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Metagenomics in malaria and non-malarial febrile illnesses

8th ICCMg Conference, Geneva, Switzerland

Christian Nsanzabana
17 November 2023

Outline

- Malaria
- Malaria Genomics
- Non-Malaria Febrile Illnesses (NMFIs)
- Why is there a need for metagenomics data in malaria endemic settings?
- Outlook

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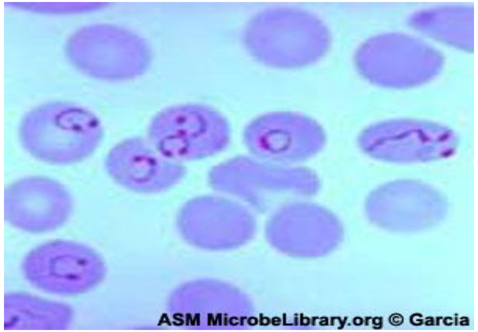
Malaria

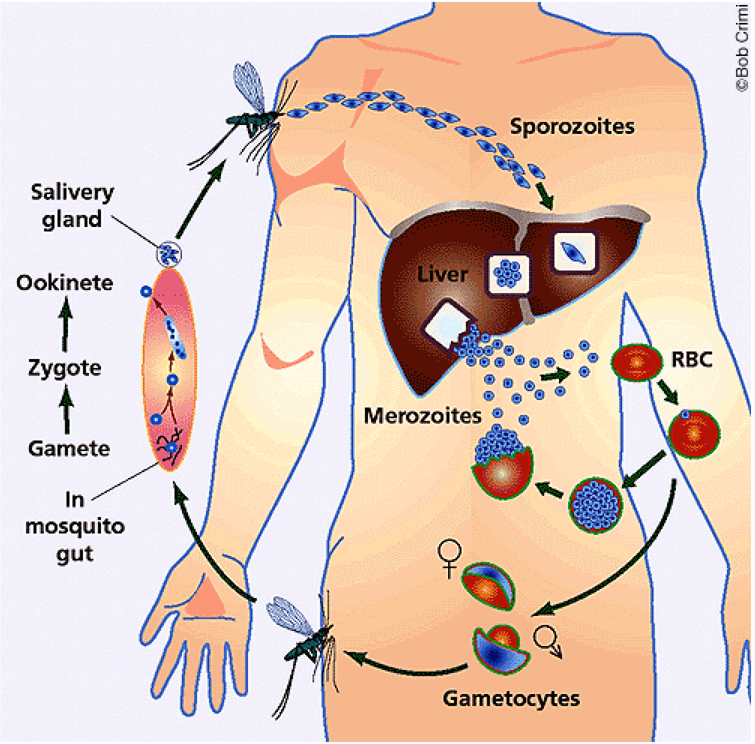
Malaria parasite cycle

- **Five** species
  - **Falciparum**
  - **Vivax**
  - **Ovale**
  - **Malariae**
  - **Knowlesi** (in Southeast Asia—the monkey malaria parasite)
- Transmitted by **Anopheline mosquito**



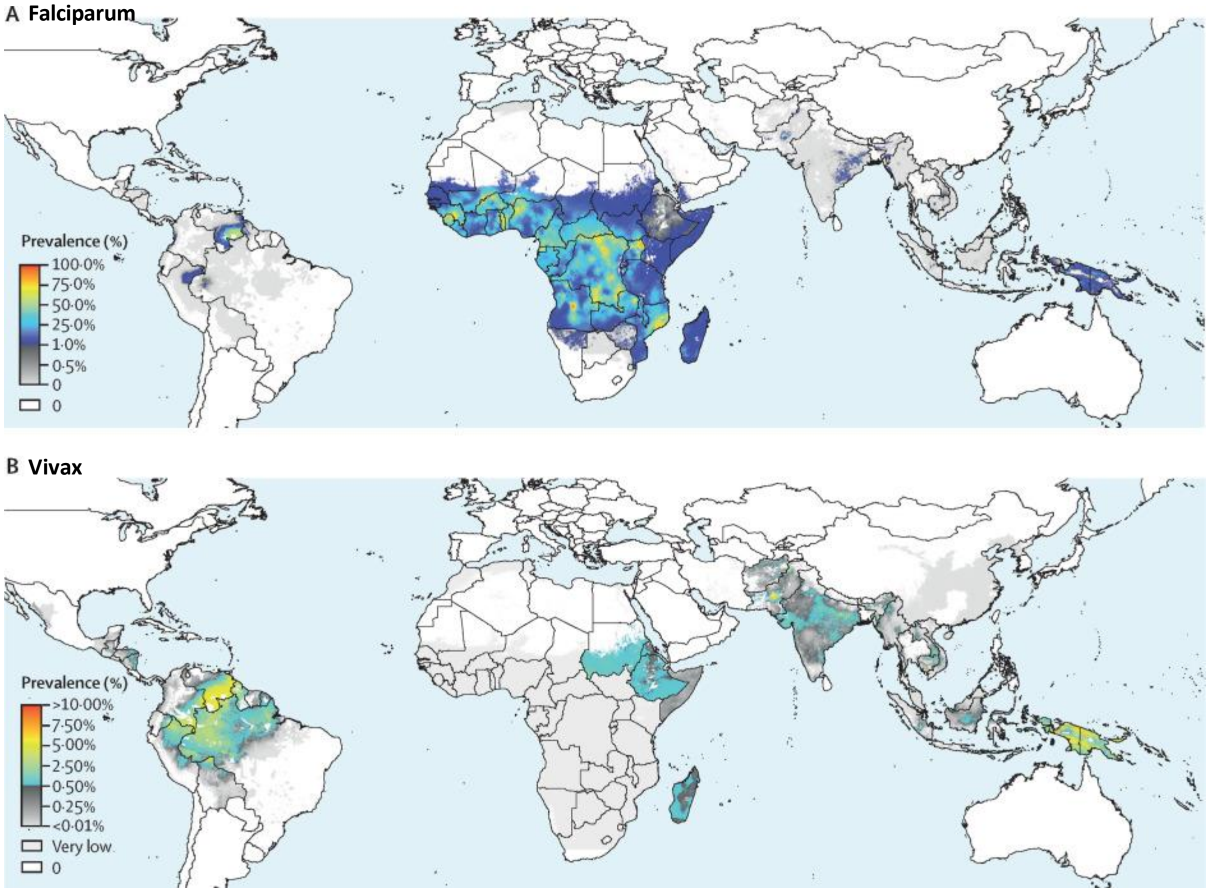
ASM MicrobeLibrary.org © Garcia

Anopheles freeborni © James Gathany / CDC



Alan F. Cowman & Brendan S. Crabb. Nature Biotechnology. 2002

Global malaria distribution

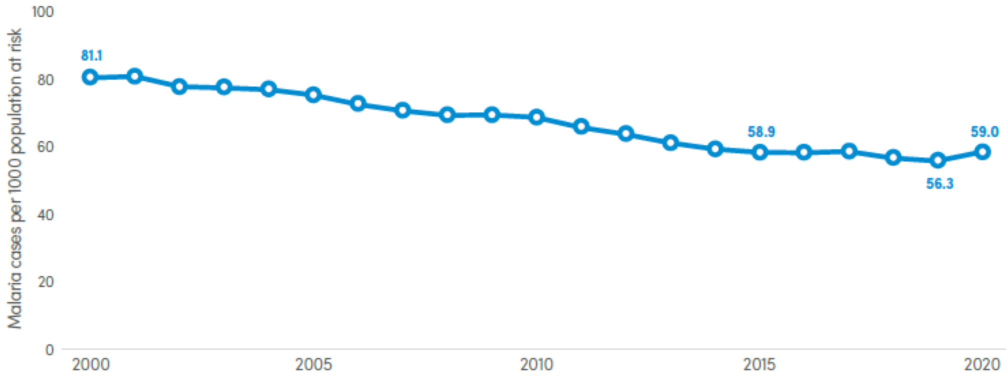


Feachem et al. 2019, Lancet ;394(10203):1056-1112

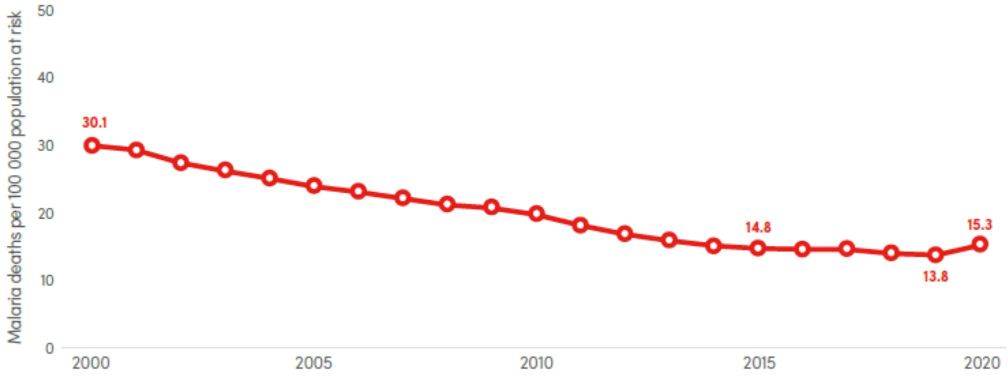
Epidemiology

- 249 millions cases in 2020 in 85 countries
- Incidence decreased by 27% between 2010 and 2015 and < 2% between 2015 and 2019
- Increase in number of cases in 2019 due to COVID 19 pandemic
- Twenty nine countries accounted for 95% of malaria cases globally
- Global mortality estimated at 627,000 in 2020
- Increase in number of deaths in 2020 due to COVID 19 pandemic (68%), and new method of calculation (32%)
- Mortality decreased by 50% between 2010 and 2019

a) **Malaria incidence**

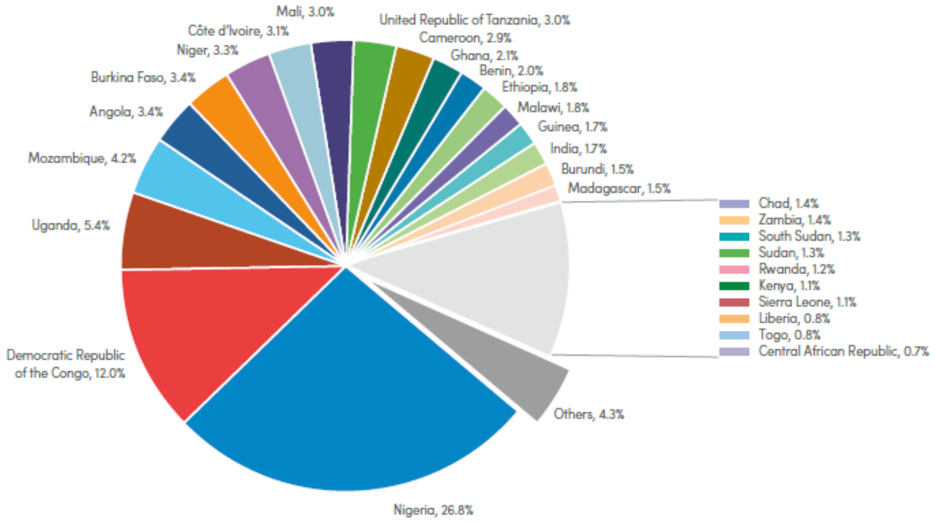


b) **Malaria mortality**



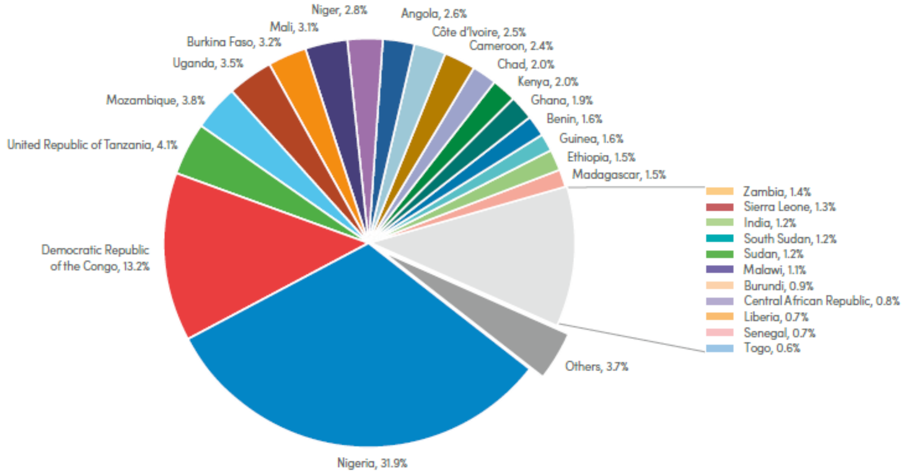
World Malaria Report, WHO, 2021

Malaria cases & deaths by country



55% malaria cases from 6 countries:
- Nigeria: 26.8%
- DRC: 12%
- Uganda: 5.4%
- Mozambique: 4.2%
- Angola: 3.4%
- Burkina Faso: 3.4%

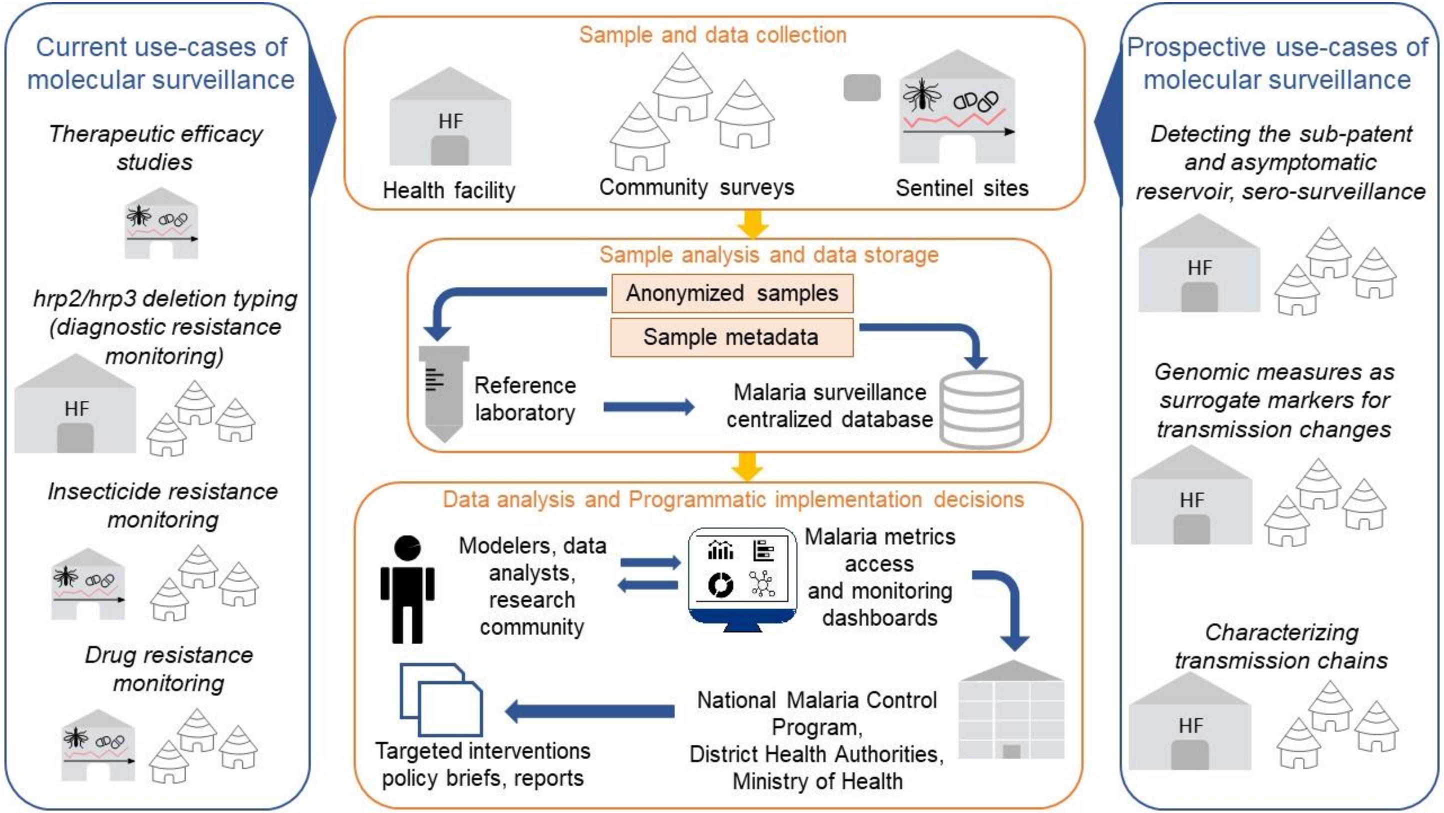
51% of malaria deaths from 4 countries:
- Nigeria: 31.9%
- DRC:13.2%
- Tanzania: 4.1%
- Mozambique: 3.8%



World Malaria Report, WHO, 2021

Malaria genomics

Use cases for malaria genomics



Current use-cases of molecular surveillance

Therapeutic efficacy studies

hrp2/hrp3 deletion typing (diagnostic resistance monitoring)

Insecticide resistance monitoring

Drug resistance monitoring

Sample and data collection

Health facility

Community surveys

Sentinel sites

Sample analysis and data storage

Anonymized samples

Sample metadata

Reference laboratory

Malaria surveillance centralized database

Data analysis and Programmatic implementation decisions

Modelers, data analysts, research community

Malaria metrics access and monitoring dashboards

National Malaria Control Program, District Health Authorities, Ministry of Health

Targeted interventions policy briefs, reports

Prospective use-cases of molecular surveillance

Detecting the sub-patent and asymptomatic reservoir, sero-surveillance

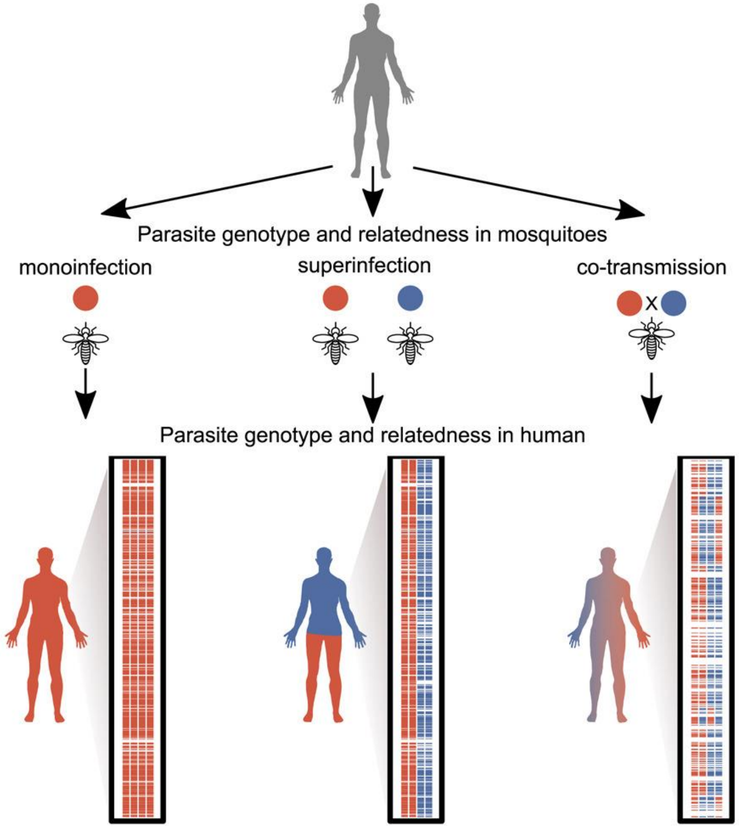
Genomic measures as surrogate markers for transmission changes

Characterizing transmission chains

Golumbeanu et al. 2023, AJTMH, in press

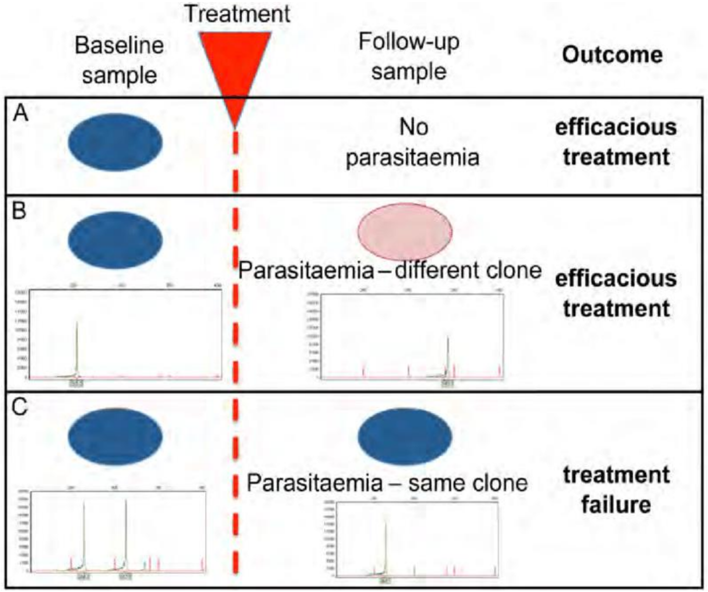
Multiplicity of infection (MOI)

- Higher MOI when using ScSeq compared to bulk sequencing
- Average MOI = 6 compared to 4
- Samples with extremely high MOI: up to 17 different clones
- Need highly diverse markers for routine surveillance with AmpSeq

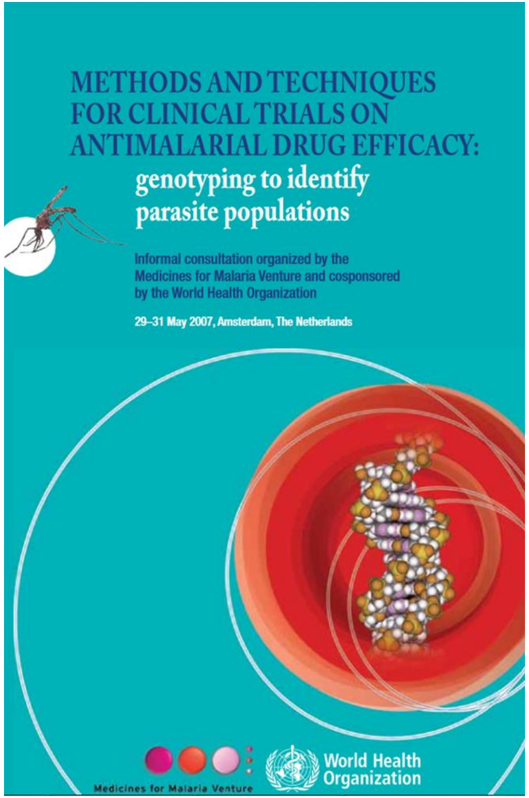


Nkhoma et al. Cell Host Microbe. 2020;27(1):93-103.e4

PCR correction



Koepfli et al. 2014; P N G Med J



METHODS AND TECHNIQUES FOR CLINICAL TRIALS ON ANTIMALARIAL DRUG EFFICACY:
genotyping to identify parasite populations

Informal consultation organized by the Medicines for Malaria Venture and cosponsored by the World Health Organization

29–31 May 2007, Amsterdam, The Netherlands

Medicines for Malaria Venture

World Health Organization

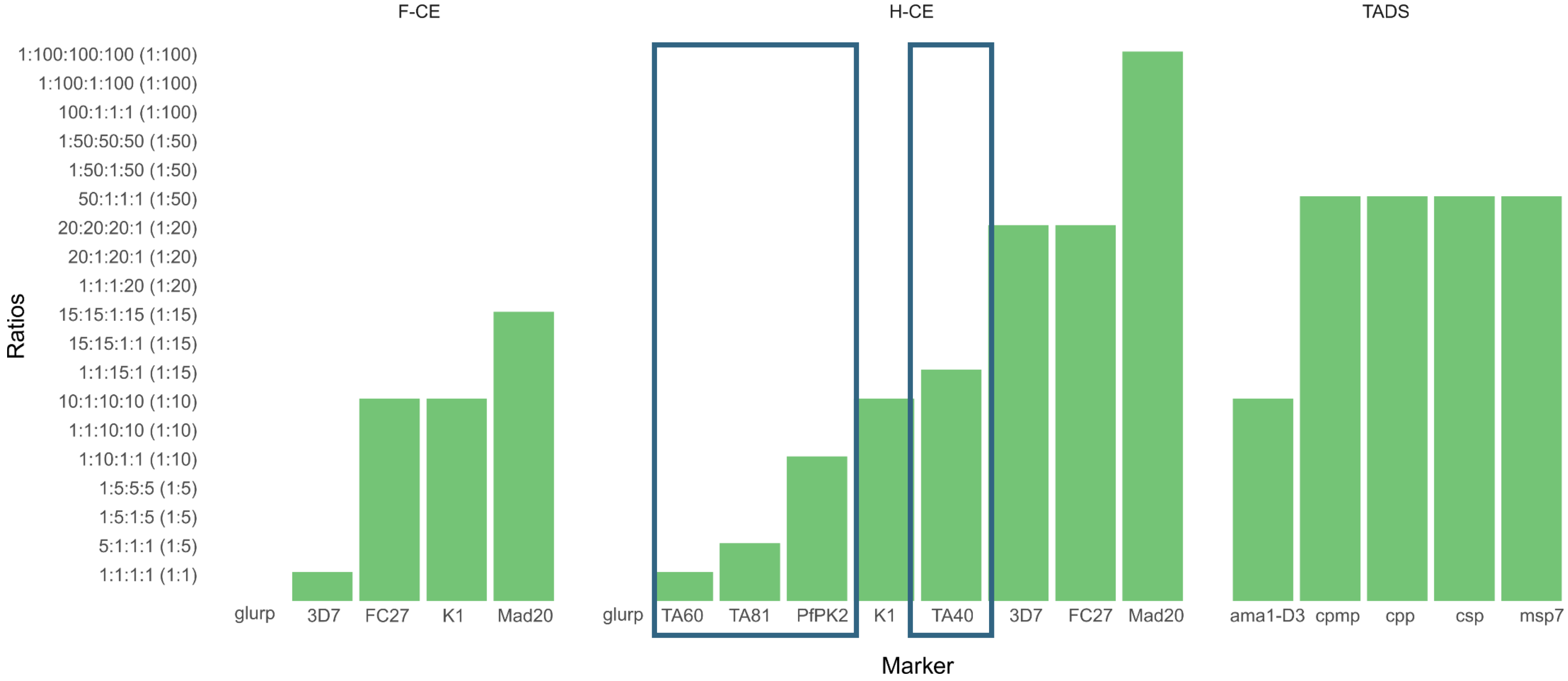
Global **Malaria** Programme

Informal consultation on methodology to distinguish reinfection from recrudescence in high malaria transmission areas

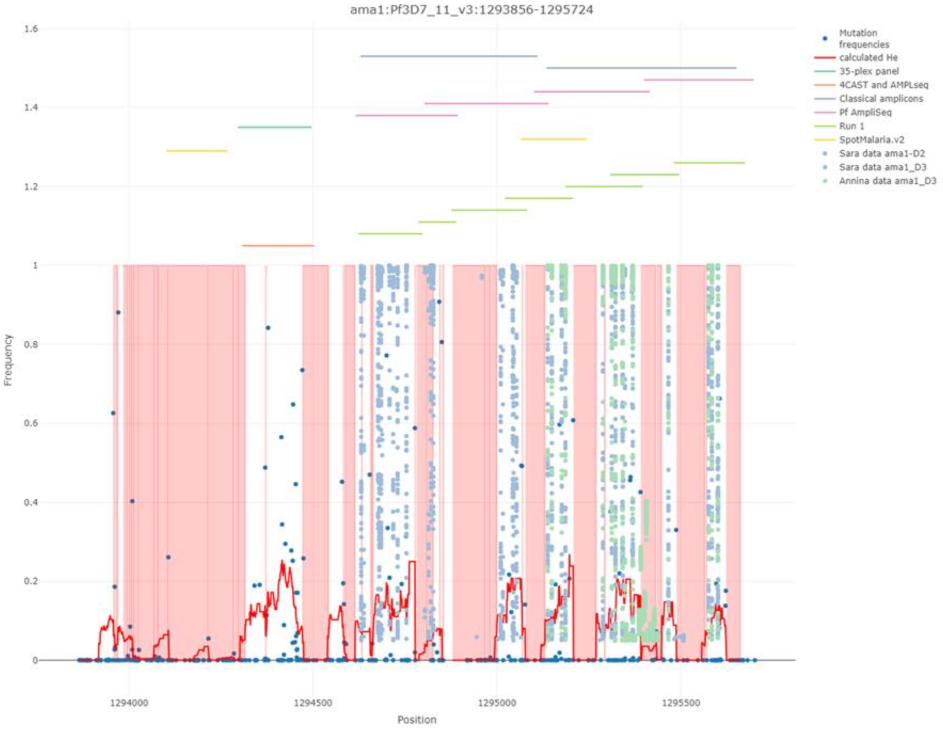
Report of a virtual meeting, 17–18 May 2021

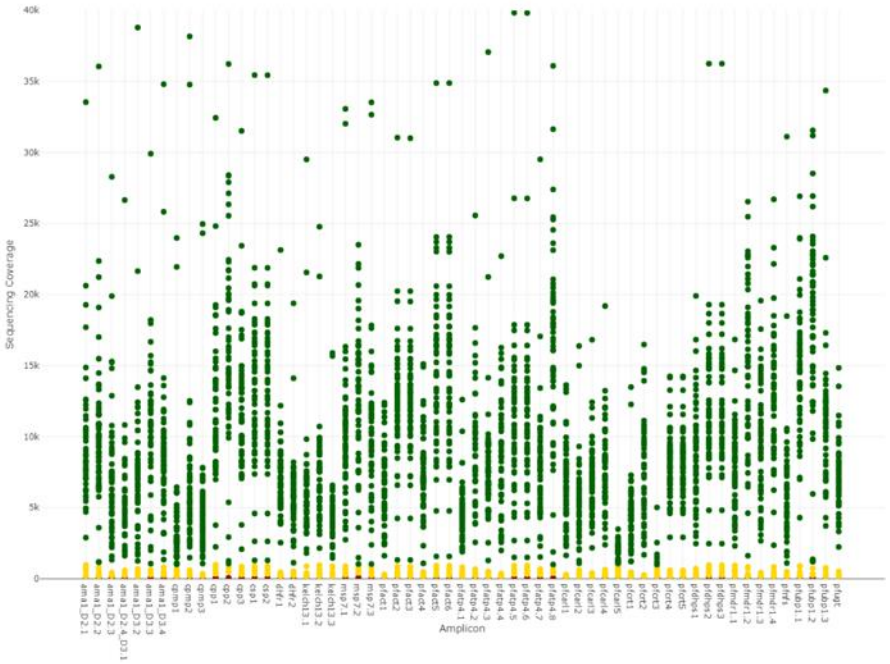
World Health Organization

Analytical sensitivity



Design molecular markers (Amplicon Sequencing)

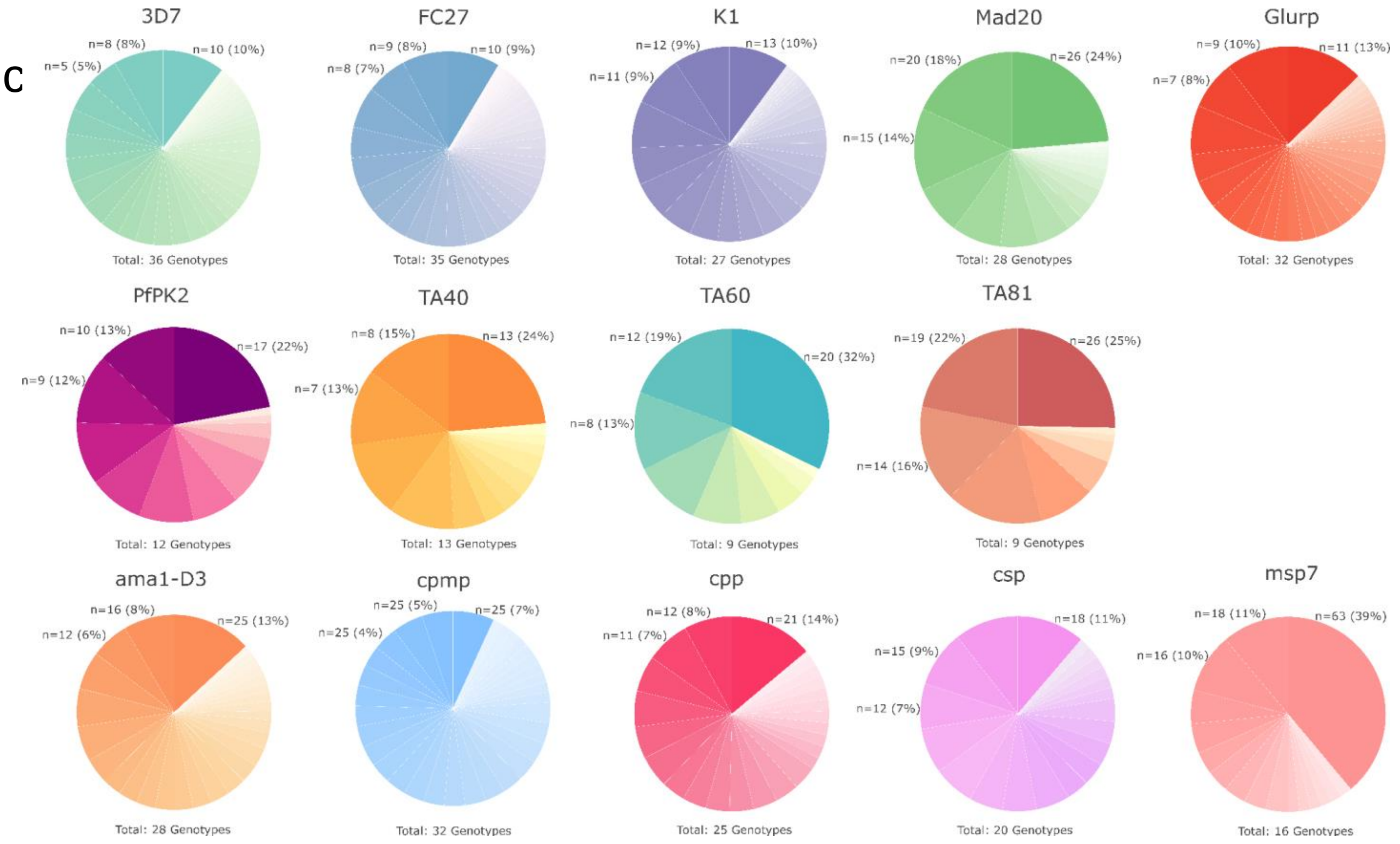




Montero Salas . et al. in development

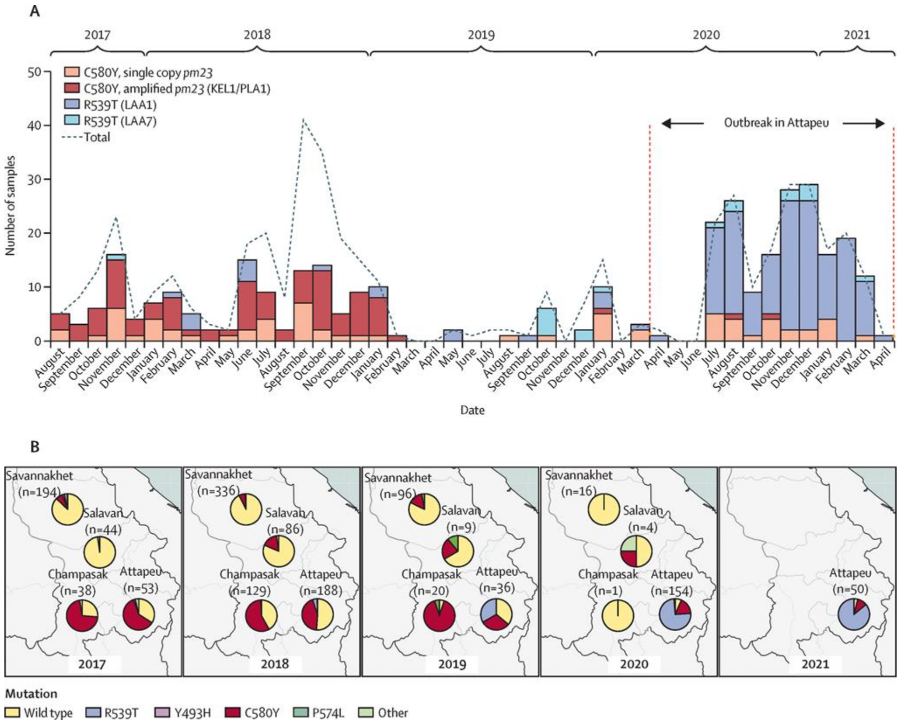
Select the optimal marker (Amplicon Sequencing)

- Length polymorphic genes by CE
- Microsatellites by CE
- SNP polymorphic genes by TADS

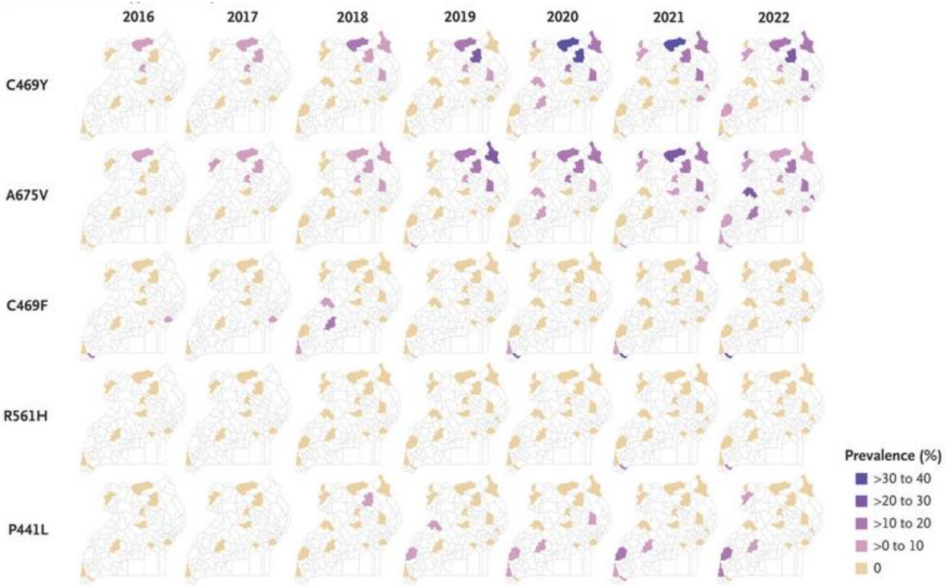


Schnoz A. et al. Submitted. https://www.biorxiv.org/content/10.1101/2023.04.24.538072v1

Monitoring the emergence and spread of resistant parasites



Wasakul et al. Lancet Infect Dis. 2023;23(5):568-577



Conrad M. et al. N Engl J Med. 2023;389(8):722-732

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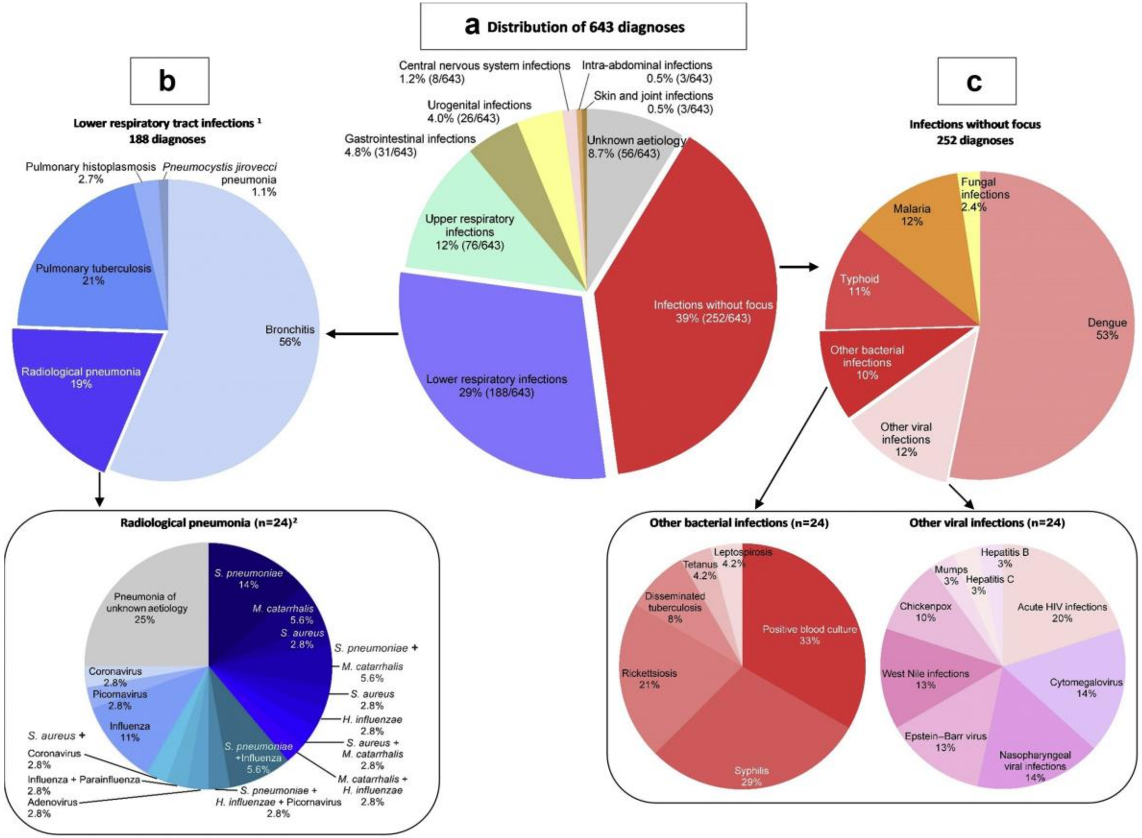
Non-Malarial Febrile Illnesses (NMFIs)

Definition

- Non-malarial febrile illnesses (NMFI) are infectious diseases affecting patients who show signs of indistinguishable fever thus necessitate use of malaria diagnostic tests, but these tests turn out to be negative for malaria

Nyaoke BA. et al. PLoS One. 2019; 14(6): e0217980

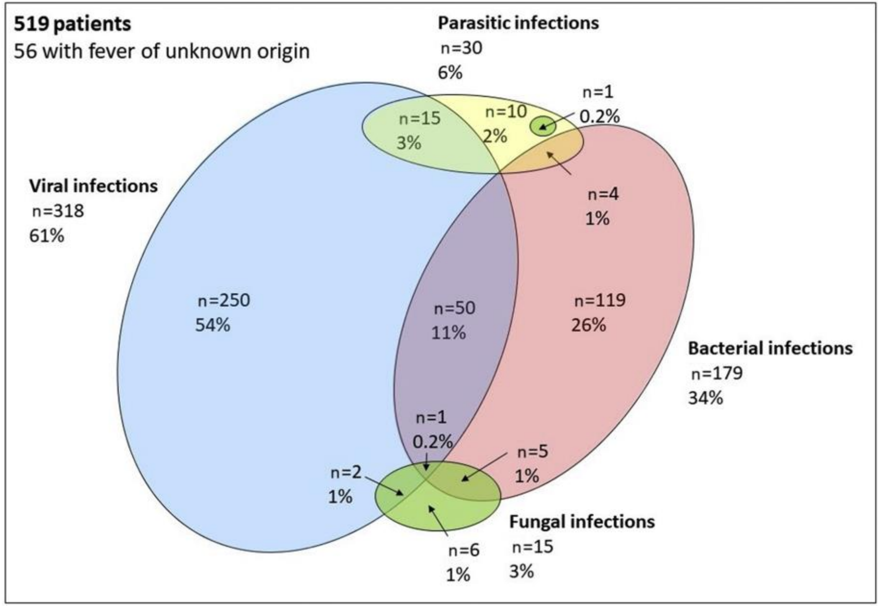
Causes of fever in Tanzania

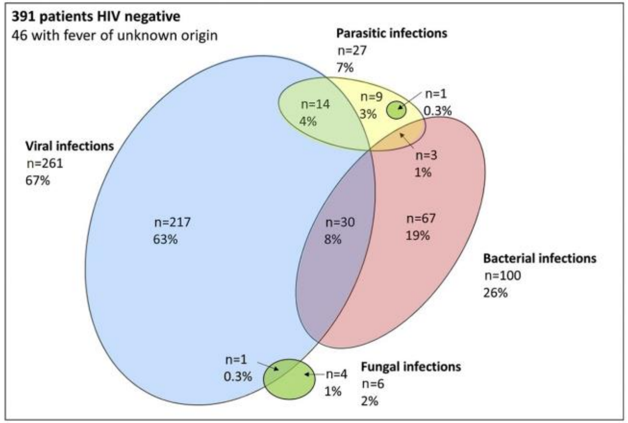


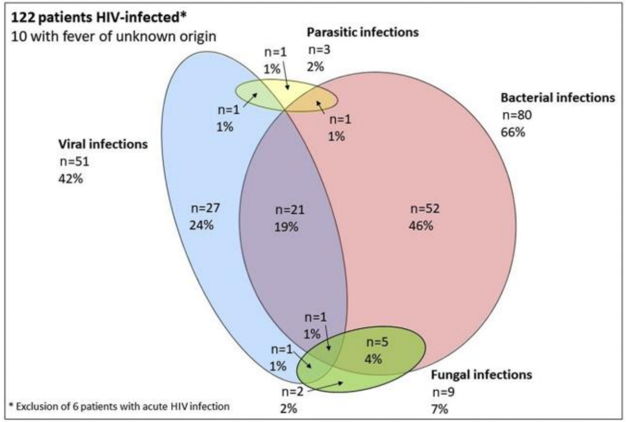
[1] Diagnoses of radiological pneumonia and bronchitis were only considered in patients without pulmonary tuberculosis and/or pulmonary histoplasmosis and/or pneumocystosis.

Boillat-Blanco N et al. Clin Microbiol Infect. 2021;27(6):913.e1-913.e7.

Causes of fever in Tanzania







* Exclusion of 6 patients with acute HIV infection

Boillat-Blanco N et al. Clin Microbiol Infect. 2021;27(6):913.e1-913.e7.

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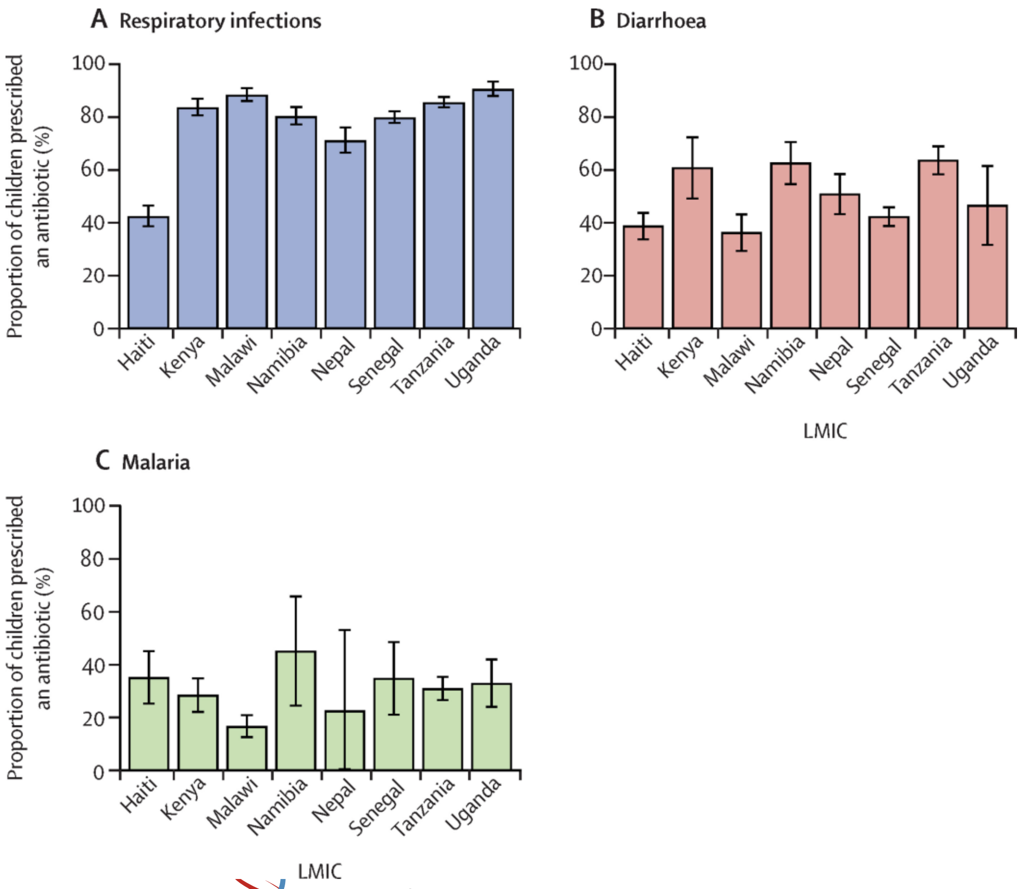
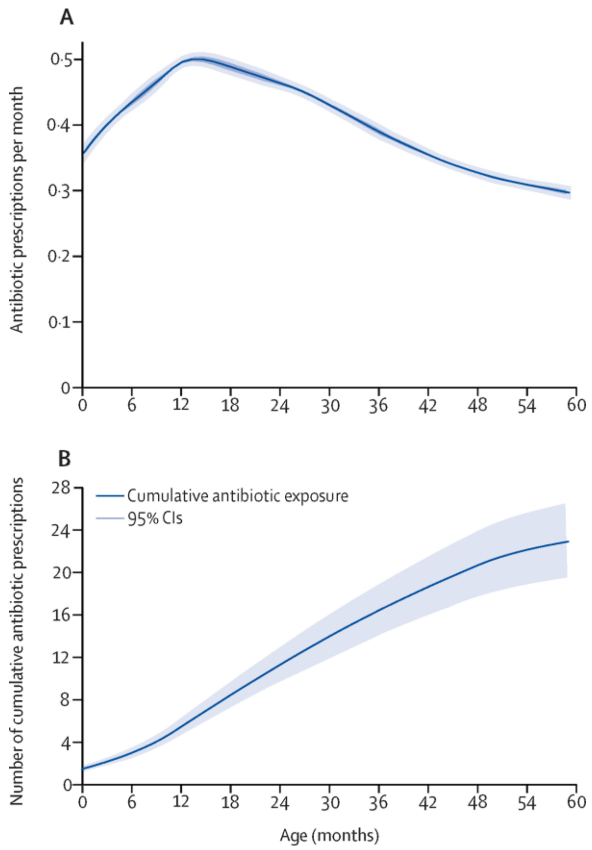
Why is there a need for metagenomics data in malaria endemic settings ?

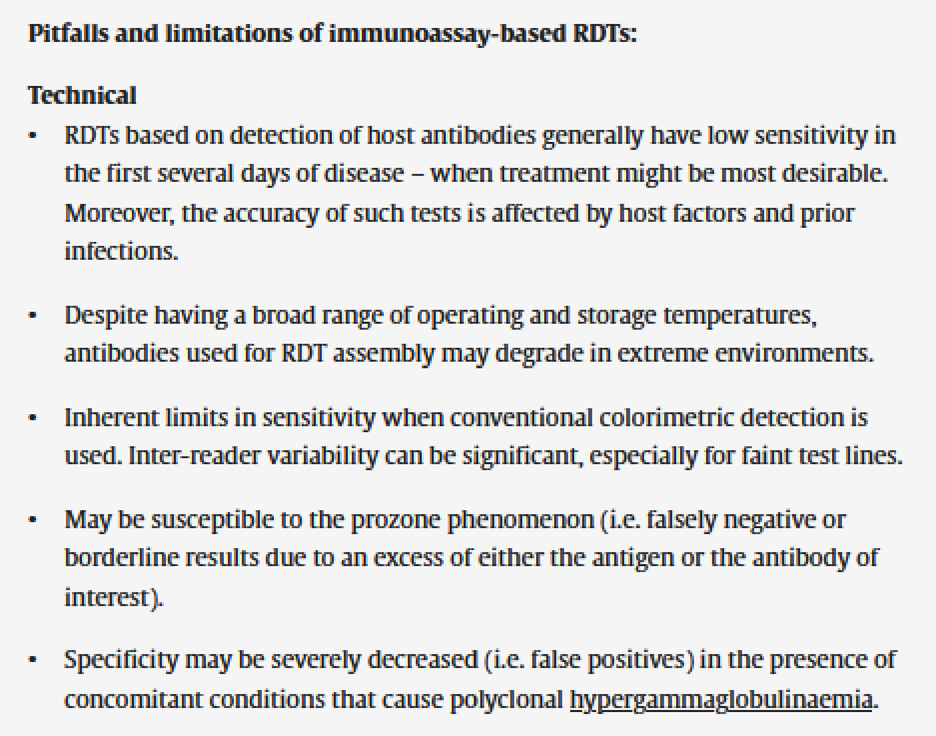
Antibiotic over prescription





Fink G et al. Lancet Infect Dis. 2020;20(2):179-187

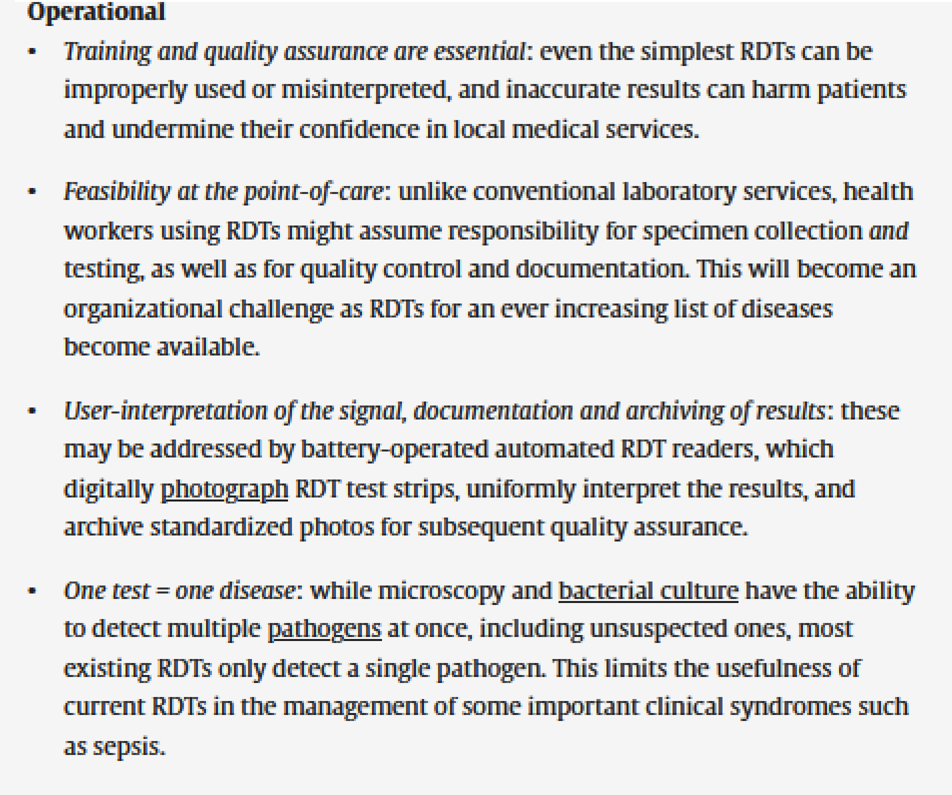
Limitations of currently used RDTs



Pitfalls and limitations of immunoassay-based RDTs:

Technical

- RDTs based on detection of host antibodies generally have low sensitivity in the first several days of disease – when treatment might be most desirable. Moreover, the accuracy of such tests is affected by host factors and prior infections.
- Despite having a broad range of operating and storage temperatures, antibodies used for RDT assembly may degrade in extreme environments.
- Inherent limits in sensitivity when conventional colorimetric detection is used. Inter-reader variability can be significant, especially for faint test lines.
- May be susceptible to the prozone phenomenon (i.e. falsely negative or borderline results due to an excess of either the antigen or the antibody of interest).
- Specificity may be severely decreased (i.e. false positives) in the presence of concomitant conditions that cause polyclonal hypergammaglobulinaemia.



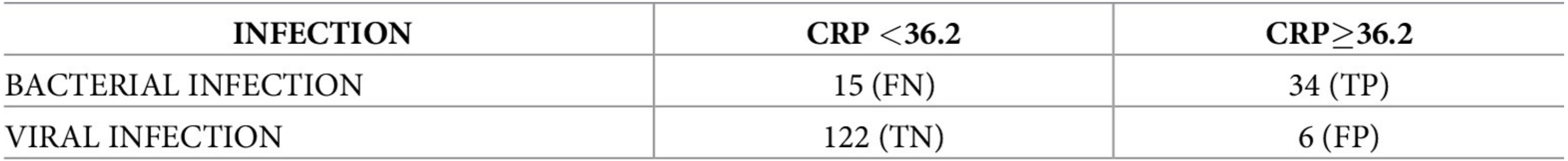
Operational

- *Training and quality assurance are essential*: even the simplest RDTs can be improperly used or misinterpreted, and inaccurate results can harm patients and undermine their confidence in local medical services.
- *Feasibility at the point-of-care*: unlike conventional laboratory services, health workers using RDTs might assume responsibility for specimen collection *and* testing, as well as for quality control and documentation. This will become an organizational challenge as RDTs for an ever increasing list of diseases become available.
- *User-interpretation of the signal, documentation and archiving of results*: these may be addressed by battery-operated automated RDT readers, which digitally photograph RDT test strips, uniformly interpret the results, and archive standardized photos for subsequent quality assurance.
- *One test = one disease*: while microscopy and bacterial culture have the ability to detect multiple pathogens at once, including unsuspected ones, most existing RDTs only detect a single pathogen. This limits the usefulness of current RDTs in the management of some important clinical syndromes such as sepsis.

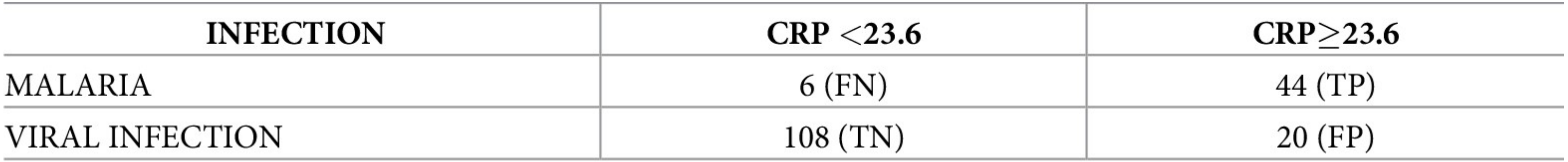
Chappuis F et al. Clin Microbiol Infect. 2013;19(5):422-31.

Use of host biomarkers

- CRP can effectively exclude malaria and bacterial infection in febrile children
- Development of CRP/malaria combined RDT



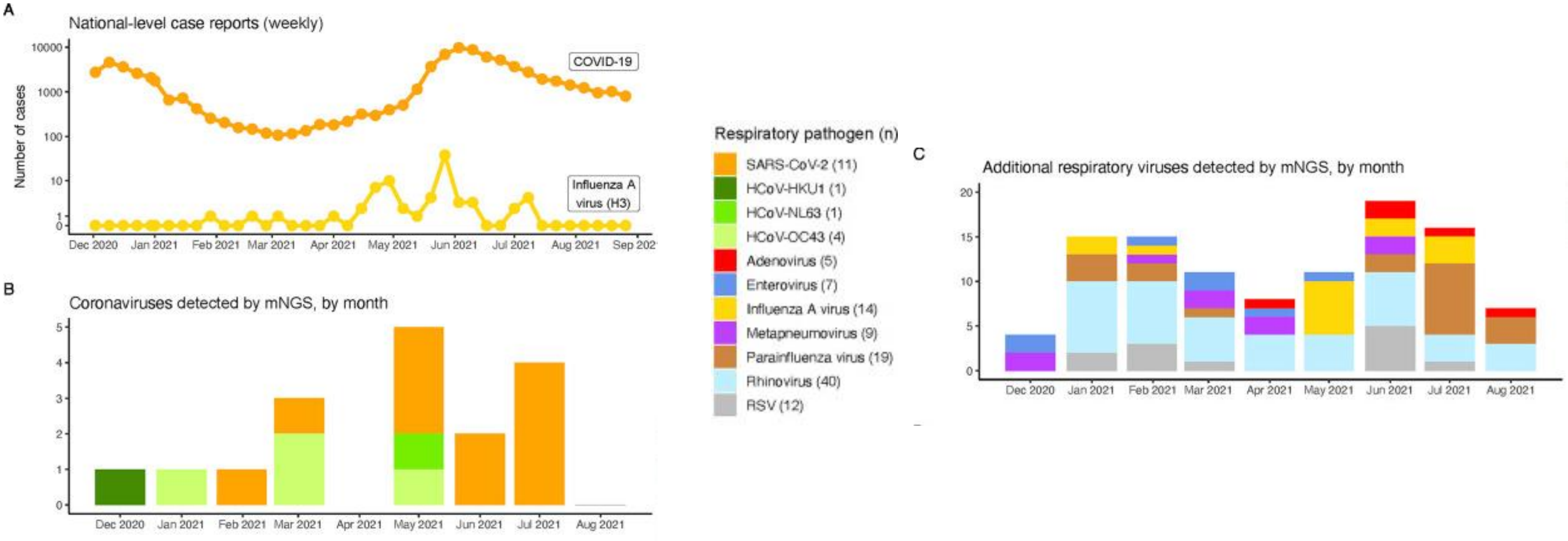
INFECTION	CRP <36.2	CRP≥36.2
BACTERIAL INFECTION	15 (FN)	34 (TP)
VIRAL INFECTION	122 (TN)	6 (FP)



INFECTION	CRP <23.6	CRP≥23.6
MALARIA	6 (FN)	44 (TP)
VIRAL INFECTION	108 (TN)	20 (FP)

Ngewngi Y. Et al.PLoS One. 2023;18(7):e0289012.

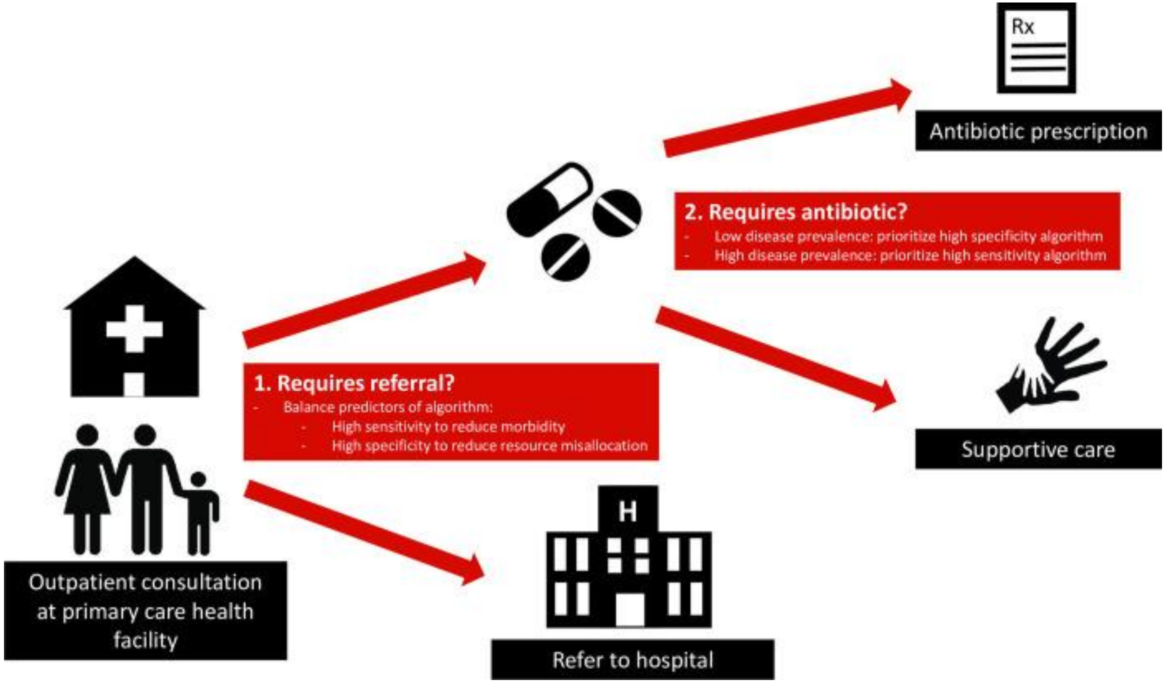
Metagenomics surveillance in LMICs



Mwakibete L. et al. PLOS Glob Public Health. 2023 May 3;3(5):e0001675.

Use of clinical algorithm assisted with AI

- Most of fevers managed at community level/small health facilities
- Lack of adequate laboratory capacity
- Clinical algorithm for patients triage
  - Clinical symptoms
  - Host biomarkers
  - Genomic surveillance data



Outpatient consultation at primary care health facility

1. Requires referral?
- Balance predictors of algorithm:
  - High sensitivity to reduce morbidity
  - High specificity to reduce resource misallocation

Refer to hospital

2. Requires antibiotic?
- Low disease prevalence: prioritize high specificity algorithm
- High disease prevalence: prioritize high sensitivity algorithm

Antibiotic prescription

Supportive care

Tan R. et al. PLOS Digit Health. 2023 Jan19;2(1):e0000170.

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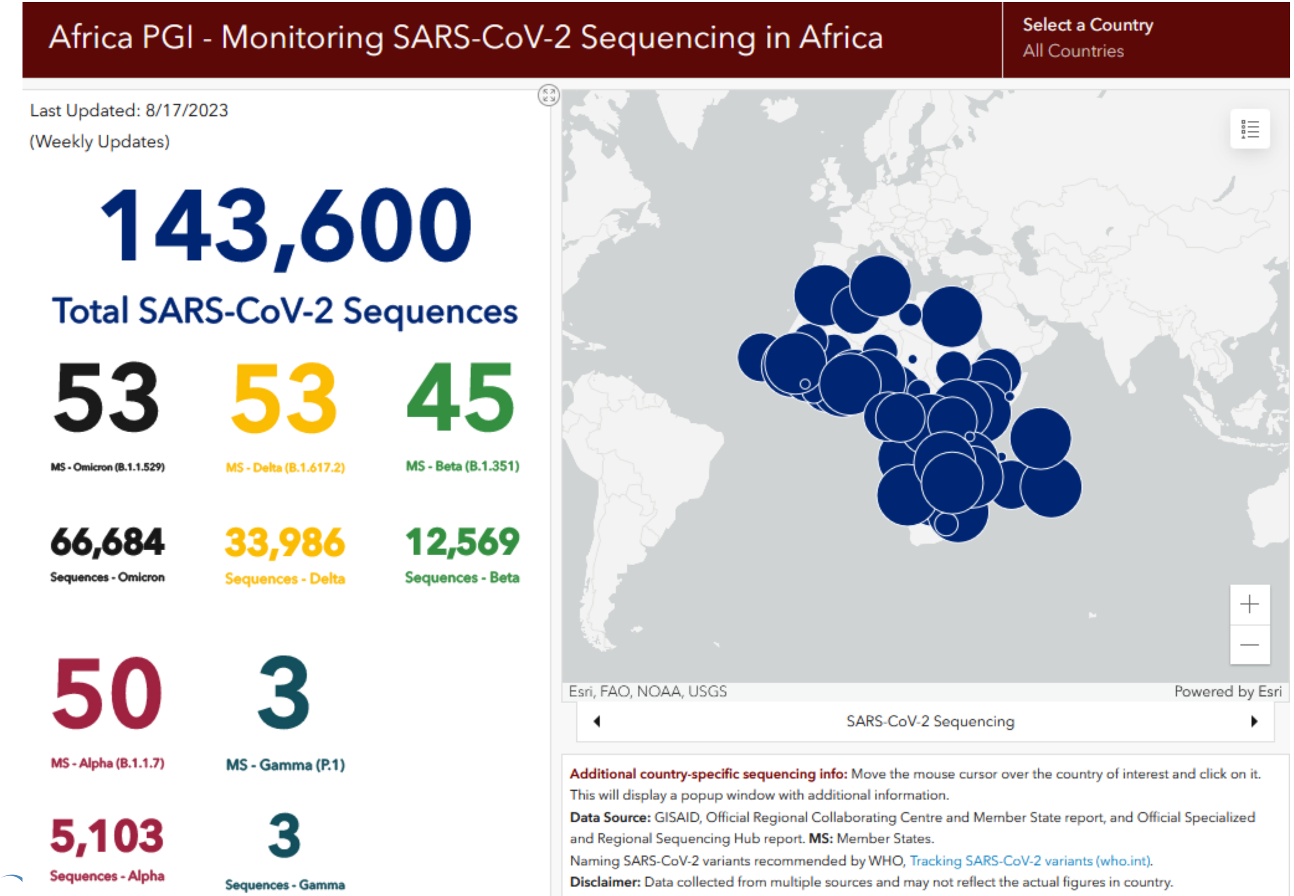
Outlook

COVID-19 as an accelerator ?



Africa PGI - Monitoring SARS-CoV-2 Sequencing in Africa

Select a Country
All Countries

Last Updated: 8/17/2023
(Weekly Updates)

143,600

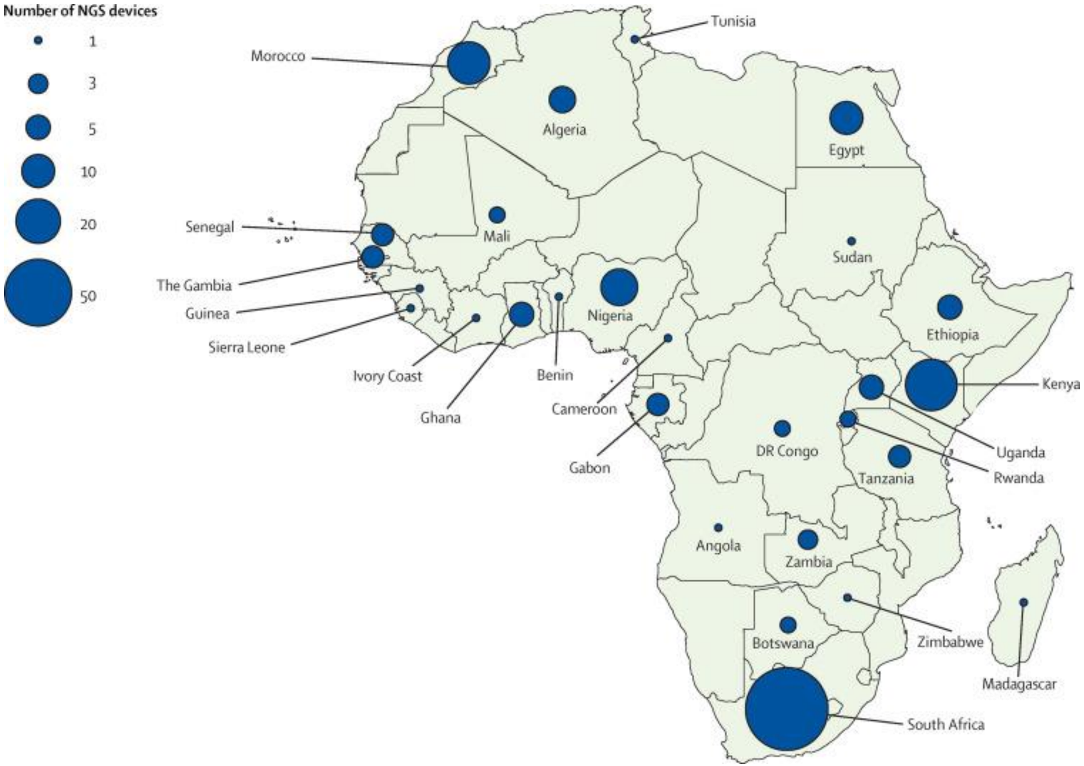
Total SARS-CoV-2 Sequences

53	**53**	**45**
MS - Omicron (B.1.1.529)	MS - Delta (B.1.617.2)	MS - Beta (B.1.351)
66,684	**33,986**	**12,569**
Sequences - Omicron	Sequences - Delta	Sequences - Beta
50	**3**	
MS - Alpha (B.1.1.7)	MS - Gamma (P.1)	
5,103	**3**	
Sequences - Alpha	Sequences - Gamma	

Additional country-specific sequencing info: Move the mouse cursor over the country of interest and click on it. This will display a popup window with additional information.
Data Source: GISAID, Official Regional Collaborating Centre and Member State report, and Official Specialized and Regional Sequencing Hub report. **MS:** Member States.
Naming SARS-CoV-2 variants recommended by WHO, Tracking SARS-CoV-2 variants (who.int).
Disclaimer: Data collected from multiple sources and may not reflect the actual figures in country.

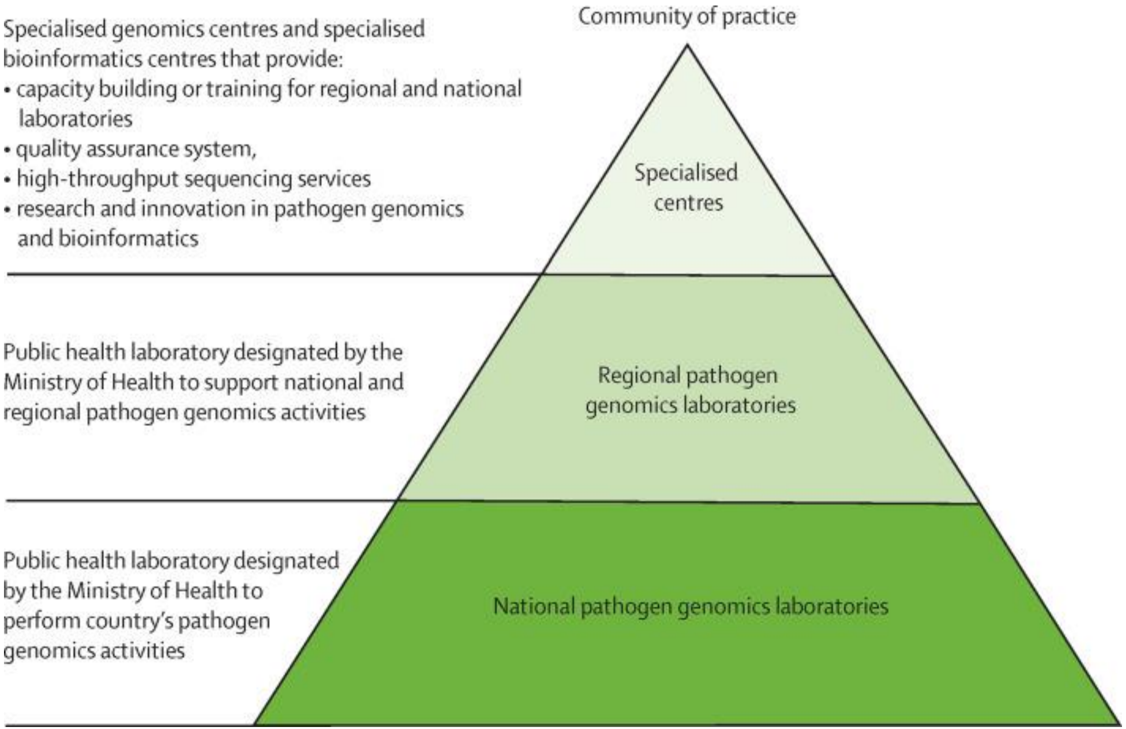
https://africacdc.org/institutes/ipg/ Accessed 16.11.2023

NGS capacity in Africa



Inzaule et al. Lancet Infect Dis. 2021;21(9):e281-e289

Genomics laboratory and bioinformatics network structure: Africa CDC



Specialised genomics centres and specialised bioinformatics centres that provide:
- capacity building or training for regional and national laboratories
- quality assurance system,
- high-throughput sequencing services
- research and innovation in pathogen genomics and bioinformatics

Public health laboratory designated by the Ministry of Health to support national and regional pathogen genomics activities

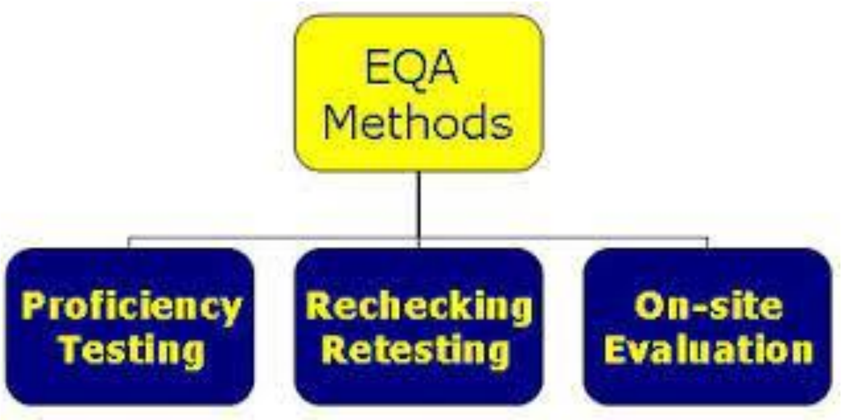
Public health laboratory designated by the Ministry of Health to perform country's pathogen genomics activities

Inzaule et al. Lancet Infect Dis. 2021;21(9):e281-e289

Way forward

- Training laboratory staff in genomic analyses
- Training in bioinformatics
- External quality assurance programs
- ISO certification

International Organization for Standardization
ISO
17025
ACCREDITED LABORATORY


EQA Methods

Proficiency Testing

Rechecking Retesting

On-site Evaluation

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Thank you for your attention !

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Department of Medicine
Malaria genotyping group