Potential Opportunities and Challenges of Deploying Next Generation Sequencing and CRISPR-Cas Systems to Support Diagnostics and Surveillance Towards Malaria Control and Elimination in Africa

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Recent developments in molecular biology and genomics have revolutionized biology and medicine mainly in the developed world. The application of next generation sequencing (NGS) and CRISPR-Cas tools is now poised to support endemic countries in the detection, monitoring and control of endemic diseases and future epidemics, as well as with emerging and re-emerging pathogens. Most low and middle income countries (LMICs) with the highest burden of infectious diseases still largely lack the capacity to generate and perform bioinformatic analysis of genomic data. These countries have also not deployed tools based on CRISPR-Cas technologies. For LMICs including Tanzania, it is critical to focus not only on the process of generation and analysis of data generated using such tools, but also on the utilization of the findings for policy and decision making. Here we discuss the promise and challenges of NGS and CRISPR-Cas in the context of malaria as Africa moves towards malaria elimination. These innovative tools are urgently needed to strengthen the current diagnostic and surveillance systems. We discuss ongoing efforts to deploy these tools for malaria detection and molecular surveillance highlighting potential opportunities presented by these innovative technologies as well as

challenges in adopting them. Their deployment will also offer an opportunity to broadly build in-country capacity in pathogen genomics and bioinformatics, and to effectively engage with multiple stakeholders as well as policy makers, overcoming current workforce and infrastructure challenges. Overall, these ongoing initiatives will build the malaria molecular surveillance capacity of African researchers and their institutions, and allow them to generate genomics data and perform bioinformatics analysis in-country in order to provide critical information that will be used for real-time policy and decision-making to support malaria elimination on the continent.

OVERVIEW

Significant progress was made between 2005 and 2015 in malaria control globally but progress has stalled in recent years (World Health Organization, 2020). Changes in malaria burden were attributed to different interventions that target both mosquito vectors and parasites, which have been scaled-up in the past two decades. Recent evidence shows an increase in malaria cases globally from 2017 to 2019 compared to previous years, but the number of deaths remained relatively unchanged from 2017 to 2019. However, in 2020, malaria deaths increased from 2019 levels, with the majority of both cases and deaths involving children and pregnant women from sub-Saharan Africa (SSA) (World Health Organization, 2021). The increase in malaria cases can be partially explained by the emergence and spread of insecticide resistance in *Anopheles* populations (Kisinza et al., 2017 and antimalarial resistance in parasites (Dondorp et al., 2009; Haldar et al., 2018; Ippolito et al., 2021) both of which threaten the effectiveness of the major malaria interventions, long-lasting insecticide treated bed-nets (LLINs), indoor residual spraying (IRS) and antimalarial drugs (Tawe et al., 2018; Apinjoh et al., 2019; Morgan et al., 2020). Increased malaria cases in 2020 were also attributed to the COVID-19 pandemic, which was believed to have disrupted malaria control activities (World Health Organization, 2021). However, other potential factors such as transmission from asymptomatic reservoirs (Andolina et al., 2021), climate change, and other environmental changes caused by human activities are thought to be responsible for the resurgence of malaria (Upatham et al., 1988; Chaumeau et al., 2019; Mitchell et al., 2022).

In 2020, 29 countries in Africa contributed about 96% of all reported malaria deaths globally, despite consistent implementation of the major malaria interventions since 2002 (World Health Organization, 2021). Malaria control in Africa relies mainly on the use of LLINs and effective antimalarial drugs, predominantly artemisinin-based combination therapy (ACTs) for routine case management and sulphadoxine/pyrimethamine (SP) for intermittent preventive therapy in pregnant women (iPTp) (World Health Organization, 2021). Despite the detection of insecticide resistance and occurrence of mutations in *PfKelch13* gene (associated with resistance to artemisinins) in Rwanda, Uganda and Eritrea, treatment failure rates remain below 10%, hence both ACT and vector control measures are still considered highly effective (World Health Organization, 2021). However, a robust surveillance and response strategy is urgently needed to detect any early failures in the current interventions and facilitate effective response to ensure malaria elimination by 2030.

At present, malaria case management and routine surveillance rely on traditional diagnostic methods based on microscopy and rapid diagnostic tests (RDTs). These methods are widely deployed by the National Malaria Control Programmes (NMCPs) in most malaria endemic countries in Africa (Tawe et al., 2018; Abbas et al., 2019; Tessema et al., 2019b; Morgan et al., 2020). Despite their short turnaround time, these methods have major limitations (Apinjoh et al., 2019 (**Table 1**). In areas of low transmission and those closer to malaria elimination, microscopy and RDTs are less sensitive and unable to detect transmissible but very low parasite densities (Nsanzabana, 2019; Andolina et al., 2021). Since most of the widely used RDTs are based on the detection of histidine rich protein 2 (HRP2), the situation is further exacerbated by the emergence and spread of *Plasmodium falciparum* with histidine rich protein 2 and 3 (*pfhrp2* and *pfhrp3*) gene deletions (Thomson et al., 2019; Feleke et al., 2021). To strengthen the current surveillance system, innovative methods of malaria diagnosis and population surveillance based on advanced molecular techniques are urgently needed to support effective case management (Ishengoma et al., 2019.

Two key technologies that are slated to have a large impact on surveillance and diagnosis of malaria are Next Generation Sequencing (NGS) and clustered regularly interspaced short palindromic repeats (CRISPR). NGS, which is also termed high-throughput or massive parallel sequencing, allows for thousands to billions of DNA or RNA fragments to be sequenced and analyzed in either a targeted or whole genome approach (Deurenberg et al., 2017; Talundzic et al., 2018). Routine malaria surveillance programs could benefit from deployment of NGS technologies, as they provide a scalable, cost-effective means of surveillance in large numbers of samples (Juliano et al., 2010; Van Tyne et al., 2011; Manske et al., 2012; Lerch et al., 2017; Ngondi et al., 2017; Tawe et al., 2018; ; Moser et al., 2021).

CRISPR and CRISPR associated protein (Cas) derived from type II CRISPR bacterial immune systems, provide the basis for a new family of assays that detect nucleic acid targets with high sensitivity and specificity. While CRISPR-Cas is mostly known to enable easy gene editing, it also has enormous diagnostic potential (Jiang et al., 2013. Indeed, CRISPR-Cas systems have

TABLE 1 | Various methods used for diagnostic and surveillance of malaria.

Method	Target	Sensitivity (percentage of true positives detected)	Specificity (percentage of true negatives detected)	Limit of detection	Cost per sample (USD)	Time	Advantages	Limitations	Reference
Microscopy	N/A	95%	98%	50–200 parasites/μL of blood	$0.12–$0.40	60 min	Identification of parasite morphologies, species and stage	Requires trained personnel and microscopes	(Hopkins et al., 2013; Pham et al., 2018; Mbanefo and Kumar, 2020)
Rapid Diagnostic Test (RDT)	PfHRP2, PfLDH	85% to 94.8%	95.2% to 99%	50–200 parasites/μL of blood	$0.60–$2.50	15–30 min	Fast and easy to use	Mutation in pfhrp-2 leading to false negatives, Unable to quantify parasitaemia, can produce false-positive results well after resolution of infection	(Cordray and Richards-Kortum, 2012; Hopkins et al., 2013; Pham et al., 2018; Mbanefo and Kumar, 2020)
Polymerase Chain Reaction (PCR)	18S rRNA, cox3, TARE-2, varATS and Pfs25	98% to 100%	88% to 94%	0.5–5 parasites/μL of blood	$0.35–$5.00	1-2 h	Low limit of detection makes it easier to detect low parasitaemia, High throughput, detects drug-resistant parasites, mixed infections	Requires expensive instruments and reagents and is not able to quantify parasitaemia	(Tangpukdee et al., 2009; Cordray and Richards-Kortum, 2012; Hopkins et al., 2013; Pham et al., 2018Mbanefo and Kumar, 2020)
High-volume quantitative PCR (qPCR)	Plasmodium sp. 18S RNA	100%	99.75%.	0.1 parasite/μl of blood	$0.50	45 min-2h	Low limit of detection makes it easier to detect low parasitaemia	Requires expensive instruments and reagents, Requires trained personnel	(Kamau et al., 2013; Imwong et al., 2014; Haanshuus et al., 2019)
Nucleic Acid Sequence-Based Amplification (NASBA)	18S mRNA	97.4–100%	80.9–94%	0.01–0.1 parasites/μL of blood	$5-$20	1–2 h	No thermocycler needed	Requires highly trained personnel, expensive	(Cordray and Richards-Kortum, 2012; Pham et al., 2018; Mbanefo and Kumar, 2020)
Loop-mediated Isothermal Amplification (LAMP)	18S rRNA, mDNA	98.3% to 100%	94.3% to 100%	1–5 parasites/μL of blood	$0.28-$5.31	30–60 min	Low limit of detection, faster reaction time than PCR, no thermocycler needed, high throughput	Easily susceptible to contamination	(Cordray and Richards-Kortum, 2012; Pham et al., 2018; Mbanefo and Kumar, 2020)
Serological test	Detection of antibodies against parasites	69.9%	100%	50–200 parasites/μL of blood	$0.50-$5.50	30-60 min	Useful for epidemiologic surveys, Relatively cheap, Provides retrospective confirmation of malaria infection	Not suitable for the diagnosis of acute malaria, Cannot discriminate species	(She et al., 2007; Tangpukdee et al., 2009; Tusting et al., 2014; Goh et al., 2021)

cox3, Mitochondrial cytochrome c oxidase III; TARE-2, Telomere associated repetitive element; varATS, Var. gene acidic terminal sequence; PfLDH, P. falciparum lactate dehydrogenase; PfHRP2; Plasmodium falciparum histidine-rich protein 2.

*Sensitivity and specificity estimates come from (Boonma et al., 2007; Han, 2013) cost estimates come from (Han, 2013) except NASBA (Mbanefo and Kumar, 2020) and serology (Tusting et al., 2014; Goh et al., 2021).

been utilized to detect and differentiate arbovirus strains (Gootenberg et al., 2017, to diagnose SARS-CoV-2 infections (Guo et al., 2020, to distinguish pathogenic bacteria (Strich and Chertow, 2019, to diagnose cancers (Yin et al., 2019, and indeed to diagnose malaria (Lee et al., 2020; Cunningham et al., 2021).

This review discusses ongoing efforts to deploy advanced molecular tools for the diagnosis and surveillance of malaria,

developing the capacity for pathogen genomics in Africa, and highlights both potential opportunities presented by these innovative technologies as well as challenges facing these efforts.

Epidemiological Applications of NGS

Our ability to detect and track the spread of malaria is potentially improved by advances in malaria genomics (particularly those

FIGURE 1 | Current and potential application of NGS in studies of parasite populations, drug resistance and surveillance of hrp2/3 gene deletions.

based on NGS) and associated bioinformatics tools in combination with epidemiological data (Escalante et al., 2015; Kwiatkowski, 2015; Watson et al., 2021). These tools can facilitate different studies of parasite populations, antimalarial drug resistance, surveillance of hrp2/3 gene deletions, and monitoring the impact of current and future interventions (**Figure 1**). Studies utilizing these methods are urgently needed and if scaled-up, can be critical for monitoring transmission to achieve proper control and the ultimate goal of malaria elimination.

Monitoring Malaria Transmission
Historically, transmission intensity and dynamics of malaria burden were measured using tools like entomological inoculation rates (EIR), case incidence and prevalence. These tools have helped to generate maps of mortality rates, prevalence and incidence as well as the overall global burden of malaria (Weiss et al., 2019; Neafsey et al., 2021), but recently have become less sensitive especially in areas of very low transmission. Due to their inherent limitations, they are unable to detect genetic signatures and parasite population structure indicative of modified transmission in response to interventions. For example, higher incidence could indicate a decline in the effectiveness of malaria interventions, even though the increase might actually be due to increased transmission rates from a source which was not addressed in the initial interventions (Mohd Abd Razak et al., 2016). Furthermore, decreased transmission limits outcrossing, influencing high inbreeding rates within the population and triggering population fragmentation. As such, conventional surveillance methods may result in misinterpreting the underlying processes shaping malaria epidemiology. Advances in NGS technologies supported by bioinformatic tools and increased availability of epidemiological data have made it possible to generate high quality and sensitive data which may help detect changes in transmission intensity, identify multi-genomic infections, evaluate intervention effectiveness, and identify potential

deficiencies in malaria control programmes in both high and low transmission areas (Watson et al., 2021). This improved understanding can be achieved by determining the number of parasite strains, genetic diversity (e.g. within-host diversity index (F_{ws}) as well as relatedness and genetic structure of parasites in an infection.

Monitoring Parasite Populations
Polymorphic genes such as merozoite surface protein (*msp1*), merozoite surface protein 2 (*msp2*) and glutamate rich protein (*glurp*) have been used extensively to study parasite genetic diversity and population structure, mostly to detect complexity/multiplicity of infection (COI/MOI), wherein multiple distinct genotypes co-occur in a single host (Lalremruata et al., 2017). Studies using these methods have demonstrated associations between MOI and such factors as host age, clinical severity, and transmission intensity (Mayengue et al., 2009; Lalremruata et al., 2017). However, these PCR-based genotyping methods have limited inter-laboratory reproducibility and also lack sensitivity, hence may underestimate allelic diversity in co-infected hosts (Assefa et al., 2014). Highly sensitive NGS tools, by contrast, can illuminate not only MOI but also parasite transmission history, genetic structure, recombination, and evolutionary history.

The use of these tools to study evolutionary history can improve our understanding of the parasite-host interactions involved in the emergence of new parasites in the region (Su et al., 2020, as well as evolutionary responses to selective pressures caused by intensified malaria elimination interventions, such as antimalarial drugs (Neafsey and Volkman, 2017). It is even possible to estimate recombination events that may lead to evasion of control and detection efforts (Miles et al., 2016). In addition, NGS data facilitates population genetic and evolutionary metric calculations, such as linkage disequilibrium (LD), effective population size (N_e), site frequency spectrum (SFS), identity by descent (IBD), identity by state (IBS), haplotype diversity (H),

principal component analysis (PCA), F_{WS}, and F_{ST}. The extent of parasite genomic data now available provides opportunities to expand and refine the global genome database and public resources, which in turn will greatly expand our understanding of parasite biology and accelerate the discovery of new interventions.

Parasite Connectivity and Importation

Traditionally, case incidence was used in combination with human migration data to link parasite populations across geographical and transmission gradients, which would inform intervention strategies. However, this method lacks the precision of NGS-based methods.

Genetic relatedness inferred from genomic data provides much higher geographic resolution of transmission (Tessema et al., 2019a) and can be quantified between 0 (completely unrelated parasites) and 1 (identical clones). Genetic relatedness between parasite samples is positively correlated with reduced transmission, and therefore can be used as a proxy measure of transmission level (Daniels et al., 2015; Cerqueira et al., 2017). Using advanced NGS tools, it is also possible to infer malaria transmission networks over a given timescale in a population (Ajibola et al., 2021). Importation of malaria parasites from higher to lower transmission areas, particularly those in the elimination phase, remains a major challenge to malaria elimination, and is difficult to detect using traditional methods. However, NGS methods are able to do so, as was demonstrated in Zanzibar (Morgan et al., 2020). Strengthening malaria molecular surveillance (MMS) using advanced genomic tools will reveal the number of secondary cases arising from each imported infection, allowing NMCPs to determine what interventions are required to prevent re-establishment of transmission. The data derived from effective MMS systems will also help to resolve the contribution of superinfection and co-transmission (Nair et al., 2014; Nkhoma et al., 2018).

Using NGS to Address Challenges in Malaria Control and Elimination
Antimalarials and Drug Resistance

Antimalarial resistance is a major obstacle to the effective control and elimination of malaria. Malaria parasites have evolved resistance to all widely deployed antimalarial drugs, leading to withdrawal of some of the drugs such as chloroquine and SP (Ishengoma et al., 2019). Antimalarial drug resistance has spread globally, although it remains rare and localized in some areas with lower transmission and therefore lower selective pressure (Haldar et al., 2018). We can clearly use these methods to retrospectively understand how and why resistance emerged and spread. However, their prospective use by NMCPs through enhancing genomic surveillance infrastructure can identify signatures of emerging drug resistance both when resistance mutations are known and unknown (Neafsey et al., 2021.

Antimalarial efficacy monitoring is usually done in therapeutic efficacy studies (TES) which enroll infected patients and genotype those with recurrent infection after treatment to distinguish between recrudescence (drug failure) and reinfection (World Health Organization, 2009). Traditional methods rely on the polymorphic markers mentioned above (World Health Organization, 2007) or microsatellites (Greenhouse et al., 2006; Malvy et al., 2018). While these methods are widespread, they are limited by their low throughput, poor resolution, and technical challenges regarding analysis, scoring and interpretation of results. Specifically, these methods under-estimate MOI or misclassify infections as either recrudescent or reinfection (Shaukat et al., 2012). However, new NGS methods using amplicon sequencing (Rao et al., 2016 or SNP barcodes are being optimized and may potentially replace the current methods, which would improve estimates of antimalarial efficacy especially in low transmission areas with limited diversity and polymorphisms in parasite populations.

NGS provides a robust, high throughput tool for identifying and tracking molecular markers of drug resistance (Ishengoma et al., 2019). Currently available markers can be used for spatial and temporal monitoring of resistance to different antimalarials, including both current drugs and those which were used in the past (Rao et al., 2016; Levitt et al., 2017; Nag et al., 2017; Talundzic et al., 2018). This monitoring is especially critical in the context of partial artemisinin resistance, which has already been reported in South-East Asia (SEA) (Leang et al., 2015; Takala-Harrison and Laufer, 2015). Artemisinin resistant parasites may be introduced from SEA (as drug resistance genes are known to spread between populations) (Roper et al., 2004; Dwivedi et al., 2016) or could potentially emerge independently in SSA, underscoring the necessity of this continuous surveillance.

It is important to understand the origin and flow of resistance genes in order to anticipate the diffusion of antimalarial resistance and interrupt its spread (Dwivedi et al., 2016). Current methods for doing this are expensive and less robust. For example, Sanger sequencing is considered to be useful in identification of major alleles but has low accuracy in identifying alleles with frequency less than 50% (minor alleles) (Rohlin et al., 2009). NGS based methods are promising as they are high throughput, less labor intensive, have high sensitivity in identifying all variants, even those with minor allele frequencies, and facilitate the discovery of novel markers (Tessema et al, 2019a).

Surveillance of *pfhrp2/3* Gene Deletions

Malaria rapid diagnostic tests (mRDTs) based on lateral flow detection of *P. falciparum* Histidine-Rich Protein 2 (*Pf*HRP2) have supported prompt and effective identification of *P. falciparum* malaria infections, particularly in resource poor regions. However, recent studies in various countries have reported some cases where pfHRP2 based mRDTs failed to detect malaria parasites in patients with positive results by either microscopy or PCR (Gamboa et al., 2010; Thomson et al., 2019; Bakari et al., 2020). While other factors may be involved (Murray et al., 2008, deletion of the *pfhrp2* gene (as well as the related *pfhrp3* that cross-reacts in RDTs) has also been reported to contribute to false-negative test results (Luchavez et

al., 2011; Cheng et al., 2014; Thomson et al., 2019), which has significantly compromised the performance of *Pf*HRP2-based RDTs. Thus, WHO currently recommends for all malaria endemic countries to undertake surveys to identify the prevalence and trends of the deletion in space and time (World Health Organization, 2019).

NGS data enables both the detection of emergence and monitoring of *pfhrp2*/3 gene deletion frequencies in all settings (low and high transmission) in relation to the pressure caused by the use of HRP2-based RDTs. With intensified surveillance, it will therefore be possible to identify areas which may need to change diagnostic strategies for effective malaria control. The deletion is currently detected based on initial clinical evidence (e.g. a microscopy-positive result for *P.falciparum* but negative RDT) and confirmed with molecular approaches such as PCR and antigen detection assays (Cheng et al., 2014). However, some samples can be RDT-negative but have intact *pfhrp2*/3 genes (Nsobya et al., 2021). Indeed, a study from the Democratic Republic of Congo that performed WGS on samples with suspected deletions showed that the genes were actually intact (Parr et al., 2021). However, recent data from the Horn of Africa, which utilized complementary molecular, immunological and sequencing assays, demonstrates that there have been multiple independent deletions in *pfhrp3*, which have been accompanied by strong selection for a single *pfhrp2* deletion due to pressure from RDTs (Feleke et al., 2021). As such, the MalariaGEN network has highlighted the utility of widely deployed NGS as a critical tool to detect the true *pfhrp2*/3 gene deletion prevalence (MalariaGEN et al., 2021.

Gene diversity can potentially explain the variability in the sensitivity of *Pf*HRP2-based RDTs (Baker et al., 2005; ; Wurtz et al., 2013; Li et al., 2015; Gendrot et al., 2019). Indeed, studies conducted in different geographical settings have shown that gene diversity in the *pfhrp2* gene plays a role in the sensitivity of RDTs especially among infections with low parasitaemia (≥ 200 asexual parasites/μl) (Baker et al., 2010). Given that countries such as Eritrea (Berhane et al., 2018 and Ethiopia (Golassa et al., 2020 have reported abnormally high prevalence of these deletions, NGS methods will likely be necessary for further characterization and monitoring of the diversity in these genes.

NGS and CRISPR as Novel Surveillance and Diagnostic Tools

Timely and accurate diagnosis is a key element of effective malaria management. Traditional microscopy and RDT-based diagnostic methods involve detection of malaria parasites or products e.g. antigens in the blood of infected patients (Kasetsirikul et al., 2016). In other laboratories, malaria diagnosis involves molecular methods such as PCR (**Table 1**). Both microscopy and RDTs have been deployed as diagnostic tools in many malaria endemic areas, despite low sensitivity due to their high detection limits (≥ 50 parasites/μL) and limited ability to detect non-falciparum species. These deficiencies cause under-reporting of infections, specifically in areas with low transmission and for individuals with asymptomatic malaria or low density infections (Slater et al., 2019). By contrast, PCR has

high sensitivity and specificity which make it capable of detecting low parasite density of less than 5 parasites/μl. This increased sensitivity will improve our ability to diagnose very low density infections, and detect asymptomatic individuals who can unknowingly contribute to disease transmission. Furthermore, the NGS-enabled ability to scan hundreds of *Plasmodium* genomes to better understand parasite biology and for novel diagnostic markers facilitates the development of tools that are species-specific, highly sensitive and agreeable to PCR or other nucleic acid amplification techniques (Lucchi et al., 2013.

In addition to the shortcomings outlined above, currently widespread robust malaria diagnostics require substantial financial investment and equipment, along with extensive personnel training. CRISPR-based diagnostics are an exciting development, as they have similar sensitivity and specificity to PCR assays, but require fewer resources, and are fast, cost-effective, and easy to use (Kostyusheva et al., 2021). The simplicity of the CRISPR-Cas based detection system allows the rapid development of diagnostic methods of a wide array of infectious diseases (Jiang et al., 2013; Gootenberg et al., 2017; Kostyusheva et al. 2021). Four CRISPR-based diagnostic methods have particular potential to replace PCR-based methods in low-resource settings: DETECTR, SHERLOCK, CARMEN, and CRISPR-Chip (Kaminski et al., 2021; Kostyusheva et al., 2021). These methods work reliably in isothermal conditions (negating the need for an expensive thermocycler) and results can be determined by lateral flow strips at the point of care, or by fluorescence read by a fluorimeter, or in some cases, with the naked eye. The use of lateral flow strips or visual inspection of fluorescence avoids the use of more technically challenging gel electrophoresis or quantification using a qPCR machine (Kaminski et al., 2021; Kostyusheva et al., 2021). CRISPR-based methods that have the potential to be developed for malaria diagnosis are described in **Table 2**.

The deployment of CRISPR-based diagnostic methods for *P. falciparum* could revolutionize the field of malaria surveillance in Africa. The ability of this method to detect as little as one copy of target DNA per reaction will play a major role in the detection of both symptomatic and asymptomatic infections and any associated resistance mutations (Kwiatkowski, 2015. CRISPR has already been used to detect malaria strain variants in mixed infections, an important challenge for malaria control and elimination efforts and a modality that could be extended to many areas of epidemiology (Kwiatkowski, 2015. One-pot systems that are suitable for field deployment due to their minimal technological and logistical prerequisites, high diagnostic reliability, and rapid turnaround time have been developed for SARS-CoV-2 and show additional promise for the expansion of these tools in malaria-endemic countries (Nguyen et al., 2021). The development of isothermal reactions performed with lyophilized reagents and with straightforward visualization at the point-of-care are particularly revolutionary as a means to quickly, cheaply, and accurately diagnose malaria infections in low-resource and/or remote locations (Cunningham et al., 2021). While these tools are still in early

TABLE 2 | CRISPR-based diagnostics used in the context of malaria.

End Point	Method (Cas enzyme, amplification)	Advantages	Limitations	Reference
Lateral Flow	SHERLOCK Version 2 (Cas13 RPA)	Similar sensitivity to RT-PCR methods but without the need for expensive thermocyclers; highly specific species delineation capabilities; can perform drug-resistance genotyping; potential for use in mosquitoes as well as in clinical samples	Not yet ready for wide-scale field use; requires higher crRNA concentrations than SHERLOCK in other pathogens; "one-pot" approach not yet achievable; assay design costs are high	(Gootenberg et al., 2018; Kellner et al., 2019)
	SHINE Cas13 RPA)	Single step tool with high sensitivity compared to RT-qPCR. Detects virus from unextracted samples. Reduced contamination risk as amplification reaction tubes remain sealed.	Not yet validated with field samples	(Arizti-Sanz et al., 2020
	STOPCovid (Cas12 LAMP)	Sensitivity of this tool is similar to RT-qPCR. Appropriate for low-complexity clinical laboratories.	Not yet validated with field samples	(Joung et al., 2020
Fluorescence/ Colorimetry	SHERLOCK VERSION 1 (Cas9, RPA)	Similar sensitivity to RT-PCR methods but without the need for expensive thermocyclers; highly specific species delineation capabilities; can perform drug-resistance genotyping; potential for use in mosquitoes as well as in clinical samples	Not yet ready for wide-scale field use; requires higher crRNA concentrations than SHERLOCK in other pathogens; "one-pot" approach not yet achievable; assay design costs are high	(Cunningham et al., 2021
	SHERLOCK version 2 (Cas13/RPA)	Multiplexable, portable, rapid, and quantitative detection platform of nucleic acids.	Not yet validated with field samples	(Gootenberg et al., 2018; Kellner et al., 2019)
	CARMEN (Cas13, PCR/ RPA)	Detects all human-associated viruses with high sensitivity. Enables comprehensive subtyping of some viruses e.g. influenza A strains and multiplexed identification of dozens of HIV drug-resistance mutations	Not yet validated with field samples	(Ackerman et al., 2020
	NASBACC (Cas9/NASBA)	Can discriminate between viral strains with single base resolution	Long turnaround time Not yet validated with field samples Challenge in the sample preparation	(Pardee et al., 2016
	DETECTR	Higher sensitivity than SHERLOCK; functions in a single tube ("one-pot" approach); isothermal like SHERLOCK; all reaction components can be lyophilized (no need for refrigeration)	Not yet validated with field samples	(Lee et al., 2020

development, their accuracy, ease-of-use, cost-effectiveness, and rapidity give them great potential to complement currently available tools.

APPLICATION OF NGS AND CRISPR-CAS SYSTEMS IN AFRICA: CURRENT STATUS AND FUTURE PERSPECTIVES

In Africa, few studies have been undertaken to determine the diversity and other features of parasite populations using NGS methods. Previous studies utilized PCR genotyping of polymorphic genes (*msp1*, *msp2* and *glurp*) to determine parasite diversity with little focus on population structures and other population genetics metrics (Carlsson et al., 2011; Yavo et al., 2016; Metoh et al., 2020; Amoah et al., 2021). Recently, some regional studies have been conducted in Africa using genomic tools and have provided initial data on parasite population structure (Manske et al., 2012; Amambua-Ngwa et al., 2019; Abera et al., 2021; Moser et al., 2021). However, other areas, particularly those with low transmission, remain largely

unexplored (Morgan et al., 2020). In addition, the metrics that are routinely used in these studies [e.g. F_{WS}, F_{ST} (the fixation index), COI/MOI, IBD, inbreeding coefficient (F), and coefficient of uniqueness (COU)] have not been fully evaluated to be reliably informative for monitoring intervention-modulated changes in malaria transmission (Abera et al., 2021; Onyango et al., 2021). Therefore, further intensive study and metric development is required. The integration of traditional epidemiological data with NGS data will likely improve malaria surveillance in service of elimination strategies. While CRISPR-based diagnostics could revolutionize the field of malaria surveillance in malaria endemic countries in Africa, they have not yet been deployed or tailored to the specific needs of NMCPs.

Meanwhile, there are ongoing collaborative projects between African researchers, NMCPs, and partners in the USA and Europe to establish local molecular, genetic, and genomic laboratory and analytic capacity to support MMS. These projects are located in several countries in SSA including Senegal, Mozambique, Tanzania, Mali, The Gambia, Ghana, Gabon, Madagascar, Cameroon, Ivory Coast, Burkina Faso, Ethiopia and Uganda (Amambua-Ngwa et al., 2019; MalariaGEN et al., 2021). In Tanzania, previous efforts were

made with the support of different funding agencies and partners and set-up the stage for the current initiatives (Amambua-Ngwa et al., 2019; MalariaGEN et al., 2021). Our current collaboration (Molecular Surveillance of Malaria in Tanzania, MSMT) is funded by the Bill and Melinda Gates Foundation and focuses on six key aims to attain the bold and ambitious goal of establishing local capacity for in-country generation and analysis of genomics data to support policy and decision making by the NMCP and its stakeholders. To that end, Tanzanian stakeholders identified three scientific aims for MSMT: 1) unraveling parasite population genetics, 2) country-wide drug resistance monitoring, and 3) country-wide surveys of *pfhrp2/3* gene deletion. In addition, the project will address three supportive aims with a focus on training for capacity building, career development, and science leadership for sustainability of MMS. The project will also focus on developing a reliable model for effective engagement with NMCP and other stakeholders, and developing use cases for MMS in Tanzania. Lastly, the project will develop and implement a framework and platforms for storage, management, analysis, and sharing of the genomic data and associated metadata.To successfully adopt and transfer NGS and bioinformatics tools to Africa and build local capacity, a robust strategy of capacity building is critical. The MSMT team has developed a "counterpart model" of capacity building. This model involves pairing Tanzanian researchers with US-based expert (counterparts) collaborators with the role ensuring successful technology transfer.

To ensure key local stakeholders are effectively engaged, the MMS projects should bring on board all local stakeholders and ensure they are fully engaged in the project. In Tanzania, the MSMT project established an advisory committee which is under the Chief Medical Officer of the Tanzanian Ministry of Health with the role of providing oversight and guiding the implementation and sustainability of the project. The project is run by the joint implementation committee, which includes the study team, NIMR, NMCP and the Ministry in charge of the local governments (which owns and runs all health facilities in Tanzania). The technical implementation committee involves NIMR researchers, NMCP and collaborating partners who are advised by a committee of experts with experience and track records in MMS and capacity building particularly in Africa.

Thus far, a genomics laboratory has been established at NIMR in Dar es Salaam and will soon be equipped with state-of-the-art equipment to ensure genomics data are generated in-country and analysed by local experts from the second year of the project in 2022. The Tanzanian researchers have sequenced over 8,500 field collected samples in laboratories in the US as part of their training, and future analysis will take place in the NIMR laboratory with further training and coaching from the US-based collaborators. It is anticipated that our training and capacity building model and the laboratory capacity to be built in Tanzania will pave a way to a sustainable MMS for supporting not only malaria but other pathogens as part of the broader pathogen genomics initiative.

CHALLENGES ASSOCIATED WITH NGS APPLICATION IN AFRICA

African researchers seeking to answer biological questions using NGS face many challenges, ranging from funding to procurement to lack of trained personnel (**Table 3**). The long-standing imbalance between collaborators in the global North and South has resulted in unsustainable patterns of research practice that must be broken if African researchers are to be free to initiate and execute NGS-dependent research studies. For the wide application of NGS technology in malaria surveillance, African countries need substantial investment to establish genomic capacity (Haanshuus et al., 2019 including human capacity, ancillary equipment for library preparation, quality control and sequencing (Escalante et al., 2015.

STUDY DESIGN AND SAMPLE COLLECTION

Historical imbalances are visible beginning with the funding and design of research programs. Too often, minimal intellectual input is sought from African researchers during this phase, and donors must critique their funding calls to address this
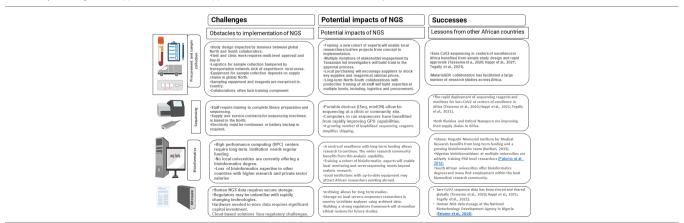
TABLE 3 | Challenges and opportunities for NGS application in a resource constrained country such as Tanzania.

	Challenges	Potential impacts of NGS	Successes
	Obstacles to implementation of NGS	Potential impacts of NGS	Lessons from other African countries
Procurement and sample collection	•Study design impacted by tensions between global North and South collaborators. •Field and clinic work requires multi-level approval and buy-in •Logistics for sample collection hampered by transportation network, lack of expertise in rural areas. •Equipment for sample collection depends on supply chains in global North. •Sampling equipment and reagents are overpriced in-country. •Collaborations often lack training component	•Training a new cohort of experts will enable local researchers to drive projects from concept to implementation. •Multiple iterations of stakeholder engagement by Tanzanian led investigators will build trust in the approval process. •Local purchasing will encourage suppliers to stock key supplies and reagents at rational prices. •Long-term North-South collaborations with productive training of all staff will build expertise at multiple levels, including logistics and procurement.	•Sars-CoV2 sequencing in centers of excellence in Africa benefited from simple study design and rapid approvals (Tessema et a., 2020; Happi et al., 2021; Tegally et al., 2021). •MalariaGEN collaboration has facilitated a large number of research studies across Africa.
Sequencing	•Staff require training to complete library preparation and sequencing. •Supply and service contracts for sequencing machines is based in the North. •Electricity must be continuous or battery backup is required.	•Portable devices (iSeq, minION) allow for sequencing at a clinic or community site. •Computers to run sequencers have benefitted from rapidly improving GPU capabilities. •A growing number of lyophilized sequencing reagents simplifies shipping.	•The rapid deployment of sequencing reagents and machines for Sars-CoV2 at centers of excellence in Africa (Tessema et al., 2020; Happi et al., 2021; Tegally et al., 2021). •Both Illumina and Oxford Nanopore are improving their supply chains in Africa.
Bioinformatics	•High performance computing (HPC) centers require long-term funding •No local universities are currently offering a bioinformatics degree. •Loss of bioinformatics expertise to other countries with higher research and private sector salaries	•A centre of excellence with long-term funding allows research to continue. The wider research community benefits from this analysis capability. •Training a cohort of bioinformatics experts will enable local mentoring and serve bioinformatic needs beyond malaria research. •Local institutions with up-to-date equipment may attract African researchers working abroad.	•Ghana: Noguchi Memorial Institute for Medical Research benefits from funding and a growing bioinformatics team (Karikari, 2015). •Nigerian bioinformaticians at multiple universities are actively training PhD level researchers (Fatumo et al., 2014) •South African universities offer bioinformatics degrees and many find employment within the local biomedical research community.
Biobque	•Human NGS data requires secure storage. •Regulators may be unfamiliar with rapidly changing technologies. •Hardware needed to store data requires significant capital investment. •Cloud-based solutions face regulatory challenges.	•Archiving allows for long term studies. •Storage on local servers empowers researchers in country to initiate analyses using archived data. •Building a strong regulatory framework will streamline ethical reviews for future studies.	•Sars-CoV2 sequence data has been stored and shared globally (Tessema et al., 2020; Happi et al., 2021; Tegally et al., 2021). • Human NGS data storage at the National Biotechnology Development Agency in Nigeria (Fatumo et al., 2014).

imbalance. For large-scale studies where samples are collected from many districts within a country, buy-in from a large number of authorities is needed. The need to meet funding deadlines for an overseas collaborator may create tension during this phase. African procurement services and scientific suppliers to furnish basic sample collection necessities are often limited or unavailable, creating further imbalance between collaborators. To overcome this challenge, long-term partnerships that emphasize training are needed. Funding for NGS projects must be balanced, and local supply chains must be developed to support sample collection and sequencing without reliance on procurement abroad. One of the best recent examples of the rapid implementation of NGS for infectious disease research was seen in the centres of excellence for SARS-CoV-2 sequencing (Tessema et al., 2020; Happi et al., 2021; Tegally et al., 2021). To monitor the emergence of novel viral strains, several African laboratories rapidly pivoted to sequencing, taking advantage of publicly shared protocols, using kits and consumables tailored to this problem, and laboratories in Botswana and South Africa were able to quickly detect and raise awareness of the omicron variant of concern (Viana et al., 2022).

SEQUENCING

The sequencing phase of an NGS study faces many related challenges. Until sequencing becomes routine, skilled staff are difficult to find and retain. Sequencing machines, consumables and quality control reagents are challenging to procure, and are priced above the budgets of many African laboratories. Both Illumina and Oxford Nanopore Technologies have taken steps to address procurement challenges by supplying flow cells and consumables directly to several African agencies, but gaps in procurement continue to be filled by collaborating Northern institutions. Equipment and flow cells were rapidly distributed to several laboratories early in the spread of SARS-CoV-2. Robust supply chains and investment from the major suppliers of sequencing technology are needed to expand similar supply chains.

BIOINFORMATICS AND DATA STORAGE INFRASTRUCTURE

NGS studies produce large amounts of data that is analyzed with an ever-evolving suite of bioinformatics tools. Adequate data storage is a significant cost and requires in-house expertise. Few African institutions are training bioinformaticians, and those that are trained may leave for better paying jobs in the North-centered biotechnology industry. Building high performance computing clusters will require sustained investment, as will building up a cohort of bioinformaticians to work on African infectious disease sequencing projects. There is also a need to

develop local expertise for equipment installation and maintenance to prevent long delays and additional costs accrued from overseas outsourcing (Apinjoh et al., 2019). Examples of institutions that have successfully built bioinformatics systems include the Noguchi institute in Ghana (Karikari, 2015, Redeemer's University in Nigeria (Fatumo et al., 2014, and several South African universities.

LONG-TERM DATA STORAGE AND REGULATORY FRAMEWORKS

Human and pathogen sequence data is sensitive, requiring adequate long-term storage, and a regulatory framework to protect patients' information. Relatively low-cost storage exists in cloud storage systems, but privacy concerns must be addressed for this to be allowed by regulators. Hardware for long-term storage must be budgeted for in research proposals, and efficient storage formats are needed.

Skilled Workforce

Despite their potential, the initial investments in infrastructure for NGS are high, the methods require highly skilled personnel to perform sample processing, bioinformaticians, molecular epidemiologists for data analysis and interpretation, field epidemiologists, disease specialists for interpretation of the generated data, and public health specialists for adoption of the findings into policy (Escalante et al., 2015; Ishengoma et al., 2019). However, there are limited initiatives to train, retain, mentor and maintain highly skilled experts in Africa. The ongoing capacity building initiatives such as those under the African Academy of Sciences (https://www.aasciences.africa/), The human heredity and Health (H3Africa Initiative, https://h3africa.org/) and others need to be strongly supported by African countries for sustainability.

CONCLUSION

The application of advanced NGS tools and CRISPR-Cas technologies to malaria diagnosis and surveillance has great potential for malaria elimination in Africa. These tools will improve our understanding of malaria transmission dynamics, including our ability to track the spread of parasites and their associated clinically relevant mutations. With the wide scale distribution of genomics facilities and expertise, as well as state-of-the-art CRISPR-based diagnostics that substantially reduce equipment and training needs, our understanding of many aspects of malaria biology will dramatically expand. In addition to wider reaching and more accurate mobile and point-of-care diagnostics, African capacity for malaria control and ultimately elimination will result from this technological expansion. Furthermore, the deployment of these technologies and associated spread of expertise is not limited to malaria, but can also facilitate the better understanding and control of other

infectious diseases as part of the ongoing initiative of building the capacity for pathogen genomics in Africa.

Nevertheless, significant challenges remain, which need to be addressed to facilitate smooth implementation of these tools necessary for malaria surveillance and elimination. Simulation studies which consider surveillance tools as an intervention strategy can also be performed to quantitatively evaluate deployment of these tools. Available simulation models such as openmalaria can be used to optimize these tools in terms of cost-effectiveness and public health. These challenges range from technological hurdles to a lack of funding investment and sufficiently skilled workforce in the areas where malaria interventions are most desperately needed. However, should these hurdles be overcome, the potential public health implications of deploying NGS and CRISPR-Cas tools in Tanzania and elsewhere are invaluable.

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