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Differences in epidemiological characteristics and antimalarial drug-resistance marker prevalence in imported and locally acquired cases from two South African malaria-endemic districts targeting elimination, 2022–2024

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Summary

Persistent malaria importation poses a threat to malaria elimination, as it has the potential to seed secondary transmission and drive drug-resistant outbreaks by introducing drug-resistant parasites into low-transmission areas where local populations have low immunity. Malaria-eliminating districts within the South African provinces of Mpumalanga and KwaZulu-Natal routinely report high levels of imported malaria, primarily from higher-burdened neighbouring countries. This secondary analysis, using routine genomic surveillance data from these districts, compared the epidemiological characteristics and drug-resistance marker prevalence in imported and locally acquired malaria cases with the aim of informing intervention selection and targeting to stem focal residual transmission in these eliminating districts. A descriptive, comparative analysis of malaria cases reported between January 2022 and August 2024 in Ehlanzeni District in Mpumalanga and uMkhanyakude District in KwaZulu-Natal province was conducted. Socio-demographic and clinical characteristics, parasite species, and the prevalence of drug-resistance markers (mutations in the kelch13, crt, mdr1, dhfr, and dhps genes) were summarised and compared between cases classified as either imported or locally-acquired. Of the 1 901 confirmed malaria cases with genomic surveillance data, 88% (1 678/1 901) were classified as imported cases. Compared to locally acquired cases, imported cases were more frequently detected through active case detection ($p<0.001$), were mostly asymptomatic infections, and had a higher prevalence of partial artemisinin and chloroquine resistance markers. Plasmodium falciparum mono-infections were predominant (98%, 1 862), with few co-infections that were more commonly detected among imported cases (2.3%) ($p=0.011$). On the other hand, locally acquired cases were more likely to progress to severe malaria than imported cases ($p<0.001$). Irrespective of case classification, males aged 20 years and older were the most at-risk group. This study highlights the risk that malaria importation poses to elimination efforts while emphasising the importance of sustained routine active case detection in low-transmission districts targeting elimination. The prompt detection and successful treatment of all cases, irrespective of case classification, is crucial for the interruption of malaria transmission, preventing drug-resistant outbreaks, and limiting disease progression. The strengthening of cross-border collaborations and the harmonisation of incidence-reducing interventions, including surveillance and case management across shared international borders, remains critical for national and regional malaria elimination.

Introduction

Malaria is a preventable and curable mosquito-borne disease caused by *Plasmodium* parasites.[1,2] Globally, an estimated 282 million malaria cases were reported in 2024, an increase of nine million cases compared to 2023.[1] Africa continues to bear a disproportionate disease burden, accounting for 95% of these cases.[1] South Africa lies at the southern edge of malaria transmission on the African continent, with endemic transmission confined to the low-altitude, tropical border regions of the Limpopo, Mpumalanga, and KwaZulu-Natal provinces.[2,3] South Africa is among the 25 countries identified by the World Health Organization (WHO) with the potential to eliminate malaria

in the near future.[1,4] Substantial progress has been made towards attaining this goal, with the country cited in the 2025 WHO malaria report as being on track to achieve a 75% reduction in incidence and mortality in 2025 compared to 2015.[1] Despite this progress, several challenges are still impeding elimination, including high case importation, climatic, biological, and financial factors.[5] Taking these factors into consideration, the country revised its elimination timeline, now aiming to halt local transmission within its borders by 2028.[6]

One strategy currently employed by South Africa to advance its elimination agenda is a phased subnational approach.[3,7] Through this strategy, smaller geographic units such as districts are targeted with focused resources and interventions aimed at halting residual local transmission. Using this approach, the King Cetshwayo District of KwaZulu-Natal achieved subnational elimination in 2024.[6,8] Encouraged by this achievement, other low-transmission districts, including Ehlanzeni in Mpumalanga and uMkhanyakude in KwaZulu-Natal, are currently pursuing subnational elimination.

Imported malaria cases account for the majority of reported infections in low-transmission settings targeting elimination in South Africa.[9-12] These imported cases pose a significant threat to elimination efforts as they can seed and sustain focal residual transmission in receptive areas as previously seen in other countries.[13] Additionally, malaria importation can introduce drug-resistant parasites into areas with low parasite diversity where drug pressure is high, as local communities have limited malaria immunity, driving the selection of these drug-resistant parasites, potentially resulting in drug-resistant malaria outbreaks.[12,13]

Antimalarial drug resistance emerges when Plasmodium parasites acquire mutations that alter parasite protein structure and/or biological processes, allowing these mutated parasites to survive therapeutic drug concentrations, leading to treatment failure.[14] In eliminating settings, an in-depth understanding of epidemiological characteristics and drug-resistance marker prevalence of all malaria carriers is essential to guide the appropriate selection and targeting of incidence-reducing interventions. This study, therefore, aimed to determine if differences in high-risk groups, parasite species, resistance marker prevalence, and case detection methods exist between imported and locally acquired cases in two South African malaria-endemic districts, targeting elimination.

Methods

Study design and setting

We conducted a secondary, descriptive, comparative analytical study using data from routine genomic surveillance activities in Ehlanzeni District of the Mpumalanga province and uMkhanyakude District of the KwaZulu-Natal province between 2022 and 2024 (Figure 1). While these two districts share some geographical and epidemiological similarities, including bordering Eswatini and Mozambique, and a high case importation rate from neighbouring countries, they also have marked differences.[9-11] Ehlanzeni District is landlocked and is the only

malaria-endemic district in Mpumalanga, whereas uMkhanyakude District lies along the coast and is one of two malaria-endemic districts in KwaZulu-Natal. Additionally, uMkhanyakude District has an estimated population of 738 437 people living across the 12 821 km^2 district area, while Ehlanzeni District is more than twice the size in both the estimated population of 2 270 897 people, and a land area of 27 895 km^2.[15-18]

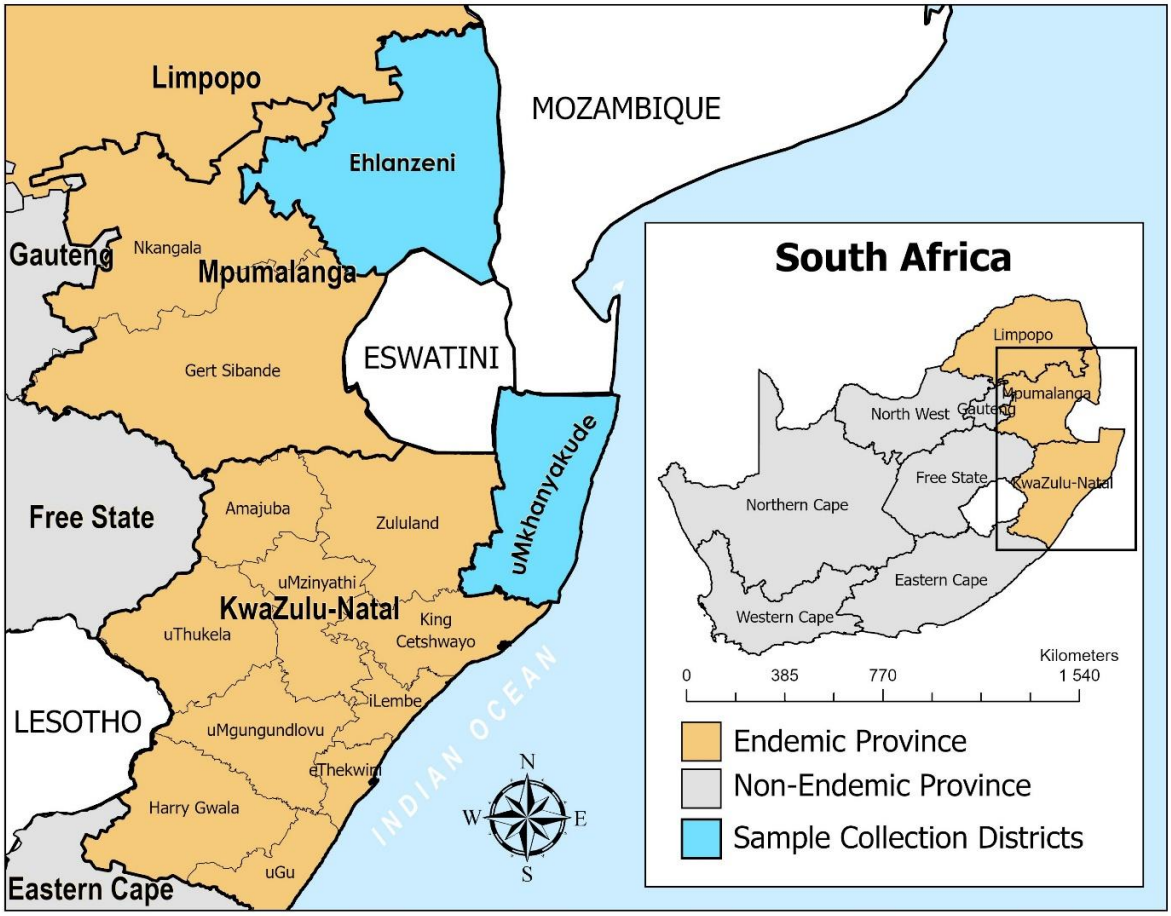

Figure 1. Location of Ehlanzeni and uMkhanyakude Districts where samples of malaria cases were collected, South Africa, 2022–2024 (ArcGIS Pro).[19]

Sample collection and analysis

Routine genomic surveillance in these two districts involves the collection of used malaria rapid diagnostic tests (RDTs) and finger-prick filter paper dried blood spots (DBSs) from RDT-positive malaria cases detected through passive and active case detection. Passive case detection involves confirming a malaria infection when testing an individual who presented to a healthcare facility on their own accord. In contrast, active case detection involves surveillance teams detecting malaria cases during test-and-treat activities in communities and at formal and informal border crossings. The collected RDTs and DBSs are routinely shipped from these districts to the malaria

molecular laboratory at the National Institute for Communicable Diseases, a division of the National Health Laboratory Service, for genomic analysis using previously published protocols.[5] In brief, parasite DNA was extracted from RDTs or DBSs using the Tween-Chelex method and subjected to a quantitative polymerase chain reaction.[20,21] Samples with a parasite concentration of ≥10 parasites per µl of blood were deep sequenced using the Multiplex Amplicons for Drug, Diagnostic, Diversity, and Differentiation Haplotypes using High-throughput Targeted Resequencing (MAD4HatTeR) protocol to confirm parasite species and identify molecular markers of drug resistance.[5]

Statistical analysis

R Studio (R version 4.3.2) was used for data cleaning, management, and analysis of the epidemiological and genomic data. Parasite genomic resistance profiles were linked to cleaned individual patient data extracted from the District Health Information System 2 (DHIS2) using unique sample barcodes. Where barcodes were missing, incomplete, or incorrectly captured, probabilistic fuzzy matching was applied using other identifying variables to link patient and genomic data. The DHIS2 dataset shared by the National Malaria Programme included patient demographic characteristics, method of diagnosis, case detection method, treatment given, recent travel history to endemic areas, and case classification based on travel history.

Imported cases were defined as malaria cases with a recent travel history to a malaria-endemic area outside South Africa, and symptom onset or diagnosis within seven to 21 days of return to South Africa. Locally acquired cases were defined as those with no recent travel to any endemic areas outside South Africa, where the source of infection can be traced to an endemic area within South Africa's borders. Infection severity was categorised as uncomplicated or severe based on treatment given and admission status. Cases treated with oral artemether-lumefantrine as outpatients, generally at primary healthcare facilities, were defined as uncomplicated malaria cases, while those admitted to secondary or higher-level healthcare facilities and treated with an intravenous antimalarial (artesunate or quinine) were classed as severe cases. Socio-demographic characteristics, the severity of infection, and parasite species were summarised and compared using descriptive statistics. Univariate analysis using Pearson's chi-square test or Fisher's exact test was conducted to assess statistically significant differences ($p<0.05$).

Mutation prevalence was calculated as the proportion of samples carrying a specific mutation among all samples successfully sequenced. Comparisons were performed for prevalence's of mutations in the kelch13 gene (associated with partial artemisinin resistance), the chloroquine resistance transporter (crt) and multidrug resistance 1 (mdr1) genes (associated with chloroquine resistance but lumefantrine susceptibility), and the dihydrofolate reductase (dhfr) and dihydropterate synthase (dhps) genes (associated with pyrimethamine and sulfadoxine resistance, respectively).[22,23] Mutation results were presented as either wild-type, mutant, or mixed-mutant (both mutant and wild-type genotypes are present).

Results

Patient demographic data

A total of 1 901 confirmed malaria cases, reported between January 2022 and August 2024, that met the case definition were successfully linked to their parasite genomic profiles and used in this analysis. The Ehlanzeni District in Mpumalanga accounted for 78% (1 483 /1 901) of the analysed cases, with the majority reported from Nkomazi Municipality (86%, 1 268/1 483). The 418 cases from uMkhanyakude District in KwaZulu-Natal were predominantly from the uMhlabuyalingana Municipality (46%, 191/418). Although imported cases constituted the majority in both districts, there was a significantly higher proportion of imported cases in uMkhanyakude District (93%, 389/418) compared to Ehlanzeni District (87%, 1 289/1 483, p<0.001) (Table 1).

Across both districts, imported cases were more frequently detected through active case detection activities compared to locally acquired cases (p<0.001), while infections in locally acquired cases were more likely to progress to severe disease (p<0.001). Although most infections in both locally acquired and imported cases were due to *Plasmodium falciparum* mono-infections (98%, 1 862), when *P. falciparum* co-infections were present, they were more prevalent among imported cases (2.3%) compared to locally acquired cases (p=0.011). Even though the median age of imported cases was slightly higher than that of locally acquired cases, 27 years (interquartile range [IQR]: 19–38 years), and 25 years (IQR: 13–41 years), respectively, the difference was not statistically significant. Males aged 20 years and older were the most affected group, irrespective of case classification (Figure 2).

Table 1. Socio-demographic characteristics of imported and locally acquired malaria cases reported from Ehlanzeni District, Mpumalanga, and uMkhanyakude District, KwaZulu-Natal, South Africa, 2022–2024.

Socio-demographic Characteristic		Imported N = 1 678	Locally acquired N = 223	P-value
Gender	Female	470 (39%)	73 (47%)	0.068
	Male	721 (61%)	82 (53%)	
	Unknown	487	68	
Age (Years)	Median age (IQR)	27 (19–38)	25 (13–41)	0.309
District	Ehlanzeni	1 289 (77%)	194 (87%)	**<0.001**
	uMkhanyakude	389 (23%)	29 (13%)	
Severity of infection	Severe	32 (1.9%)	18 (8.1%)	**<0.001**
	Uncomplicated	1 646 (98%)	205 (92%)	
Parasite species	*P.falciparum*	1 639 (98%)	223 (100%)	0.166
	P.falciparum + *P.malariae*	28 (1.7%)	0 (0%)	
	P.falciparum + *P.ovale*	8 (0.5%)	0 (0%)	
	P.falciparum + *P.ovale* + *P.malariae*	3 (0.2%)	0 (0%)	
Infection type	Co-infection	39 (2.3%)	0 (0%)	**0.011**
	Mono-infection	1 639 (98%)	223 (100%)	
Detection method	Active case detection	578 (36%)	20 (9%)	**<0.001**
	Passive case detection	1 044 (64%)	201 (91%)	
	Unknown	56	2	

Unknown: Missing data or incomplete information not included in the analysis; IQR: inter-quartile range;
Bold p-values are statistically significant.

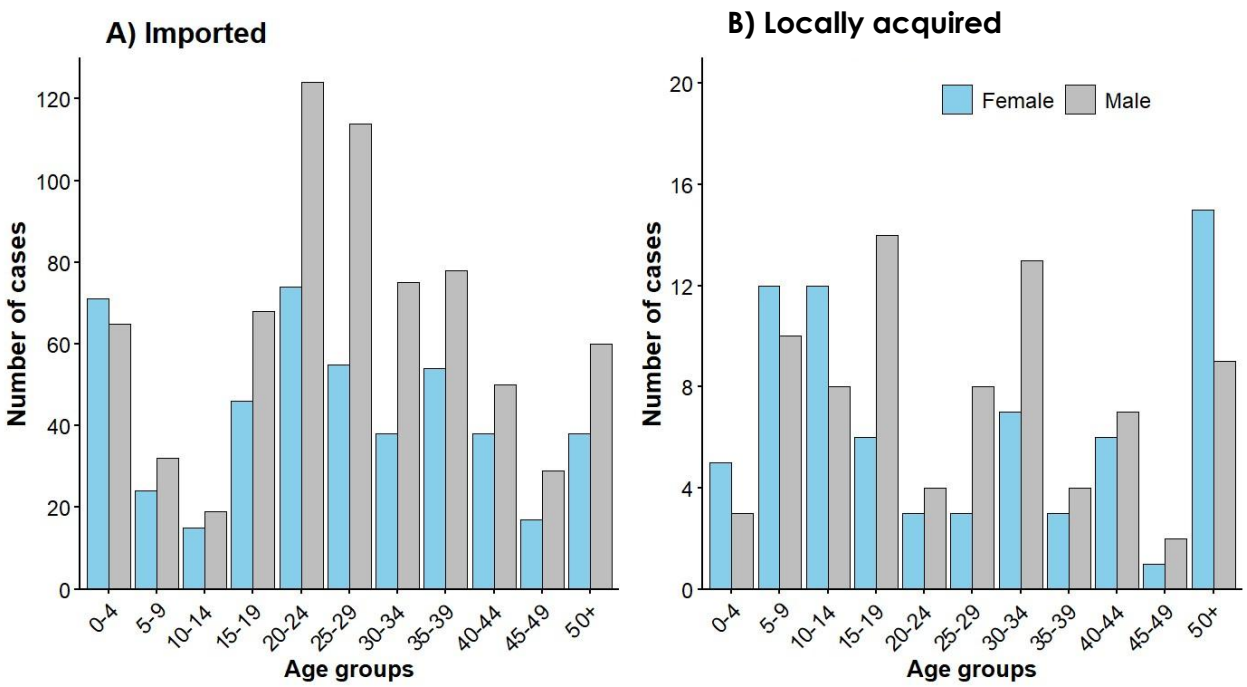

Figure 2. Age and gender distribution of A) imported and B) locally acquired malaria cases reported from Ehlanzeni District, Mpumalanga, and uMkhanyakude District, KwaZulu-Natal, South Africa, 2022–2024.

The number of reported cases of both imported and locally acquired cases followed a similar seasonal pattern, with case numbers increasing from mid-September, peaking in January and April–May each year. The number of imported cases peaked sharply in January 2023, May 2023, and January 2024. However, the number of locally acquired cases remained low during the two January peaks and only showed a notable increase in May 2023 (Figure 3).

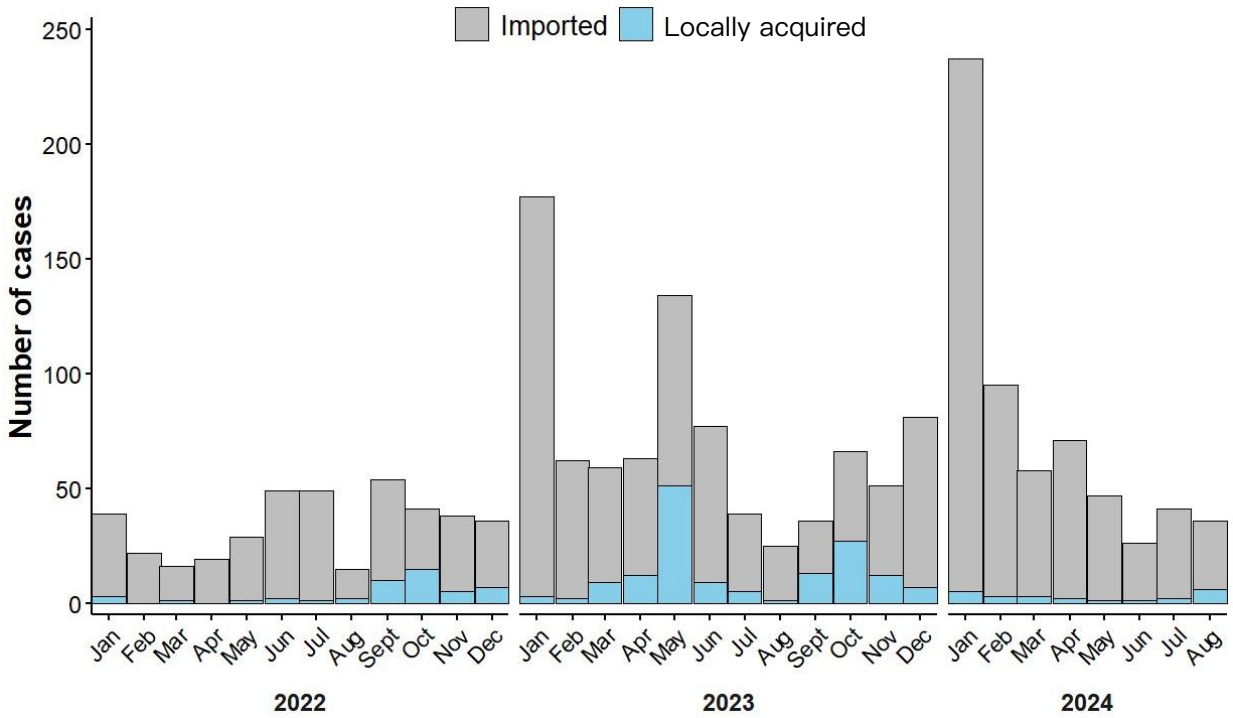

Figure 3. Number of imported and locally acquired malaria cases by month of diagnoses from Ehlanzeni District, Mpumalanga, and uMkhanyakude District, KwaZulu-Natal, South Africa, 2022–2024.

Drug resistance prevalence

Mutations (validated and associated) in the *kelch13, crt,* and *mdr1* genes were rare, occurring at a prevalence below one per cent in both imported and locally acquired cases (Table 2). However, when mutations in these three genes were present, they were generally detected in imported cases as opposed to locally acquired cases (Table 2). Markers of artemisinin partial resistance (V494I and P574L) and chloroquine resistance (CVMNK 72-76 CVIET) were only present in imported cases. In contrast, mutations in the *dhfr* (*dhfr* triple) *and dhps* (*dhps* double) genes, associated with resistance to pyrimethamine and sulfadoxine, respectively, were present in over 90% of the imported and locally acquired cases analysed.

Table 2. Prevalence of mutations in the *kelch13, crt, mdr1, dhfr,* and *dhps* genes among imported and locally acquired malaria cases reported from Ehlanzeni District, Mpumalanga, and uMkhanyakude District, KwaZulu-Natal, South Africa, 2022–2024.

Gene*	**Marker/Mutation**	**Interpretation for antimalarial treatment**	**Imported** (% Prevalence**)**	**Locally acquired** (% Prevalence)
kelch13	V494I (Mutant)	Associated marker (artemisinin partial resistance)	0.85	0.00
kelch13	P553L (Mutant)	Validated marker (artemisinin partial resistance)	0.00	0.61
kelch13	P574L (Mutant)	Validated marker (artemisinin partial resistance)	0.3	0.00
crt	CVMNK 72-76 CVIET (Wild-type)	Associated marker (chloroquine susceptibility, reduced lumefantrine susceptibility)	0.23	0.00
mdr1	N86F+N (Mixed-mutant)	Associated marker (chloroquine susceptibility, reduced lumefantrine susceptibility)	0.21	0.00
mdr1	N86F+N+Y (Mixed-mutant)	Associated marker (chloroquine susceptibility, reduced lumefantrine susceptibility)	0.96	0.73
mdr1	N86N+Y (Mixed-mutant)	Associated marker (chloroquine susceptibility, reduced lumefantrine susceptibility)	0.11	0.00
dhfr	Triple mutant	Very high-level pyrimethamine resistance marker	99.59	98.8
dhps	Double mutant	High-level sulfadoxine resistance marker	97.52	93.53
dhps	Triple mutant	Very high-level sulfadoxine resistance marker	0.55	0.00
dhps-dhfr	Quintuple mutant	High-level sulfadoxine-pyrimethamine resistance marker	97.22	92.75
dhps-dhfr	Sextuple mutant	Very high-level sulfadoxine-pyrimethamine resistance marker	0.46	0.00

**Chloroquine resistance transporter (crt), Multidrug resistance 1 (mdr1), Dihydrofolate reductase (dhfr), Dihydropterate synthase (dhps),* Haplotypes reported for *dhfr, dhps,* and *dhps-dhfr* genes

Discussion

This study found significant differences in epidemiological characteristics between imported and locally acquired cases reported from two South African malaria-endemic districts targeting elimination. Imported cases were generally detected through active case detection activities and were normally asymptomatic, while locally acquired cases were more commonly identified through passive case detection following symptomatic self-presentation to healthcare facilities. Imported cases, especially among mobile migrant adult males, tend to be asymptomatic infections, as the source of infection is normally higher-burden countries.[9,24] This suggests that these individuals have some level of acquired immunity, reducing the likelihood of symptomatic presentation. However, research has shown that even when symptomatic, mobile and migrant populations delay presenting to healthcare facilities as they face numerous barriers, including unfamiliarity with the local health system, communication challenges, and concerns related to immigration documentation status.[24,25] Given the threat that untreated, imported malaria poses to extremely low-transmission receptive areas targeting elimination, every effort must be made to promptly detect and treat all malaria carriers with standard treatment and the transmission-blocking antimalarial (single low-dose primaquine) to ensure a cure and limit the chances of onwards transmission.[26]

The observed increased risk of uncomplicated malaria progressing to severe disease among locally acquired malaria cases was concerning. This is likely an unfortunate consequence of South Africa's impressive gains against malaria over the years. The reduced malaria risk in many formerly high-burden areas across South Africa's endemic provinces has been accompanied by a declining index of suspicion for malaria among both healthcare workers and affected communities.[27] The early non-specific malaria symptoms such as fever, headache, and muscle pains tend to be initially associated with other more common conditions like influenza, COVID-19, or pneumonia, with malaria only considered when symptoms worsen.[25,28] Raising malaria awareness among healthcare workers and communities in areas targeting malaria elimination must be prioritised to reduce the chances of onwards transmission and, more importantly, progression to severe malaria and possible death.

Previous findings of an extremely low prevalence of molecular markers associated with artemisinin partial resistance in South Africa were congruent with our study, supporting the continued use of artemisinin-based combinations for malaria treatment.[29,30] Although artemisinin partial-resistance markers were rare, when present, they were generally detected among imported cases. Additionally, markers of chloroquine resistance, an antimalarial last used in South Africa in the late 1980s, were present only in imported cases, suggesting higher artemisinin and chloroquine drug pressure in regions outside of South Africa.[31,32]

Although sulfadoxine-pyrimethamine (SP) has not been used to treat malaria in KwaZulu-Natal since 2001 and in Mpumalanga since 2007, molecular markers associated with SP resistance and SP treatment failure were present in almost all locally acquired cases from both provinces. [31-33] This may reflect regional SP drug pressure as previously seen with SP or cross-resistance associated with the large-scale use of cotrimoxazole as prophylaxis or treatment of

bacterial and/or opportunistic infections in individuals living with HIV and/or tuberculosis.[5,34] The high prevalence of SP resistance markers precludes the use of SP in any drug combination for the treatment of malaria in South Africa.[26]

As only *falciparum*-confirmed infections were used in this analysis, the prevalence of non-falciparum infections could not be accurately estimated. However, previous studies have shown that over 90% of the reported cases in South Africa are a result of *P. falciparum* mono-infections, with *P. ovale*, *P. malariae,* and *P. vivax* infections rare and generally co-occurring with *P. falciparum*.[5,35] This was consistent with our study findings, as no isolated non-falciparum infections were found. Furthermore, no *P. falciparum-P. vivax* co-infections were observed, and mixed-species infections of *P. falciparum* co-occurring with either *P. ovale* or *P. malariae* were rare, especially among locally acquired cases. Isolated *P. vivax* infections have been reported in the Limpopo province and in neighbouring Botswana.[36,37] Further studies are still required to confirm the presence and estimate the prevalence of *P. vivax* malaria in South Africa.

This study had a few limitations. Firstly, malaria-eliminating districts in the Limpopo province were not included in the study. As a result, the generalisability of the findings and recommendations to similar settings within Limpopo is limited. Given the province's distinct epidemiological context and its contribution to the national malaria burden, a similar study in Limpopo is needed. Only malaria samples detected by the recommended point-of-care diagnostic in South Africa, falciparum-specific RDTs, were used to generate the genomic profiles. This may have resulted in non-*falciparum* mono-infections being undetected, resulting in an underestimation of the true burden of non-*falciparum* infections in the study areas. Lastly, the large sample size difference between imported and locally acquired cases resulted in an imbalanced comparison that may have limited the statistical power of the study findings.

Conclusion

This study identified demographic characteristic differences between imported and locally acquired cases in two low-transmission South African districts targeting malaria elimination that can be exploited to stem malaria transmission in these districts. Routine, sustained, active test-and-treat activities along known migratory pathways of mobile and migrant populations remain essential for reducing the risk of untreated imported cases sustaining residual transmission and/or introducing drug-resistant malaria parasites into non-immune populations residing in these low-transmission settings. Additionally, malaria awareness in communities residing in areas targeting elimination needs to be strengthened to improve early treatment-seeking behaviours, thereby reducing progression to severe disease. Healthcare professionals in these areas must maintain a high index of suspicion for malaria. The early detection and effective treatment of all malaria carriers are critical for malaria elimination.

Recommendations

- Strengthening malaria awareness through tailored communication for individuals living in low-transmission areas, encouraging early treatment-seeking behaviour when experiencing flu-like illness.
- Maintaining a high index of suspicion for malaria amongst healthcare professionals, particularly those in malaria-endemic risk areas, irrespective of transmission intensity.
- Strengthening and appropriate targeting of community-based test-and-treat activities to promptly detect and treat asymptomatic malaria carriers before they seed transmission. In low-transmission receptive areas targeting elimination, all eligible individuals should also receive a single low dose of primaquine to prevent onwards transmission.
- Targeted interventions that focus on the most affected high-risk groups, such as mobile migrant adult males, using communication methods tailored and proven effective for them.
- Use of malaria chemoprevention for individuals travelling to high-risk areas within and outside South Africa, which is available from pharmacies and travel clinics without a prescription. Doxycycline for malaria chemoprevention is now available at no cost in the South African public sector.
- Maintaining and strengthening existing cross-border collaborations, with harmonisation of malaria control and elimination interventions across borders.
- Strengthening of routine malaria genomic surveillance in South Africa for prompt detection and responses to emerging antimalarial drug resistance.

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Ethical considerations

The study is nested within the broader surveillance study approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M201124), the Mpumalanga Provincial Department of Health (MP_2015RP53_229), the KwaZulu-Natal Provincial Department of Health (KZ_202010_035), the South African National Department of Health and the University of California, San Francisco Institutional Review Board (350074).

Conflicts of interest

The authors declare no conflicts of interest regarding the authorship and publication of this article.

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