 2013 Aug 29;103(10):779–83.
9. SADC Elimination 8. SADC Malaria Elimination Eight Regional Surveillance Quarterly bulletin: Malaria incidence along E8 border districts. Windhoek, Namibia; 2020.
10. Boussaroque A, Fall B, Madamet M, Camara C, Benoit N, et al. Emergence of Mutations in the K13 Propeller Gene of *Plasmodium falciparum* Isolates from Dakar, Senegal, in 2013-2014. 2015;
11. Tola M, Ajibola O, Idowu ET, Omidiji O, Awolola ST, et al. Molecular detection of drug resistant polymorphisms in *Plasmodium falciparum* isolates from Southwest, Nigeria. *BMC Res Notes*. 2020 Dec 1;13(1).
12. Uwimana A, Legrand E, Stokes BH, Mangala Ndikumana J-L, Warsame M, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat Med*. 2020;26:1602–8.
13. Nzoumbou-Boko R, Gildas Panté-Wockama C-B, Ngoagoni C, Petiot N, Legrand E, et al. Molecular assessment of kelch13 non-synonymous mutations in *Plasmodium falciparum* isolates from Central African Republic. *Malar J*. 2020;19:191.
14. Bwire GM, Ngasala B, Mikomangwa WP, Kilonzi M, Kamuhabwa AAR. 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*. 2020;10(3500 (2020)).
15. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana S-I, et al. Evidence of Artemisinin-Resistant Malaria in Africa. <https://doi.org/101056/NEJMoa2101746>. 2021 Sep 22;385(13):1163–71.
16. WWARN. Artemisinin Molecular Surveyor [Internet]. 2021 [accessed 2021 Aug 21]. Available from: <http://www.wwarn.org/molecular/surveyor/k13/index.html?t=201608031200#0>
17. Straimer J, Gnädig NF, Witkowski B, Amaratunga C, Duru V, et al. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. *Science* (80-). 2015;347(6220).
18. Chenet SM, Akinyi Okoth S, Huber CS, Chandrabose J, Lucchi NW, et al. Independent Emergence of the *Plasmodium falciparum* Kelch Propeller Domain Mutant Allele C580Y in Guyana. *J Infect Dis*. 2016 May 1;213(9):1472–5.
19. Mathieu LC, Cox H, Early AM, Mok S, Lazrek Y, et al. Local emergence in Amazonia of *Plasmodium falciparum* k13 C580Y mutants associated with in vitro artemisinin resistance. *Elife*. 2020 May 1;9:1–21.
20. 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*. 2019;17(1).
21. Olivera MJ, Guerra AP, Cortes LJ, Horth RZ, Padilla J, 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(5):1056.

22. Ndwiga L, Kimenyi KM, Wamae K, Osoti V, Akinyi M, et al. A review of the frequencies of *Plasmodium falciparum* Kelch 13 artemisinin resistance mutations in Africa. *Int J Parasitol Drugs drug Resist*. 2021 Aug 1;16:155–61.
23. World Health Organization. A framework for malaria elimination. Geneva; 2017.
24. World Health Organization. Malaria surveillance, monitoring & evaluation: a reference manual. Geneva; 2018.
25. Huang F, Liu H, Yan H, Zhou S, Xia Z. Antimalarial Drug Resistance Surveillance in China, 2016–2020. *China CDC Wkly*. 2021;3(17):366.
26. Sudathip P, Saejeng A, Khantikul N, Thongrad T, Kitchakarn S, et al. Progress and challenges of integrated drug efficacy surveillance for uncomplicated malaria in Thailand. *Malar J* 2021 201. 2021 Jun 9;20(1):1–16.
27. Kagoro FM, Barnes KI, Marsh K, Ekapirat N, Mercado CEG, et al. Mapping genetic markers of artemisinin resistance in *Plasmodium falciparum* malaria in Asia: a systematic review and spatiotemporal analysis. *The Lancet Microbe*. 2022 Jan;
28. Raman J, Kagoro FM, Mabuza A, Malatje G, Reid 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*. 2019 Aug 22;18(1).
29. Ndiaye M, Sow D, Nag S, Sylla K, Tine C R, 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(5):1593–6.
30. Jacob CG, Thuy-Nhien N, Mayxay M, Maude RJ, Quang HH, et al. Genetic surveillance in the Greater Mekong subregion and South Asia to support malaria control and elimination. *Elife*. 2021 Aug 10;10.
31. Maude RJ, Mercado CEG, Rowley J, Ekapirat N, Dondorp A. Estimating malaria disease burden in the Asia-Pacific. *Wellcome Open Res*. 2019 Apr 1;4:59.
32. Barr-Walker J. Evidence-based information needs of public health workers: a systematized review. *J Med Libr Assoc*. 2017 Jan;105(1):69–79.
33. Silal SP, Little F, Barnes KI, White LJ. Towards malaria elimination in Mpumalanga, South Africa: a population-level mathematical modelling approach. *Malar J*. 2014 Dec 3;13(1):297.
34. Snow RW, Marsh K, Le Sueur D. The Need for Maps of Transmission Intensity to Guide Malaria Control in Africa. *Parasitol Today*. 1996;12(12).
35. 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. p. 44–7.
36. Carroll LN, Au AP, Todd Detwiler L, Fu T, Painter IS, et al. Visualization and analytics tools for infectious disease epidemiology: A systematic review. *J Biomed Inform*. 2014;51:287–98.

37. NICD. National guidelines for the treatment of malaria, South Africa 2018 (Updated January 2019). Pretoria; 2019.
38. Sharp BL, le Sueur D. Malaria in South Africa—the past, the present and selected implications for the future. *S Afr Med J*. 1996 Jan;86(1):83–9.
39. Libiseller C, Grimvall A. Performance of partial Mann–Kendall tests for trend detection in the presence of covariates. *Environmetrics*. 2002 Feb 1;13(1):71–84.
40. Silverman BW. Density estimation for statistics and data analysis. In: *Monographs on Statistics and Applied Probability*. Chapman and Hall; 1986. p. 1–22.
41. Pohlert T. Non-Parametric Trend Tests and Change-Point Detection [R package trend version 1.1.4]. 2020 Sep 17;
42. Statistics South Africa. Census 2011, Statistical release (Revised). 2011.
43. Barnes KI, Durrheim DN, Little F, Jackson A, Mehta U, et al. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLoS Med*. 2005 Nov;2(11):e330.
44. Stepniewska K, Humphreys GS, Gonçalves BP, Craig E, Gosling R, 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*. 2020 Aug 11;
45. Taylor WR, Naw HK, Maitland K, Williams TN, Kapulu M, 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*. 2018 Jan 18;16(1).
46. Raman J, Allen E, Workman L, Mabuza A, Swanepoel H, 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*. 2019 Jun 24;18(1).
47. Barnes K, Mabuza A, Frean PJ, Magagula R, Bridget M, et al. Smart surveillance towards malaria elimination in Mpumalanga , South Africa (SS4ME): novel approaches for mapping antimalarial resistance (Protocol). 2017.
48. Centre for Humanitarian Data. Humanitarian Data Exchange [Internet]. 2021 [accessed 2021 May 12]. Available from: <https://data.humdata.org/>
49. Padley D, Moody AH, Chiodini PL, Saldanha J. Use of a rapid, single-round, multiplex PCR to detect malarial parasites and identify the species present. *Ann Trop Med Parasitol*. 2003 Mar 18;97(2):131–7.
50. Sutherland CJ, Haustein T, Gadalla N, Armstrong M, Doherty JF, et al. Chloroquine-resistant *Plasmodium falciparum* infections among UK travellers returning with malaria after chloroquine prophylaxis. *J Antimicrob Chemother*. 2007 Jun;59(6):1197–9.
51. Price RN, Uhlemann A-C, Brockman A, McGready R, Ashley E, et al. Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. *Lancet (London, England)*. 2004 Jul 31;364(9432):438–47.

52. Talundzic E, Chenet SM, Goldman IF, Patel DS, Nelson JA, et al. Genetic Analysis and Species Specific Amplification of the Artemisinin Resistance-Associated Kelch Propeller Domain in *P. falciparum* and *P. vivax*. PLoS One. 2015 Aug 20;10(8).
53. Talundzic E, Okoth SA, Congpuong K, Plucinski MM, Morton L, et al. Selection and Spread of Artemisinin-Resistant Alleles in Thailand Prior to the Global Artemisinin Resistance Containment Campaign. PLoS Pathog. 2015 Apr;11(4):e1004789.
54. Team R. stats-package: The R Stats Package. 2009.
55. World Health Organization. Report on antimalarial drug efficacy, resistance and response 10 years of surveillance (2010 - 2019). Geneva; 2020.
56. Nsanzabana C, Djalle D, Guérin PJ, Ménard D, González IJ. Tools for surveillance of anti-malarial drug resistance: an assessment of the current landscape. Malar J. 2018 Feb 8;17(1):75.
57. Chen H, Hailey D, Wang N, Yu P. A Review of Data Quality Assessment Methods for Public Health Information Systems. OPEN ACCESS Int J Environ Res Public Heal. 2014;11:11.
58. World Health Organization. Data Quality Review (DQR) Toolkit [Internet]. [accessed 2020 Jan 14]. Available from: https://www.who.int/healthinfo/tools_data_analysis/dqr_modules/en/
59. Diakité SAS, Traoré K, Sanogo I, Clark TG, Campino S, 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 Nov 12;18(1):361.
60. World Health Organization. Malaria Threat Map [Internet]. 2019 [accessed 2020 Aug 26]. Available from: <https://apps.who.int/malaria/maps/threats/>
61. Baraka V, Mavoko HM, Nabasumba C, Francis F, Lutumba P, 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 Feb 1;13(2):e0191922.
62. Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, 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 Oct 1;91(4):833–43.
63. Ljolje D, Dimbu PR, Kelley J, Goldman I, Nace D, 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. 2018 Feb 20;17(1).
64. Davlantes E, Dimbu PR, Ferreira CM, Florinda MJ, Pode D, 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. 2018 Apr 3;17(1).
65. Gansané A, Moriarty LF, Ménard D, Yerbanga I, Ouedraogo E, 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 Dec 1;20(1):48.

66. Wu Y, Soe MT, Aung PL, Zhao L, Zeng W, et al. 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).
67. Bretscher MT, Dahal P, Griffin J, Stepniewska K, Bassat Q, et al. The duration of chemoprophylaxis against malaria after treatment with artesunate-amodiaquine and artemether-lumefantrine and the effects of pfmdr1 86Y and pfcr1 76T: a meta-analysis of individual patient data. *BMC Med*. 2020 Feb 25;18(1).
68. Conrad MD, Rosenthal PJ. Antimalarial drug resistance in Africa: the calm before the storm? *Lancet Infect Dis*. 2019 Oct 1;19(10):e338–51.
69. Davlantes E, Dimbu PR, Ferreira CM, Florinda Joao M, Pode D, 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*. 2018 Apr 3;17(1).
70. Dimbu PR, Horth R, Cândido ALM, Ferreira CM, Caquece F, et al. Continued low efficacy of artemether-lumefantrine in Angola in 2019. *Antimicrob Agents Chemother*. 2021 Feb 1;65(2).
71. Plucinski MM, Dimbu PR, Macaia AP, Ferreira CM, Samutondo C, et al. Efficacy of artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisinin-piperaquine for treatment of uncomplicated *Plasmodium falciparum* malaria in Angola, 2015. *Malar J*. 2017 Feb 2;16(1).
72. Baloyi RE, Shandukani MB, Graffy R, Misiani E, Mayet N, 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*. 2018 Aug 23;17(1).
73. Githinji S, Oyando R, Malinga J, Ejersa W, Soti D, et al. Completeness of malaria indicator data reporting via the District Health Information Software 2 in Kenya, 2011-2015. *Malar J*. 2017 Aug 17;16(1).
74. 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*. 2020 Dec 1;20(1).
75. Bhattacharya AA, Umar N, Audu A, Allen E, Schellenberg JRM, 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 Jan 1;14(1).
76. World Health Organization. World Malaria Report. Geneva; 2016.
77. 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.
78. World Health Organization. World malaria report 2019. Geneva; 2019.
79. Hyde E, Bonds MH, Ithantamalala FA, Miller AC, Cordier LF, 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*. 2021 Dec 1;20(1).

Figures

Figure 1

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, mRDT - Malaria Rapid Diagnostic Test, PCR - Polymerase Chain Reaction, MMR - Molecular Markers of Resistance)

Figure 2

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 malaria filter paper dried blood spots of mRDT positive malaria patients. (MMR=Molecular markers of resistance results, mRDT=malaria rapid diagnostic test and Pf=Plasmodium falciparum)

Figure 3

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 4

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.

Figure 5

Figure 5. 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*

Figure 6

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 Supplemental Tool S1 were conducted in October 2018, January, March and August 2019

Figure 7

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 x 5 km grid, artemisinin *Plasmodium falciparum* K13 (left) and lumefantrine (right) *mdr186ASN/crt76LYS* molecular markers of “resistance”, denoted by their susceptibility in Nkomazi Sub-district, Ehlanzeni, Mpumalanga (March 2018 - Feb 2020)

Supplementary Files

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- [SupplementarydocumentsMDM31Jan2022.docx](#)