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By Kristen Bastug

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Utilization of Oxford Nanopore Technology for Human Infectious Diseases Detection and Surveillance in Africa: A Scoping Review

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Utilization of Oxford Nanopore Technology for Human Infectious Diseases Detection and Surveillance in Africa: A Scoping Review

1.1 Author names

Kristen Bastug*[1], Uzal Umar*[2], Brooke Olson[3], Tina Slusher[4], Christopher Faulk[5], Mark Okolo[6], Stephen Oguche**[7], Beth K. Thielen**[8]

*Co-first authors contributed equally to the work

**Co-last authors contributed equally to supervising the work

1.2 Affiliation(s) and ORCiD ID

1. University of Minnesota, Department of Pediatrics, Division of Pediatric Infectious Diseases, Minneapolis, MN. ORCiD ID 0000-0003-4655-0377

2. University of Jos, Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Jos Plateau State, Nigeria, P.M.B 2076. ORCiD ID 0000-0003-2805-6420

3. University of Minnesota, Health Sciences Libraries, Minneapolis, MN. ORCID iD 0009-0005-3300-1602

4. University of Minnesota, Department of Pediatrics and Division of Critical Care, Minneapolis, MN and Hennepin County Medical Center, Department of Pediatrics and Division of Critical Care, Minneapolis, MN. ORCiD ID 0000-0002-5798-5774

5. University of Minnesota, Department of Animal Science, College of Food, Agricultural and Natural Resources Science, Saint Paul, MN. ORCiD ID 0000-0002-9749-9658

6. University of Jos and Jos University Teaching Hospital, Department of Medical Microbiology, Faculty of Basic Clinical Sciences, College of Health Sciences, Jos, Plateau State, Nigeria, P.M.B 2076. ORCiD ID 0000-0002-1745-5189

7. University of Jos and Jos University Teaching Hospital, Department of Paediatrics, Faculty of Clinical Sciences, College of Health Sciences, Jos, Plateau State, Nigeria, P.M.B 2076. ORCiD ID 0000-0002-6223-027X

8. University of Minnesota, Department of Pediatrics, Division of Pediatric Infectious Diseases, Minneapolis, MN. ORCiD ID 0000-0001-5922-0241

1.3 Corresponding author and email address

Kristen Bastug: bastu004@umn.edu and kabastug@gmail.com

1.4 Keywords

Molecular diagnostics, molecular epidemiology, nanopore sequencing, rapid diagnostic tests, clinical laboratory techniques

2. Abstract

Background:

Nanopore-based sequencing by Oxford Nanopore Technologies (ONT) offers rapid, cost-effective, and portable sequencing. As an emerging technology, ONT must be evaluated for efficacy and practical application in both high- and low-resource settings. This scoping review (SR) aimed to: **1)** describe how nanopore technology is used in Africa for surveillance and diagnosis of human infectious diseases, **2)**

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describe how nanopore technology aids in the real-time detection of infectious pathogens in Africa, and **3)** identify challenges and opportunities for utilizing nanopore technology in Africa to study infectious diseases.

Methods: This SR followed the Joanna Briggs Institute Reviewer's Manual framework for SRs. English-language studies published from January 1, 2008, to April 30, 2024 that used ONT on human specimens collected in Africa and targeted ≥1 microbial agent were included. Searches were performed in Embase, Medline, PubMed, CINAHL, and the Cochrane Library. The protocol was publicly available on the Open Science Framework (1) prior to data collection. Two independent reviewers screened studies using Covidence, and data was extracted using a custom REDCap instrument. Descriptive statistics and data visualization were performed in Microsoft Excel.

Results: 1162 studies were identified and 93 (8%) underwent full-text review. The portable MinION Mk1B was the most common ONT device (65% of studies). Eighty-eight studies analyzed specimens from a single African country. Of these, 45% were sequenced in the same country, 7% in a different African Country, 11% in a non-African country, and 32% did not specify location. Specimen types included direct patient specimens (62%) and cultured isolates (35%), or a combination of both. Blood, serum, or plasma was most common (35%), followed by naso- or oropharyngeal specimens (27%). Forty-four studies used ONT during an active infectious diseases outbreak, 25 of which studied SARS-CoV-2. Seventy-two studies used ONT for genomic surveillance of infectious pathogens or antibiotic resistance genes, and one study used ONT for a direct clinical application. African-affiliated authors were included as first, middle, and last authors in 46% of studies, and 15% were published by entirely African-affiliated teams. Ten studies published information on workflow timeline and five studies published the per-specimen cost.

Conclusions: ONT can enable timely and affordable sequencing in African countries as demonstrated through a small number of studies that accomplished these goals individually. Improved reporting of ONT-specific methodology is needed, including timelines, cost, barcoding, flow cell model, and the use of negative controls. Publications that provide these details will enhance reproducibility and support the development of new studies using ONT for the diagnosis and surveillance of infectious diseases in low resource settings.

3. Impact statement

Molecular diagnostic approaches for the detection of infectious pathogens have advanced and now include long-read nanopore sequencing developed by Oxford Nanopore Technologies (ONT). This technology offers portability, the potential for reduced cost, and faster turnaround time from sample collection to results compared to existing methodologies. These factors may facilitate expansion of molecular sequencing into low resource settings, but the feasible and practical use of ONT in Africa has not been described. This scoping review describes the current use of ONT sequencing in Africa for surveillance and diagnosis of human infectious diseases in regard to common pathogens, study populations, and laboratory approaches. Researchers seeking to use ONT in resource limited settings for infectious diseases research will gain insights into current applications and methodologic approaches for using this technology. The research gaps identified in this review form a strong framework to guide future studies using ONT in resource-limited settings.

4. Data summary

The authors confirm all supporting data, code, and protocols have been provided within the article or through supplementary data files.

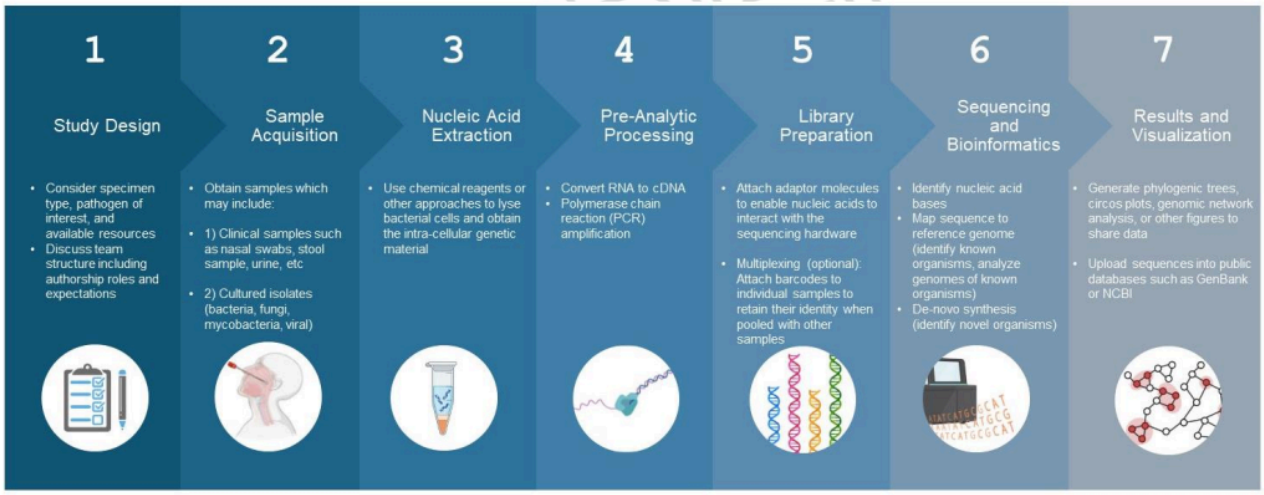
5. Introduction

Genomic sequencing technology has significantly advanced since the development of Sanger sequencing in the 1970s. Whereas Sanger sequencing analyzed DNA fragments generated from a single DNA

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template, next-generation sequencing (NGS) approaches simultaneously sequence millions of nucleic acid molecules. Nucleotide sequences from many organisms within the same sample can be analyzed through a process termed “metagenomic” NGS. The general NGS workflow **(Figure 1)** begins with consideration of the study design, followed by sample acquisition (e.g. a direct patient specimen or cultured isolate). RNA or DNA is extracted from the sample via different approaches, which may include the use of chemical reagents to lyse open cells and obtain intra-cellular material. Pre-analytic processing is then performed, which may include the conversion of RNA into cDNA, and/or polymerase chain reaction (PCR) amplification to increase the abundance of the nucleic acid target of interest. The cDNA then undergoes “library preparation” which can involve attaching adapter molecules to facilitate interaction with the sequencing platform, and/or attaching unique sequences termed “barcodes” to track individual samples within a larger pool of specimens. The sample or pooled specimen is then placed on the sequencer to determine the sequence of nucleotide bases, which can be done using florescence-based approaches (Sanger, PacBio, and Illumina sequencing) or identifying unique electrical signals (nanopore sequencing). Bioinformatics tools are used either simultaneously during sequencing or in post-processing analysis. This may include 1) mapping the obtained sequences to existing reference genomes of known organisms to detect the presence of an organism or investigate its whole genome sequence, or 2) using the sequence data to construct a new genome from scratch in a process referred to as “de-novo assembly.” The genome sequences can be uploaded into public databases such as GenBank or the European Nucleotide Archive, and results can be visualized in the form of phylogenic trees, circos plots, genome network analyses, and more.

Figure 1: Typical steps in a next-generation sequencing workflow. The approach begins with study design (left side) and ends with generation of results (right side).



The options for NGS sequencing have expanded due to the development of newer technologies that can sequence long, continuous stretches of nucleic acids. Two prominent long-read sequencing approaches include nanopore sequencing (developed by Oxford Nanopore Technology, ONT) and single-molecule real-time technology (SMRT, developed by PacBio). ONT offers potential advantages for use in low- and middle-income countries (LMICs) including real-time data generation and a low-cost, portable device designed for handheld use.

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Nanopore sequencing offers a unique approach for NGS.

The underlying mechanism of nanopore sequencing and its associated workflow offers customization options that enable flexibility for different settings and experimental goals. ONT sequencing functions by passing individual nucleic acid molecules through microscopic pores (termed "nanopores") as a continuous strand, generating an electrical signal that is unique to each nucleotide as it passes through the nanopore. Thousands of these nanopores are embedded in a synthetic membrane within a flow cell, allowing simultaneous sequencing of many individual molecules. Different flow cells have been developed to handle either DNA or RNA molecules, and some flow cell models contain a higher number of pores which increases the sequencing power at a higher financial cost. A computer then converts the electric signal into the corresponding nucleotide, with the ONT company reporting speeds of 400-450 base pairs per second for DNA sequencing, consistent with findings from independently published studies (2). The maximum length of a nucleic acid sequence that can be analyzed is limited to the length of the input material, with no theoretical upper limit. Read lengths up to 1 million base pairs have been reported (3), contrasting with the 100-400 base pair length that is typical for short-read technology such as Illumina. Importantly, ONT technology continues to advance, and readers are advised to seek the most up-to-date information when planning experiments.

Within the ONT platform, there are experimental options at the pre-analytic processing, library preparation, and sequencing steps that influence the yield and cost of a sequencing experiment.

At the pre-analytic processing stage, enrichment strategies such as PCR can be used to amplify sequences of interest within the sample, particularly for specimen types that have a relatively low abundance of the sequence of interest compared to host DNA or environmental micro-organisms. Hybrid-capture is another approach whereby targeted, single-stranded oligonucleotide probes bind to sequences of interest in a DNA sample, while concurrently binding to magnetic beads. The remainder of specimen which is not bound to the beads is discarded, increasing the abundance of the sequence of interest within the sample. A higher abundance of the target sequence typically leads to higher-quality sequencing results and may also extend the usability of flow cells by reducing unnecessary, non-specific sequences from exhausting the nanopores.

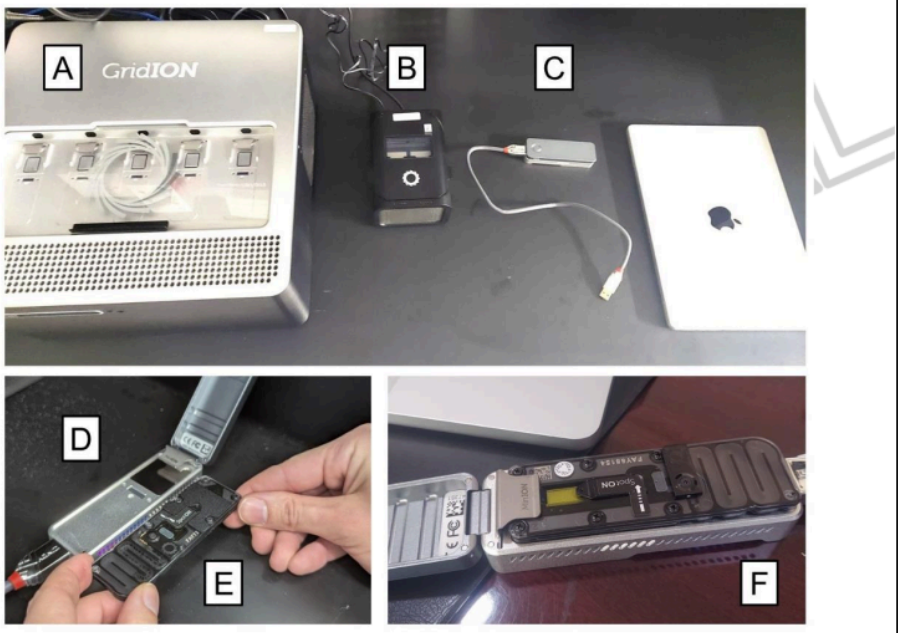
Library preparation for ONT offers ways to customize and optimize experimental approaches. The attachment of an ONT-specific molecular adapter is necessary to allow the nucleic acid sequences to move through the nanopores and ONT offers several chemistry kits to accomplish this task. One is the "Rapid Sequencing Kit (RSK)," whereby transposons are used to insert the adaptors into the middle of nucleic acid sequences. Another chemistry kit is the "Ligation Sequencing Kit (LSK)", which uses blunt-end ligation to attach adaptors to the ends of nucleic acid sequences. The RSK requires fewer reagents and is a faster workflow, making it potentially more useful in LMICs. Both the RSK and LSK are currently available to use with or without PCR amplification (PCR-free approaches can be used if the input gDNA concentration is high enough), and both can be used with barcoding kits to analyze up to 96 unique barcodes in one sequencing run. ONT offers other specialized kits including the "16s Rapid Barcoding Kit" for bacterial identification, which uses the RSK in combination with PCR to amplify the 16s ribosomal RNA gene in a single workflow. Supply chain, storage requirements, third party reagents, and experimental goals are all considerations when selecting the ONT sequencing kit for an experiment.

The sequencing portion of the workflow requires both a reusable sequencing device and a consumable flow cell component, both of which offer flexibility in sequencing capacity and portability. Available ONT sequencing devices include the Minion Mk1B (released in 2015), Gridion (2017), Promethion (2018), and Minion Mk1C (2020). The MinION Mk1B (also referred to simply as the "MinION", **Images 1C, 1D, and 1F**), is a portable device that requires a connection to a separate computer to perform sequencing. The most recently developed portable model, the MinION Mk1C (not pictured), is an "integrated" device that contains

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built-in computational ability. However, the Mk1C will be discontinued in 2025 and replaced by the MinION Mk1D with an associated connection to an iPad for portable sequencing. The GridION machine (**Image 1A**) is an integrated benchtop-sized device that can process five MinION flow cells at once. The PromethION device uses higher capacity flow cells to enable higher-coverage sequencing and is available as modular devices (such as the Promethion P2 Solo, **Image 1B)** or fully integrated devices. Laptops used with the MinION Mk1B must contain enough computational power to perform the sequencing. Access to computationally sufficient computers and the internet may impact the experimental approach and bioinformatics analysis by restricting access to bioinformatics tools.

Image 1: Photographic images of three ONT devices with an adjacent laptop for size reference. A) Gridion, B) PromethION P2 Solo, C) MinION (closed), D) MinION (open), E) MinION flow cell, F) MinION flow cell placed into MinION sequencer



Selection of the flow cell model depends on the sequencing machine and experimental goals. Introduced in 2019, the Flongle flow cell is the lowest-cost, has the lowest number of nanopores and most limited sequencing capacity, and must be used with the MinION device. The MinION flow cell (**Images 1E and 1F**) is compatible with both the MinION and GridION and contains a higher number of nanopores than the Flongle flow cell. The PromethION flow cell (not pictured, but compatible with the P2 Solo in **Image 1B**) has a higher number of nanopores than the MinION flow cell. Flow cells can be re-used after a sequencing run via a washing procedure that removes the prior specimen and re-sets the nanopores, though the number of available sequencing pores tends to decrease over time with each flow cell use and flow cells cannot be re-used indefinitely. Flow cell design has improved over time with improved accuracy, with the most recent MinION flow cell (model 10.4.1) reported at >99% accuracy (4), much improved from the initial 15% error rate when nanopore technology was first developed.

During sequencing, nucleic acids are read in real-time which offers unique opportunities. It is possible to enrich for target sequences through “adaptive sampling” during live sequencing, whereby a computer monitors the nanopores for specific sequences of interest as they pass through the nanopores in real-time. In “enrichment mode,” the computer monitors for the presence of pre-specified targeted sequences of interest as nucleic acids pass through the nanopores. If target sequences are present, they are identified and analyzed in the reverse direction to improve read accuracy. Sequences that are not of interest are ejected from the nanopores. Similarly, “depletion mode” can select for sequences that will be automatically ejected from the nanopores. Finally, sequences are read in real-time and can be mapped to existing reference genomes while sequencing is ongoing, enabling researchers to potentially shorten the duration of sequencing when adequate results are achieved.

Nanopore sequencing has been used successfully for accurate, real-time surveillance of infectious diseases outbreaks.

Nanopore sequencing was used as part of the 2015 Ebola virus epidemic in West Africa for real-time genomic surveillance, generating results within 24 hours after sample acquisition(5). This work was done in connection with the ARTIC network, a collaboration between the University of Birmingham, the University of Edinburgh, the University of Cambridge and the Wellcome Trust, which aims to develop affordable mobile viral sequencing systems that can be used in outbreak settings worldwide (6). Subsequently, the SARS-CoV-2 pandemic created an urgent need for rapid analysis of viral variants throughout the world. The ARTIC network developed a multi-plex primer scheme and associated computer software for real-time analysis of SARS-CoV-2 on ONT platforms, and importantly, shared this approach with the international community to enable rapid data collection to monitor the evolving SARS-CoV-2 genome during the outbreak(7). One study in Germany (8) adapted this approach into a simplified laboratory and bioinformatics workflow on clinical samples that were positive for SARS-CoV-2 on real-time PCR. They performed real-time basecalling to optimize the balance between data collection and flow cell usage. The workflow time from RNA extraction to whole genome sequence result was 7 hours for an individual specimen, and 11 hours for multiplexed specimens, and they were able to use each flow cell 3 times for an estimated cost of $40 USD per sample. Nanopore sequencing for SARS-CoV-2 whole genome sequencing was found to detect single nucleotide variants with >99% sensitivity when compared to the short read sequencing platform Illumina as the gold standard (9). These methods facilitated the generation of SARS-CoV-2 genomic surveillance data in many countries, but systematic efforts are needed in order to understand how ONT sequencing is used for pathogen surveillance in LMICs.

Understanding how researchers in LMICs use ONT requires the evaluation of equity indicators in research publications.

Inequities in authorship representation persist for authors from LMICs where the studies themselves are conducted. One study evaluated publications from 2014-2018 in PubMed that were conducted in LMICs and found that 14.8% demonstrated "authorship parasitism," meaning that no authors were affiliated with the country where the study took place (10). Another study reviewed authorship guidelines described by global health-focused journals (N=45) and found that only 17.8% (N=8) of these described criteria for inclusion of local authorship for research conducted in LMICs (11). Of these, only three journals required the inclusion of authors who were affiliated with the study country. In evaluating the use of ONT worldwide, the inclusion of local authors can serve as an indicator of equitable use of this technology. Additionally, understanding if studies perform ONT sequencing in the same country where samples are collected will provide valuable insights into equitable and practical use of ONT worldwide.

Nanopore sequencing has the potential to expand laboratory capacity for molecular diagnostics in LMICs worldwide but the practical use and implementation in Africa has not been evaluated. The authors recognize that the African continent is heterogeneous with a variety of countries, communities, and healthcare settings with different levels of resource access. However, understanding the use of ONT technology in Africa to study human infectious diseases from technologic and equity standpoints can inform future research efforts within the field of molecular epidemiology in global settings. In this scoping review, we:

1) Describe the current use of Oxford Nanopore Technology (ONT) in Africa for surveillance and diagnosis of human infectious diseases in regard to common pathogens, study populations, and laboratory approaches,

2) Discuss applications of ONT for real-time detection of infectious pathogens in Africa, and

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3) Identify challenges, existing gaps, and opportunities for future research studies to design experiments utilizing ONT in Africa.

6. Methods

6.1 Study Framework

This scoping review was conducted by using the framework for scoping review as described by the Joanna Briggs Institute (JBI) Reviewer's Manual(12) in accordance with the PRISMA-ScR guidelines(13) and the JBI methodology for scoping reviews(14). Prior to data collection, the protocol was made publicly available on Open Science Framework(1).

6.2 Search Strategy

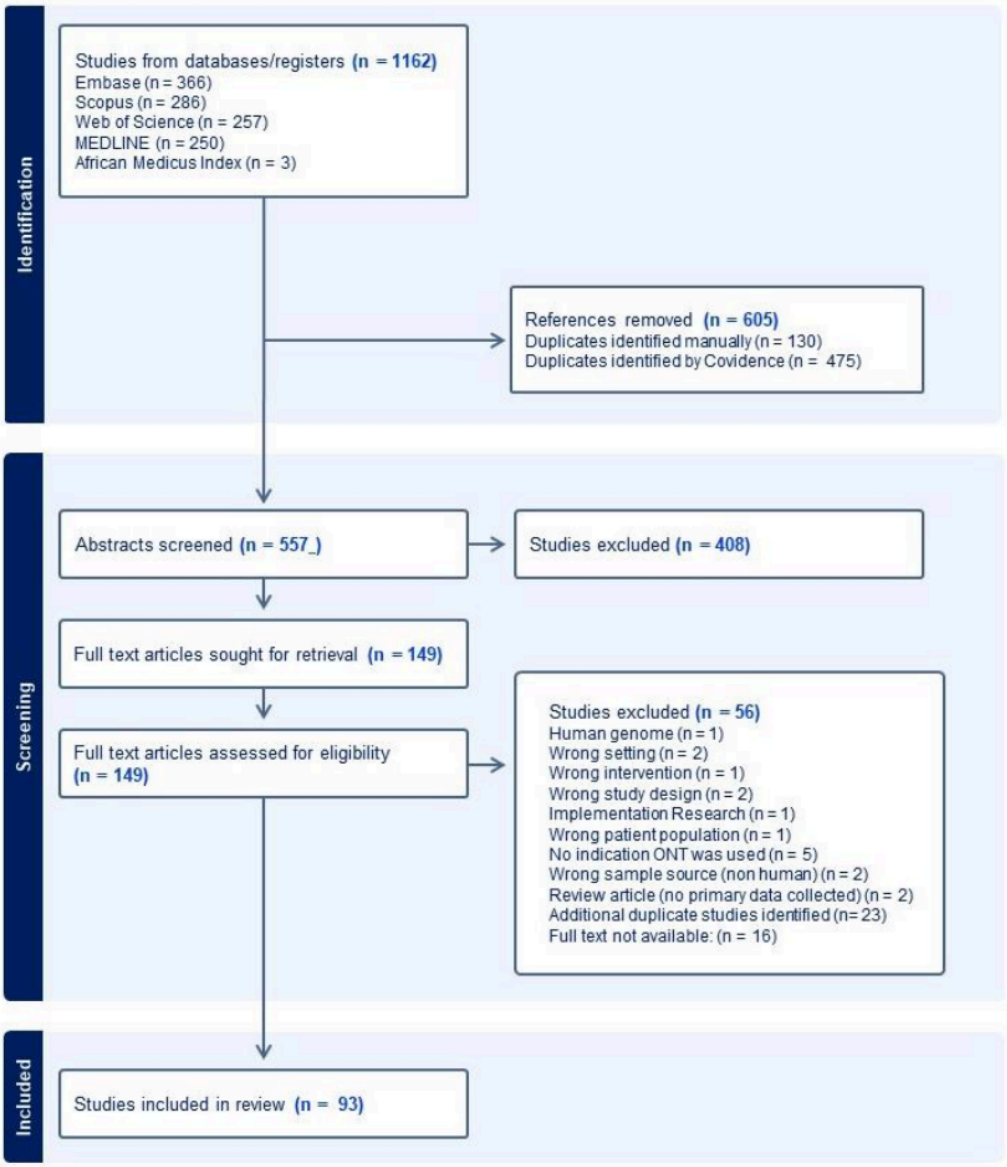
The search strategy was developed by a health sciences librarian (BO) and utilized controlled vocabulary and natural language search terms to capture the concepts of nanopore, minion, gridion, or promethion. Search hedge developed by Campbell was adapted to identify studies related to Africa (15). The search was developed for MEDLINE(R) ALL (via Ovid) and then translated to the following databases: Embase+Embase Classic (via Ovid), Scopus, Web of Science Core Collection (SCI-Expanded, SSCI, AHCI, CPCI-S, BKCI-S, BKCI-SSH, ESCI, CCR-Expanded, IC), and African Index Medicus from their inception to April 30, 2023. Search results were limited to English language and a publication date of 2008 but no other limits were applied. This date range was chosen because Oxford Nanopore Technology was trademarked in 2008, and it is not expected to find published results prior to this. The search string of the MEDLINE (Ovid) database is accessible in the Supplementary Material **(Supplementary Figure 1).** The included full-text articles were used to complete a manual search of the references as well as a forward citation search of all articles included in the study.

Included papers were limited to full text pre-print and published studies of any study type including experimental studies such as randomized and non-randomized controlled trials; prospective and retrospective cohort studies, case-control studies, surveillance studies, and cross-sectional studies; and descriptive study designs including letter to the editor, case reports, case series and cross-sectional studies. Sources such as grey literature, conference abstracts, thesis papers, text and opinion papers were excluded due to insufficient available details available to answer the study questions. Studies with at least one specimen derived from humans of any age or gender (either cultured isolates or primary specimens) that included at least one microbial target were included. Studies that only utilized non-human specimens (animal, plant, environmental) were excluded. Studies examining samples that were obtained in Africa and sequenced elsewhere were included.

Identified citations were collated and uploaded into Covidence and duplicates were both automatically and manually removed. Following a pilot test, titles and abstracts were screened by two independent reviewers (KB, UU) for assessment against the inclusion criteria for the review. Potentially relevant sources were retrieved in full, and their citation details uploaded into Covidence. The full text of selected citations was assessed in detail against the inclusion and exclusion criteria by two independent reviewers (KB, UU). Reasons for exclusion of sources that did not meet the inclusion criteria during full text review were recorded and reported. Any duplicates, such as a pre-print and published paper from the same author and study, were manually excluded. Any disagreements between the reviewers at each stage of the selection process were resolved through discussion or with an additional third reviewer (BT, MO). The results of the search and the study inclusion process are presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping review (PRISMA-ScR) flow diagram **(Figure 2)**[10].

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Figure 2: PRISMA flow chart diagram of the search strategy.



6.3 Data extraction and analysis

Data was extracted from papers that met inclusion criteria by two independent reviewers (KB, UU) using a data extraction tool developed by the reviewers in REDCap. The data extracted included specific details about the author order and affiliation, study, participants, concept, context, study methods and key findings relevant to the review questions **(Supplementary Table 1).** If a study referenced a previously existing protocol or other study for full methodology, that source was accessed if it was required to answer the study questions. Data was exported from REDCap into Microsoft Excel for analysis. Descriptive statistics such as counts and percents were used to analyze the data.

7. Results

The database search generated 1162 results. After removal of duplicates and application of inclusion and exclusion criteria, 93 studies were included in this review **(Figure 2).** Four studies were preprints at the time the search was conducted (3 preprints in 2023, 1 in 2024). In the following sections, we discuss results as they pertain to the main steps of a typical NGS workflow **(Figure 1).**

7.1 Study Design

Authorship and Conflicts of Interest: Thirty-one studies included multiple co-first authors, and 21 studies included multiple co-last authors. Fourteen studies (15%) were composed of only authors who were affiliated with African institutions. The most common authorship team structure included having an author affiliated with an African Institution (termed "African-affiliated) in all three positions of the paper, i.e. as first, middle and last authors (N=52, 56%). Other combinations included an African-affiliated author in the first and middle positions (N=16, 17%), middle only (N=15, 16%), last and middle (N=6, 6%), first and last (N=1, 1%), and first only (N=1, 1%). Two studies (2%) did not include an African-affiliated author. One study included authors who participated in the ONT MinION Access Programme and received some of the MinION devices and flow cells at free or reduced cost.

7.2 Sample Acquisition

Location: Eighty-eight studies analyzed specimens from a single African Country **(Figure 3).** Of these, five countries accounted for nearly 40% (N=35) of all publications including Kenya (N=8), Nigeria (N=7), Democratic Republic of the Congo (DRC, N=7), Senegal (N=7), and Ghana (N=6). Five studies analyzed specimens from multiple African countries, for a total of ten different countries within those five studies[1]. The location where ONT sequencing occurred was clearly specified in 65/93 studies (70%). Four studies performed ONT sequencing in more than one location, and one of these 4 studies collected samples from >1 African country.

Specimen type: The most common methodologic approach was using ONT to sequence a direct patient specimen (58/93, 62%). Two studies included both direct patient specimens and cultured isolates, and the remaining 33/93 studies (35%) sequenced cultured isolates only. For studies that analyzed direct patient specimens, the most common specimen type was either blood, serum, or plasma (N=34 studies), followed by nasopharyngeal or oropharyngeal specimens (N=25), stool (N=12), urine (N=9), cerebrospinal fluid (N=6), lower respiratory tract (N=4), vaginal (N=3), and dried blood spot (N=2). Some studies analyzed a combination of specimen types and therefore these results do not reflect an additive total.

Study Population: Some studies used de-identified patient specimens without available clinical data, and a total of 58 studies (62%) did not report the age of the person or population from whom the specimen was obtained. Children were included in the overall study design for 31 studies (33%) and ONT was clearly used to sequence their specimens in 28 of these studies. Persons living with HIV (PLWH) were included in the overall study design for 5 studies and had their specimens clearly sequenced via ONT in 4 studies. Samples from pregnant people were sequenced with ONT in 1 study. A one-health framework which included human specimens and the addition of either animal or environmental specimens occurred in 4 studies.

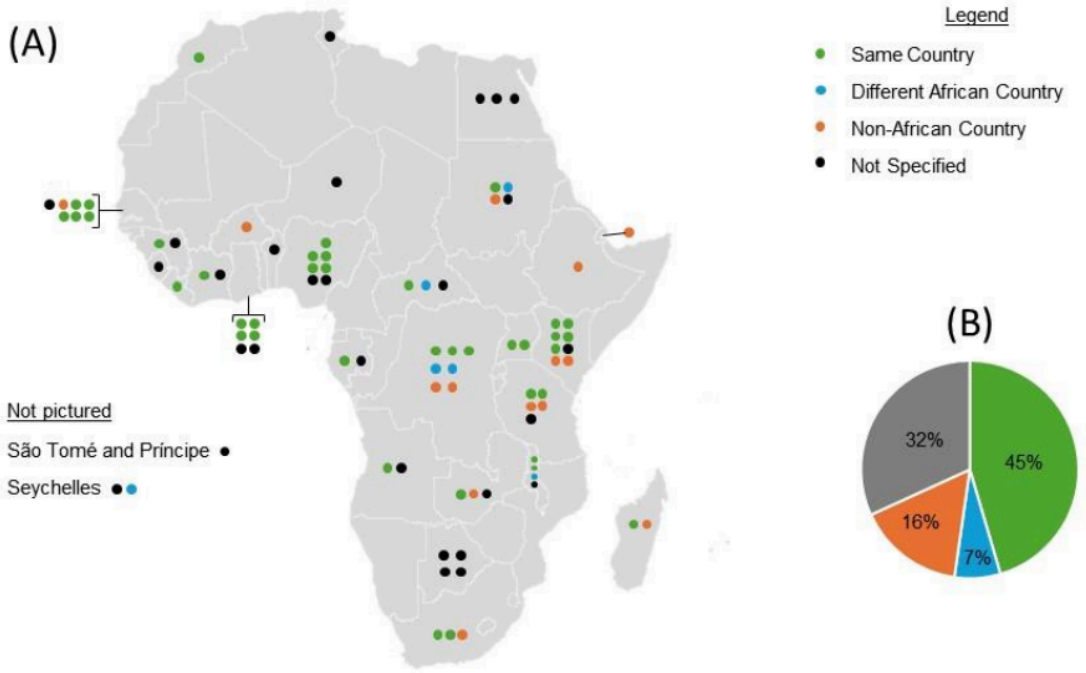
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[1] For the five studies that included samples from more than one country, sample locations were the following 1) Burkina Faso and Ethiopia, 2) Burkina Faso, Niger, Nigeria, 3) Cameroon, Niger, Nigeria, 4) Algeria, Cameroon, Niger, Senegal, Tunisia 5) Madagascar, South Africa, and England.

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Study size: The number of specimens sequenced specifically with ONT technology was discernible in 91/93 studies. Most commonly, a subset of all samples in a study were sequenced with ONT rather than the entire collection of samples. The number of ONT specimens sequenced in a study was typically small (<10 samples, N = 31) or medium (11-99 samples, N =40). Eighteen studies sequenced >100 samples, and two studies sequenced >1000 samples.

Figure 3: A) Location of ONT sequencing in studies that analyzed specimens from a single country (N=88)*. Circles denote one study each and are located within the country where specimens were collected, and are not geographically representative of location. Circle color corresponds to the location of sequencing. B) The pie chart indicates the sequencing location distribution across all eighty-eight studies.



*Angola (2), Benin (1), Botswana (4), Burkina Faso (1), Central African Republic (3), Côte d'Ivoire (2), Djibouti (1), Democratic Republic of the Congo (7), Egypt (3), Ethiopia (1), Gabon (2), Ghana (6), Guinea 2), Kenya (8), Liberia (1), Madagascar (2), Malawi (3), Morocco (1), Niger (1), Nigeria (7), São Tomé and Príncipe (1), Senegal (7) Seychelles (2), Sierra Leone (1), South Africa (3), Sudan (4), Tanzania (5), Tunisia (1), Uganda (2), Zambia (3)

Five studies are not pictured due to sample collection from multiple African countries. These included: 1) Burkina Faso and Ethiopia; 2) Burkina Faso, Niger, Nigeria; 3) Cameroon, Niger, Nigeria; 4) Algeria, Cameroon, Niger, Senegal, Tunisia; 5) Madagascar, South Africa, and England

7.3 Pre-analytic Processing

The majority of studies (84/93, 90%) first used a confirmatory method to establish the presence of a pathogen prior to the use of ONT. This commonly included real time-PCR positivity, serologic positivity, or the use of a previously identified microbial culture isolate. Fifty-seven of all studies (61%) used PCR to enrich the sample for sequences of interest prior to sequencing with ONT, of which 39/57 (68%) used a tiled-amplicon PCR approach and 2/57 used the 16s Rapid Barcoding Kit from ONT. Other studies used PCR to selectively amplify specific genes or sequences of interest. No studies used a hybrid-capture approach or adaptive sampling for enrichment.

7.4 Nucleic acid extraction

Nucleic acid extraction occurred almost exclusively through the use of commercially-available extraction kits (N=90/93). Three studies described custom methods that did not use commercial extraction kits which included alkaline lysis, salting out, and chloroform extraction approaches.

7.5 Library Preparation

Of the studies that reported the use of barcoding (N=66), twenty-six (39%) utilized the ONT Native Barcoding Kit, twenty-eight (42%) used the Rapid Barcoding Kit, two used the 16s Rapid Barcoding Kit, and one used a custom approach with nested PCR primers targeting major capsid protein VP1. Six studies used other types of commercial kits and 3 studies reported using barcoding but did not specify the exact approach. However, some studies that examined multiple samples did not specifically describe the use of barcoding in their methods. For medium-sized studies (between 11-99 samples analyzed with ONT), barcoding was not described in 7/40 studies, and for large studies (between 100-999 samples analyzed with ONT) it was not described in 7/18 studies. One of the two studies that analyzed ≥1000 samples did not describe the use of barcoding. Only eleven studies (12%) clearly reported the use of a negative control sample during the ONT sequencing run.

7.6 Sequencing and Bioinformatics

ONT Sequencing devices: The majority of studies used a single type of ONT sequencing device rather than multiple different models. The most common was the MinION (N=60, 65%), followed by the GridION (N=20, 22%), then MinION Mk1C (N=5, 6%). Six studies used a combination of ONT devices. Two studies did not specify the ONT device that was used. About half of studies (N=49, 54%) used only ONT technology without another sequencing device**.** The most common technologic combination with non-ONT devices was ONT and Illumina (N=38/93, 41%). Sanger sequencing was used in 4 studies and PacBio was used in 1 study.

Flow cells: The specific type of flow cell used for sequencing was not reported in 37/93 (40%) of studies. The most common flow cell was the previous MinION flow cell version 9.4, used in 34/93 (37%) of studies. Three studies used the most recent MinION flow cell version 10.4. Of the 84 studies that analyzed more than one sample, 19/84 (23%) reported the number of samples sequenced per flow cell, ranging from 4-96 samples. Twenty-six total studies (28%) reported the amount of time the specimen(s) was analyzed on the sequencer, with results ranging from 3 minutes to 48 hours. Only three studies discussed the number of times flow cells were re-used; two studies described that flow cells were not reused, and one study described using each flow cell for 2 sequencing runs.

Computers and bioinformatics: Only two of the studies that used the MinION machine described the associated use of an external computer, with one of those fully describing the technologic specifications of the laptop to sufficient detail for reproducibility. Almost half (48%) of papers cited a link to the website GitHub for further details on the bioinformatics approach used in the study.

Sequencing Location: For studies that analyzed specimens from a singular location (N=88), the sequencing location was clearly discernible in 60/88 (68%) of studies**.** For these studies, the sequencing location was in the same country of specimen collection for 40 studies (45%), a non-African country in 14 studies (16%), a different African country in 6 studies (7%), and unspecified in 28 studies (32%) **(Supplementary Table 2).** For all studies, 20 African countries were identified that performed ONT sequencing in-country, whether for samples collected in that country itself or a different country. The top 6 countries by publication number were Kenya (8), Nigeria (6), Ghana (5), Senegal (5), Democratic Republic of the Congo (4), and South Africa (4) **(Supplemental Figure 2)** which were responsible for 32 of the 49 instances where ONT was performed in Africa (65%). Two studies utilized a private company outside of Africa for ONT sequencing and for both of these studies, the details of the sequencing protocol were not publicly accessible. Of the studies that performed sequencing in a non-African country (N=16) the most common location was England (N=4, 16%) followed by Norway (N=3), Germany (N=2), the United States (N=2), Belgium (N=2), China (N=1), France (N=1), and Japan (N=1).

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7.7 Results and Visualization

Clinical Applications of ONT: Only one study demonstrated the use of ONT in a clinical setting to inform care for a patient. This case report used ONT to assist with stool analysis of a hospitalized, clinically ill child with dehydration and loose stools. The authors were called to travel to the hospital with the MinION device and used a portable, non-targeted metagenomics approach to analyze a stool sample. They identified the presence of *Campylobacter jejuni* in the child's stool, a finding that resulted in the prescription of erythromycin for the patient.

ONT utilization during the SARS-CoV-2 pandemic: Twenty-five total studies (27%) used ONT to study the SARS-CoV-2 virus. None of the SARS-CoV-2 related publications discussed any aspect of their workflow timeline (i.e, time from specimen acquisition to result), so it is not known if the results were used to inform public health decisions in real-time. The Illumina sequencing device was used in addition to ONT in 5/25 (20%) of SARS-CoV-2 studies. Two studies examined an additional pathogen alongside SARS-CoV-2 (Influenza virus in one study, other human coronaviruses in one study). Of the 25 publications, one study included specimens from Senegal, Niger, Tunisia, Cameroon, and Algeria and the other 24 studies examined specimens from a single country.

Pathogen detection and surveillance: Other than SARS-CoV-2 virus, the next most common pathogens studied were Dengue virus (N=7), Ebola virus (N=4), and three studies each for *Acinetobacter baumanii*, *Neisseria meningitidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Plasmodium falciparum*, Chikungunya virus, and Hepatitis B virus **(Supplementary Table 3).** Nineteen studies used ONT during an infectious diseases outbreak other than SARS-CoV-2 including (in alphabetical order) Chikungunya virus, Dengue virus, Ebola virus, Lassa virus, *P. falciparum*, Monkeypox virus, *N. meningitidis*, Polio virus, and Sudan virus **(Supplementary Table 4).** Four of these clearly reported obtaining results in real-time.

Generation of whole-genome sequences using ONT: Seventy-one of 93 studies (76%) performed whole-genome sequencing using ONT. Of these, 38/71 (54%) reported the percent of genome coverage achieved and 55/71 (77%) clearly reported uploading a new sequence into a public database. For all studies including whole genome sequencing and other approaches, 70/93 studies (75%) reported uploading a new sequence of any kind to a public database. Some authors uploaded their sequences to multiple databases, and the most common databases were the Global Initiative on Sharing All Influenza Data (GISAID, N=30 studies), GenBank (N=26 studies) and the European Nucleotide Archive (N=16 studies).

Antimicrobial resistance genes and plasmid testing: ONT was used for antimicrobial or antiviral resistance gene testing in 26 studies (28%) and plasmid sequencing was incorporated into 17 studies (18%).

Duration of time required to achieve results: A total of ten studies reported at least one additional aspect of the workflow timeline other than sequencing duration. Four studies reported the time from specimen acquisition to result with a time ranging from less than two days to 10 days. Four studies reported the time from nucleic acid extraction to result, ranging from 14 hours to 30 days. Five studies reported that a result was obtained in <7 days from the time of specimen collection. Two studies reported multiple aspects of the workflow duration, with one reporting 10 days from sample acquisition to result, with 6 hours from library preparation to result. The other reported one day from both sample acquisition to result and nucleic acid extraction to result.

Financial cost of the ONT Workflow: The minority of authors (14/93, 15%) discussed financial cost as a potential benefit for using ONT in their abstract, introduction or discussion. Five total studies reported the per-sample cost of their study results. A study by Shaw et al (2023)(16) in the Democratic Republic of the Congo compared nanopore sequencing for poliovirus detection in stool samples to the gold standard, cell-culture based detection. They reported a per-sample cost of $20 USD per sample for multiplexed runs with 96 samples on an ONT flow cell compared to $31 USD per sample for the culture-based approach. Another study by de Cesare et al (2024)(17) used nanopore sequencing for detection of malaria on dried blood spots in Zambia and reported a cost of $25 USD per sample when multiplexing 96 samples together on a flow cell, and an additional $1 USD/sample cost if Flongle flow cells were used to sequence smaller

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batches of 24 samples. They reported that a custom enrichment approach using reduced volume, and selective whole genome amplification (sWGA) saved $4 USD per sample compared to standard volume sWGA. Another study by Girgis et al (2023)(18) used ONT to analyze dried blood spots *for P. falciparum* resistance genes in Ghana calculated a per-sample cost of $35 USD. One study available as a pre-print at the time of this study by Tshiabuila et al (2024, now available as a full publication(19)) developed an ONT assay for whole-genome sequencing of Hepatitis B virus and compared it to an existing Illumina assay. They found the USD cost per sample was $10-40 with ONT and $150-250 for Illumina. A slightly older study by Tafess et al (2020)(20) investigated antibiotic resistance genes in mycobacterium tuberculosis isolates with the MinION compared to MiSeq. The MinION samples were multiplexed into 12 samples per run with a per sample USD cost of $71.56, while the MiSeq samples were multiplexed in batches of 24 with a per-sample cost of $67.83.

8. Discussion

This is the first scoping review in the published literature that describes the use of ONT to detect human infectious diseases in Africa. This report offers new insights into several aspects of the practical adoption of ONT into LMICs in recent years.

Our results support that ONT technology is being used by African scientists as supported by authorship team structures that included African-affiliated authors in all three authorship positions (first, middle, and last) in the majority of studies. Additionally, 15% of all studies were completed by an entirely African-affiliated authorship team. This is encouraging and suggests that the use of ONT in Africa does not depend on non-African partners to bring in reagents or technology from elsewhere, though this is not a proven assumption and is a topic for future investigation. In this review, only 2% of studies displayed "authorship parasitism", which is smaller than a previously reported 14.8% author parasitism rate (10). Evaluating the country of submission for genome sequences generated with ONT may represent another way to evaluate the equitable use of ONT technology, but was beyond the scope of this study. A previous study (21) examined metadata from GenBank and found that nearly 75% of all sequences were uploaded by the global north, with 70% originating from three countries, the United States, China and Switzerland. Sequences deposited into GenBank by locations in the global south contained long reads more frequently (25.7%) than those in the global north (20.2%), suggesting that long-read sequencing such as ONT may be used more frequently in certain parts of the world.

ONT sequencing is used in-country to sequence specimens collected from people living in Africa. Sequencing was performed in the same country where specimens were collected in almost half of all studies, while about one-third of studies did not provide a clear enough description to deduce where ONT sequencing occurred. The recent SARS-CoV-2 pandemic may have influenced country-specific and regional genomic sequencing capacity by incorporating ONT sequencing into existing laboratories. In 2021 the World Bank conducted a landscape analysis (22) of global networks that supported genomic surveillance and determined that the SARS-CoV-2 pandemic resulted in strengthened genomic surveillance networks in Africa, including the establishment of twelve SARS-CoV-2 specialized laboratories in partnership with Africa CDC located in Senegal, Ghana, Nigeria, Republic of the Congo, South Africa, and Uganda. Other genomic hubs were in Gambia, Mali, and the DRC. In our study, ONT sequencing occurred in all of these locations except for Mali and Gambia. However, the same report also identified inadequate surveillance capacity and heterogeneity in the region defined as Sub-Saharan Africa (SSA). Eastern and Southern SSA had higher sequencing capacity, while Central Africa had significant gaps in surveillance facilities. Some African countries had no genomic sequencing capacity, including Liberia, Namibia, South Sudan, Mauritania, Seychelles, and Tanzania. We identified one study in which specimens were both collected and analyzed in Liberia. Specimens from Seychelles were included in one study and sequenced in Kenya, while specimens from Tanzania were included in five studies with two conducting sequencing in-country, two in a non-African country, and one with sequencing location not discernible. Namibia, South Sudan, and Mauritania did not perform any sequencing, nor were specimens from the countries included in published studies. The World Bank Report (22) found that 70% of sequencing capacity was situated outside of National Public Health Institutes, and 38% of all sequencing devices in Africa were located in

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South Africa. Our study revealed that 4.3% of studies performed sequencing in South Africa, suggesting that ONT technology may be more evenly distributed than other types of NGS technology.

The broad range of pathogens, whole-genome sequencing during infectious diseases outbreaks, and ability to detect antimicrobial resistance genes reveals a broad use of applications for ONT for pathogen surveillance in LMICs. Most studies targeted a specific pathogen of interest rather than a non-targeted approach. The most common pathogen studied was SARS-CoV-2, which is consistent with the increased utilization of the portable MinION sequencer during the global SARS-CoV-2 pandemic. A broad range of pathogens and specimen types were identified, including bacteria, RNA and DNA viruses, the parasite *Plasmodium falciparum*, the fungi *Cryptococcus neoformans* and *C. gattii*, and several mycobacterial species. For the purpose of this review, surveillance activities included both surveillance for pathogens or antimicrobial resistance genes and encompassed the majority of ONT applications (77% of all studies). A total of 19 studies used ONT during infectious diseases outbreaks, though only four clearly reported generating real-time results. In all of the outbreak-related studies, ONT was not used to diagnose patients with the illness, but rather was used for genomic surveillance after a confirmatory test first established the diagnosis in a patient. Generating a full list of antimicrobial resistance genes (AMR) that were examined was beyond the scope of this paper, but examination of AMR was a common application that was done in almost one-third of all studies.

ONT sequencing was not a primary method for the clinical diagnosis of infectious diseases in humans in Africa but shows promise for clinical applications.

ONT has been used for clinical diagnosis in other settings in high-income countries, including a real-time metagenomics-based approach for diagnosis of lower respiratory tract infections in hospitalized patients(23) with a median turnaround time of 6.7 hours, and prospective detection of pathogens in bile duct fluid with results obtained within 8 hours(24). Studies using ONT in Africa continue to emerge, with one study in Eritrea using the MinION to detect drug-resistant tuberculosis in clinical samples with a turnaround time of 5-6 hours (25), and another study in Ghana using ONT to study the genetic diversity, antimicrobial resistance genes, and virulence of *Vibrio cholerae* in clinical and environmental samples (26). In our study, a single case report was identified that used ONT for a clinical application. The authors used a non-targeted sequencing approach to analyze a stool of a child while diarrhea and dehydration, which generated metagenomic data on the entirety of the organisms and host DNA detected in the child's stool. Interpreting metagenomics results such as this in a clinical setting can be challenging due to a lack of gold standard criteria to discern colonizing organisms from true pathogens, but the results of this test led to the prescription of erythromycin to target *C. jejuni* and resulted in clinical improvement.

Clinical diagnosis from direct patient specimens may be challenging due to the need for enrichment strategies to enhance for pathogens that are present in low abundance. The majority of studies in this review used pre-analytic processing to enrich samples for pathogen DNA or confirm the diagnosis of a specific pathogen prior to sequencing. Enrichment strategies may add to the per-sample cost, which is important when considering clinical utility and the ability to scale ONT for widespread use in LMICs. However, the per-sample cost was very under-reported in the papers identified in this review, reported in only five studies. Three of these compared ONT with Illumina workflows and found that ONT had a lower per-specimen cost, which supports that ONT can aid in diagnostic testing in resource-constrained areas. A unique feature of ONT sequencing is the ability to perform adaptive sampling, which can enrich for pathogens without additional reagents, but this approach requires competency in bioinformatics. The ONT bioinformatics analysis program "Epi2Me" is more user-friendly for beginners, but it requires a stable and fast internet connection in order to analyze ONT sequence data. Custom bioinformatics pipelines can be designed to run off-line, but these require additional bioinformatics skills to create and implement. Notably, all sequencing runs require internet access in order to initiate the sequencing which may create a barrier in some settings, but a sustained internet connection is not necessary. No studies used adaptive sampling, and cost savings from this approach are not known. Developing approaches that incorporate this technology into patient testing may contribute to decreased per-sample costs, but additional research is needed.

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Finally, the turnaround time from sample collection to result greatly influences clinical utility. In some centers, traditional culture-based methods may take days to weeks for results to become available, which is not a clinically-actionable timeframe. This review identified several studies that obtained results within 1-2 days, which demonstrates that ONT results can generate clinically-useful information. Overall, the lower cost and fast turnaround time of ONT sequencing shows promise for clinical diagnostics in LMICs, including for infectious pathogens and host genetic syndromes to inform point-of-care decision making. Additional work is needed to develop pipelines for clinical diagnostics in these settings.

Reproducibility of ONT-based workflows will be enhanced by increased reporting of laboratory approaches in the following topic areas:

Flow cell usage: The use of the specific model of flow cell was not described in 40% of studies. As ONT technology advances, updated flow cell technology has demonstrated successive improvements in accuracy from prior versions, and therefore including the specific flow cell version in publications is crucial. Flow cell washing and re-use is an important metric given that sequencing depth may be compromised as the number of uses increases due to a lower availability of sequencing pores, as well as the potential to decrease cost if flow cells are re-used multiple times. The number of times flow cells were used was only specified in 3/93 studies.

Multiplexing: Multiplexing samples through the use of barcoding is an important aspect of any study using NGS, particularly due to the ability to decrease the per-sample cost. In studies with at least a sample size of 11 or greater, 25% did not specify the use of barcoding. It is possible that some of these studies used barcoding without describing it specifically, but additional clarity will facilitate reproducibility.

Paired computer technology: The most commonly used device, the MinION sequencer, requires connection to a laptop with sufficient computational power to perform sequencing. However, only two studies that used the MinION specifically described the use of a laptop. While not a primary aim of this study, four papers discussed that internet access was a barrier to the implementation of ONT technology.

Web-based links for methodologic reporting: Two studies used private companies located outside of Africa to perform the sequencing and the authors of these papers cited the company website for methodology. In one case, the link the authors provided did not function. In the other, the authors provided a link to the company's GitHub for the bioinformatics methods, but there was no link to the wet-lab methodology. These examples highlight the importance of ensuring methodologic transparency to enable accurate interpretation of results and reproducibility.

There were several important limitations to this study. We reviewed only published studies, as gray literature or published abstracts were not expected to contain the level of detail required for data extraction required for this review. It is possible that more scientists are using ONT technology without resulting in publications, and therefore the use of ONT may be under-reported. Additionally, an important step of ONT sequencing is the use of bioinformatics for data analysis, but evaluating the use of different bioinformatics tools was beyond the scope of this study due to the large number of available options, customization, and heterogeneity of study needs.

Conclusion

The potential for ONT to enable timely, low cost, accurate sequencing in African countries is possible as demonstrated through a small number of studies that accomplished these goals individually, but more work is needed to improve transparency and reproducibility of ONT applications in Africa **(Table 1).** ONT was not primarily used for clinical diagnosis of infectious diseases. Most studies used ONT for genomic surveillance of pathogens or antimicrobial resistance genes, and used a PCR-based approach to amplify a nucleic acid sequence of interest prior to ONT sequencing. The use of ONT to generate results with a short interval between specimen collection and result was described in a small number of studies. There is a need for improved reporting of ONT methodology including pipeline timelines, cost, use of barcoding, flow cell

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models, and the use of negative controls. Publications that provide these details will enhance reproducibility and support the development of new studies using ONT for the diagnosis and surveillance of infectious diseases in low resource settings.

Table 1: Current gaps and proposed solutions for research studies using ONT in Africa and other LMIC

Gap	Justification	Solution (s)
Timely Results	Workflow time discussed in 10.7 % of studies	Clear reporting for standardized steps of the sequencing workflow
Cost	Sample cost discussed in 5.4% of studies	Authors report the estimated per-sample cost
Reproducibility of ONT-Specific Methodology	ONT technology is rapidly evolving, and increased methodologic transparency is needed in publications to enhance reproducibility: • Barcoding not described by 25% of studies with >11 specimens • Flow cell model not specified by 40% of studies • Flow cell re-use described in 3.2% of studies (add solution) • 12% of studies clearly reported the use of a negative control during sequencing	• Detailed description of nanopore-specific study design in methods section • Protocols are posted in publicly accessible repository (i.e, protocols.io) • Peer review process includes reviewers with expertise in nanopore sequencing
Sequence Generation	Sequencing location was not clearly discernable in 30% of studies	Clearly describing sequencing location will improve understanding of ONT use in LMICs
	Genomic data was uploaded to public databases in 77% of studies	Authors should clearly describe methodology for uploading data into public databases such as GenBank, SRA*, ENA*, or GISAID*
Clinical Utility	One study clearly used ONT to directly influence patient care	More studies using ONT for clinical applications are urgently needed in LMICs

*SRA = Sequence Read Archive, ENA = European Nucleotide Archive, GISAID = Global Initiative on Sharing All Influenza Data

Author statements

8.1 Author contributions

Kristen Bastug*[1]: Conceptualization, data curation, formal analysis, funding, investigation, methodology, project administration, resources, validation, visualization, writing original, writing review/editing

Uzal Umar*[2]: Conceptualization, data curation, formal analysis, investigation, methodology, validation, writing original, writing review/editing

Brooke Olson[3]: Conceptualization, investigation, methodology, resources, writing original, writing review/editing

Tina Slusher[4]: Supervision, writing review/editing

Christopher Faulk[5]: Supervision, writing review/editing

Mark Okolo[6]: Conceptualization, supervision

Stephen Oguche**[7]: Conceptualization, supervision, writing review/editing

Beth Thielen**[8]: Conceptualization, investigation, methodology, resources, supervision, validation, writing review/editing

8.2 Conflicts of interest

The authors declare no conflicts of interest for this work.

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8.5 Consent for publication – Not applicable

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SUPPLEMENTARY MATERIAL is available for download.

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Supplementary Material - PDF (posted with preprint and Version of Record)

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Supplementary Figure 1: Search terms used in the search strategy.

Ovid MEDLINE(R) ALL

1 exp Africa/

2 (Africa* or sarhara or Maghreb or Algeria or Angola or Benin or Botswana or "Burkina Faso" or Burundi or "Cabo Verde" or "Cape Verde" or Cameroon or "Central African Republic" or Chad or Comoros or Congo or "Cote d'Ivoire" or "Ivory Coast" or Djibouti or Egypt or "Equatorial Guinea" or Eritrea or Eswatini or Ethiopia or Gabon or "Gabonese Republic" or Gambia or Ghana or Guinea or "Guinea-Bissau" or Kenya or Lesotho or Liberia or Libya or Madagascar or Malawi or Mali or Mauritania or Mauritius or Morocco or Mozambique or Namibia or Niger or Nigeria or Rwanda or "Sao Tome" or Principe or Senegal or Seychelles or "Sierra Leone" or Somalia or "South Africa" or Sudan or Swaziland or Tanzania or Togo or "Togolese Republic" or Tunisia or Uganda or Zaire or Zambia or Zimbabwe or DRC or DR Congo or Congo Kinshasa or Democratic Republic of the Congo).ti,ab,kf,kw.

3 (Algerian* or Angolan* or Beninese or Botswanan* or Burkinabe* or Burundian* or "Cape Verdean*" or "Cabo Verdean*" or Cameroonian* or "Central African*" or Chadian* or Comorian* or Congolese or Ivorian* or Djiboutian* or Egyptian* or "Equatorial Guinean*" or Equatoguinean* or Eritrean* or Swazi or Swazis or Swati or Ethiopian* or Gabonese or Gabonaise or Gambian* or Ghanaian* or Guinean* or "Bissau Guinean*" or Kenyan* or Mosotho or Basotho or Liberian* or Libyan* or Madagascan* or Malawian* or Malian* or Mauritanian* or Mauritian* or Moroccan* or Mozambican* or Namibian* or Nigerien* or Nigerian* or Rwandan* or Rwandese or Ruandan* or Ruandese or "Sao Tomean*" or Santomean* or "Sierra Leonean*" or "South African*" or "South Sudanese" or Sudanese or Tanzanian* or Togolese or Tunisian* or Ugandan* or Zambian* or Zimbabwean*).mp. [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms, population supplementary concept word, anatomy supplementary concept word]

4 1 or 2 or 3

5. or exp Nanopores/

6 nanopore?.ab,ti,kf,kw.

7 5 or 6

8 4 and 7

9 (minion or gridion or promethion).ti,ab,kf,kw.

10 7 or 9

11 4 and 10

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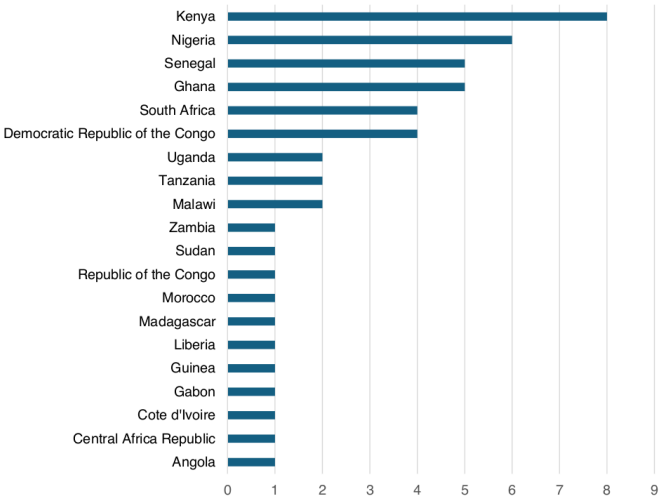
Supplementary Table 1: Detailed data extraction approaches	
Publication Information	Title, year, author names, author affiliations, author order, co-authorship, involvement by Oxford Nanopore Technology (funding, employees, investigative donations), journal
Participant Information	Age, involvement of special populations (children, pregnant women, persons living with HIV), total number, number sequenced via ONT
Study Approach	Specific pathogen of focus, plasmid sequencing, antimicrobial resistance gene, whole genome sequencing, location of sequencing, purposes for outbreak or surveillance activity, direct use in clinical care
Specimen Information	Organism (human required; any additional animal or environmental samples), total number of specimens in study, number of samples selected for sequencing via ONT and the number that were successfully sequenced, type of specimen (direct human specimen or cultured isolate), selection of sample for ONT based on previous lab study (i.e, known PCR-positive, serology positive, positive amplicons seen on gel), type of specimen (body fluid or source)
Specimen preparation	Reverse transcription approach if applicable, nucleic acid extraction approach, library preparation approach, barcoding kit, the use of PCR enrichment prior to ONT, the use of other specific enrichment techniques (hybrid capture probe, tiled amplicon PCR, 16s or 28 s PCR), the use of flow cells (model type, number of uses, number of washes), the use of DNA fragmentation
Sequencing Device	ONT device (flongle, MinION Mk1B, Mk1C, Gridion, Promethion), use of an additional sequence device (Illumina, Sanger, IonTorren, PacBio)
Basecalling and Bioinformatics	Length of sequencing time, link to a GitHub, use of external computer
Results	Time from sample acquisition to analysis or any steps within the NGS workflow, report of use of negative and positive controls, upload of genomic data to public database, result used clinically for a patient, discussion of cost by authors (in introduction and/or results)

Supplemental Table 2: Location of sequencing for studies that examined specimens collected in a single country (N=88 total). The country of specimen collection is shown in the first column in alphabetical order.

Country	Total Number of Studies	Sequencing Location			
		Same Country	Other African Country	Non-African Country	Not specified
Angola	2	1	0	0	1
Benin	1	0	0	0	1
Botswana	4	0	0	0	4
Burkina Faso	1	0	0	1	0
Central African Republic	3	1	1	0	1
Cote d'Ivoire	2	1	0	0	1
Djibouti	1	0	0	1	0
Democratic Republic of the Congo	7	3	2	2	0
Egypt	3	0	0	0	3
Ethiopia	1	0	0	1	0
Gabon	2	1	0	0	1
Ghana	6	4	0	0	2
Guinea	2	1	0	0	1
Kenya	8	5	0	2	1
Liberia	1	1	0	0	0
Madagascar	2	1*	0	1	0
Malawi	4	2	1	0	1
Morocco	1	1	0	0	0
Niger	1	0	0	0	1
Nigeria	7	5	0	0	2
Sao Tome and Principe	1	0	0	0	1
Senegal	7	5	0	1	1
Seychelles	2	0	1	0	1
Sierra Leone	1	0	0	0	1
South Africa	3	2	0	1	0
Sudan	4	1	1	1	1
Tanzania	5	2	0	2	1
Tunisia	1	0	0	0	1
Uganda	2	2	0	0	0
Zambia	3	1	0	1	1
Total	**88**	**40**	**6**	**14**	**28**

*Sequencing occurred in both Madagascar and India but is counted once as occurring within the same country

Supplemental Figure 2: The number of studies sequenced in each African country. Twenty different African countries performed ONT sequencing. Some singular studies performed sequencing in multiple locations, and therefore the additive total differs from the total number of studies.



Supplementary Table 3: Non-SARS-CoV pathogens detected using ONT in Africa . Results are grouped by pathogen type (column 1), then sub-categorized by genus (for bacteria, fungi, and parasites) or illness-category (for viruses) in column 2. Individual studies are listed according to author (column 4), year (column 5) and study size (column 6). Results are listed in alphabetical order. Authors designated with an asterisk indicates the study examined more than one pathogen, and therefore the author and associated study is listed more than one time in the table.

Pathogen Type	Genus or Category	Species	Author	Year	Study Size
Bacteria	*Acinetobacter*	*A. baumanii*	Moyo	2021	<10
			Odih	2023	11 to 99
			Sanchez-Urtaza	2023	<10
		A. nosocomialis	Snyman	2021	<10
	Bordetella	*B. trematum*	Tchan*	2023	<10
	Clostridioides	*C. difficile*	Darkoh	2022	<10
	Escherechia	*E. coli*	Milenkov	2023 (pre-print)	11 to 99
			Tegha	2021	11 to 99
			Lupande-Mwenebitu*	2021	<10
	Helicobacter	*H. pylori*	Mwangi	2020	<10
	Klebsiella	*K. pneumoniae*	Ahmed	2021	<10
			Ofosu-Appiah	2024	<10
			Pedersen	2020	<10
	Neisseria	*N. gonorrhoeae*	Agbodzi	2024	11 to 99
			Juma	2021	11 to 99
		N. meningitidis	Brynildsrud	2018	<10
			Brynildsrud	2019	<10
			MacAlasdair	2021	Not specified
	Providencia	*P. vermicola*	Lupande-Mwenebitu*	2021	<10
	Pseudomonas	*P. aeruginosa*	Olalekan	2023	<10
			Kiyaga	2022	<10
		P. qingdaonensis	Ogoti	2022	<10
	Salmonella	*S. enterica* serovar Typhimurium	Van Puyvelde	2022	<10
	Staphylococcus	Methicillin-resistant *S. aureus*	Monecke	2023	<1
	Vibrio	*V. cholerae*	Ekeng	2021	11 to 99
Fungi	*Cryptococcus*	*C. neoformans, C. gattii*	Kebabonye	2023	11 to 99
Mycobacteria	*Mycobacterium*	*M. avium subsp. paratuberculosis*	Elmagzoub	2022	11 to 99
		M. tuberculosis	Hall	2023	≥ 100
			Nilgiriwala	2023	11 to 99
			Tafess	2020	≥ 100
		M. ulcerans	Tchan*	2023	<10
Parasite	*Plasmodium*	*P. falciparum*	de Cesare	2024	11 to 99
			Girgis	2023	≥ 100
			Hosch	2024	11 to 99
Virus	Arboviruses	Chikungunya virus	Bower	2021	11 to 99
			Dieng	2023	11 to 99
			Vairo, 2020	2020	<10
		Dengue virus	Dieng	2022	≥ 100
			Dieng	2023	11 to 99
			Dieng	2024	<10
			Hafsia	2023	<10
			Hill	2019	<10
			Lazaro	2024	<10
			Neto	2022	11 to 99
		Yellow fever virus	Shaibu	2023	11 to 99
	Viral Hemorrhagic Fever	Ebola virus	Hoenen	2016	11 to 99
			Mbala-Kingebeni	2019	<10
			Quick	2016	≥ 100
			Wawina-Bokalanga	2019	<10
		Lassa virus	Kafetzopoulou	2019	≥ 100
		Sudan virus	Balinandi	2023	≥ 100
	Other	Adenovirus	Lambisia	2023	11 to 99
		Hepatitis B virus	Phinius	2024	≥ 100
			Phinius	2023	11 to 99
			Tshiabuila, preprint	2024 (pre-print)	≥ 100
		Hepatitis C virus	Postigo-Hidalgo	2022	11 to 99
		Monkeypox virus	Vandenbogaert	2022	<10
		Norovirus	Makhaola	2021	11 to 99
		Poliovirus	Shaw	2023	Not Specified

Supplementary Table 4: Summary of studies using ONT for pathogen detection other than SARS-CoV-2 during infectious diseases outbreaks. Studies are grouped by pathogen in alphabetical order.

Pathogen	Author	Year	Sequencing Device	Sample Size	Sequencing Location	Other device used in addition to ONT
Chikungunya virus	Bower	2021	Minion	11 to 99	Same Country	None
	Dieng	2023	Gridion	11 to 99	Same Country	Illumina
	Vairo	2020	Minion	<10	Not Specified	Sanger
Dengue virus	Dieng	2022	Minion	≥ 100	Same Country	None
	Dieng	2024	Mk1c	<10	Same Country	None
	Hill	2019	Minion	<10	Same Country	None
	Lazaro	2024	Mk1c	<10	Not Specified	None
	Neto	2022	Minion	11 to 99	Same Country	None
Ebola virus	Hoenen	2016	Minion	11 to 99	On site	Sanger
	Mbala-Kingebeni	2019	Minion	<10	Same Country	None
	Quick	2016	Minion	≥ 100	On site	None
	Wawina-Bokalanga	2019	Minion	<10	Same Country	None
Lassa virus	Ashcroft	2022	Minion	11 to 99	Same Country and Non-Africa	None
	Kafetzopoulou	2019	Minion	≥ 100	Same Country	Illumina
P. falciparum	de Cesare	2024	Minion and Flongle	11 to 99	Same Country and Non-Africa	None
Monkeypox virus	Vandenbogaert	2022	Minion	<10	Same Country	Illumina
Neisseria meningitidis	Brynildsrud	2018	Minion	<10	Non-Africa	Illumina
Polio virus	Shaw	2023	Minion, Mk1c, gridion	Not Specified	Same Country	Sanger
Sudan virus	Balinandi	2023	Minion	≥ 100	Same Country	Illumina

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