Nanopore sequencing technology for clinical diagnosis of infectious diseases where laboratory capacity is meager: A case report

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ABSTRACT

In resource-limited settings, patients are often first presented to clinical settings when seriously ill and access to proper clinical microbial diagnostics is often very limited or non-existing. On February 16th, 2022 we were on a field trip to test a completely field-deployable metagenomics sequencing set-up, that includes DNA purification, sequencing, and bioinformatics analyses using bioinformatics tools installed on a laptop for water samples, just outside Moshi, Tanzania. On our way to the test site, we were contacted by the nearby Machame hospital regarding a child seriously ill with diarrhea and not responding to treatment. Within the same day, we conducted an onsite metagenomics examination of a fecal sample from the child, and *Campylobacter jejuni* was identified as the causative agent. The treatment was subsequently changed, with almost immediate improvement, and the child was discharged on February 21st.

1. Introduction

The fast turn-around time, portability, and real-time data analysis provided by Oxford Nanopore Technologies (ONT) give the potential of it becoming the future technology to be utilized in clinical diagnostics, especially in areas where laboratory set-ups are meager [1]. Combined with portable laboratory accessories, such as bento-lab (Portable DNA Laboratory.), metagenomics-sequencing has been used to explore previously uncharted microbial genomes [2]. Metagenomics has shown promise as a diagnostic tool especially where microbial culture is impossible and urgent interventions are needed, for example during outbreaks or when the organisms

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cannot be recovered through microbial culture [3–7]. A major obstacle is however, the access to easy-to-use lap-top based bioinformatics analyses, that allow a completely flexible, and field-deployable workflow integrating sample preparation, sequencing, and analyses. As part of a new research project, we have developed a completely mobile set-up, that combines a portable PCR machine, a microcentrifuge, gel electrophoresis, and a transilluminator. We intended to test it using environmental water samples at a field trip on February 16, 2022 outside Moshi, Tanzania. However, in the morning on our way, we got a call regarding a seriously ill child from the nearby Machame hospital.

2. Case presentation

On February 15, 2022 a male toddler 3 years of age, was brought to Machame Lutheran Hospital located in Hai district in Kilimanjaro region with a history of diarrhea and vomiting for the past week. The patient has been vomiting three times a day and passing out watery stool occasionally with blood stains. This was accompanied by on-and-off fever, especially during the night. The patient had been sent to a nearby health center before coming to Machame Lutheran Hospital. At the previous health center, the patient was treated (medication could not be tracked) with no improvement and hence referred to Machame Lutheran Hospital for further management. The patient had no history of admission due to the current illness.

On examination, the toddler was looking ill, clinically pale, with no jaundice observed, wasted, unhappy, weak with a swollen face, and not dehydrated with a body temperature of 38.5 °C. The provisional diagnosis declared acute watery diarrhea with no dehydration, amoebic dysentery, acute malnutrition, and severe anemia. The management plan was as follows admission at the children's ward and the following parameters should be checked; provider-Initiated HIV Testing and Counseling.

(PITC), random blood glucose (RBG), hemoglobin (HB) stool analysis, and malaria rapid diagnostic test (MRDT). The plan was to put the patient on mineral supplements and some antibiotics prior to laboratory findings (empirical management). However, this initial examination was further reviewed by a senior clinician on the same day while the patient was admitted to the children's ward at Machame Lutheran Hospital. The senior clinician reviewed the 3years old toddler with a body weight of 9 kg, who presented with diarrhea for more than a month, vomiting for more than 3 days accompanied by an on-and-off fever. On examination, the patient was observed to be pale++ (severely pale), wasted, weak, with fur-grey-like hair, not interested in the environment, and dehydrated. Provisional diagnosis (i) chronic diarrhea with marasmus, (ii) severe anemia with secondary malnutrition. On February 16, 2022 a watery stool sample was collected for further examination, on a mobile laboratory setup for metagenomics sequencing using Oxford Nanopore Technology. Two hundred microliters (200ul) of watery stool sample were subjected to DNA extraction using Quick-DNA HMW MagBead Kit (Catalog No. D6060) from ZYMO RESEARCH as per manufacturer protocol. The quantity of 400ng of purified DNA was confirmed on Qubit 2.0 fluorometric assay. Library preparation was done using Rapid Sequencing (SQK-RAD004) Version: RSE_9046_v1_rev_14Aug2019 sequencing kit and metagenomics sequencing were done on a MinION for 4 hours. Then MinION data were based called using Guppy 5.0.16.

2.1. Routine laboratory and sequence analysis

Routine laboratory tests performed at the hospital were PITS, MRDT, HB 4.9 g/dl, RBG 7.6 mmol/L, and stool analysis which did

Table 1
Relative abundance of bacterial species identified.

Bacterial species	Number of bases	Relative abundance
Escherichia coli*	1103065	22.66%
Comamonas kerstersii	904446	18.58%
BIfidobacterium kashiwanohense	499159	10.25%
Desulfovibrio vulgaris	341562	7.02%
Parabacteroides distasonis	311669	6.40%
Collinsella aerofaciens	274892	5.65%
Lactobacillus ruminis	189420	3.89%
Sutterella wadsworthensis	156074	3.21%
Roseburia hominis	130953	2.69%
Olsenella sp.	127369	2.62%
Bifidobacterium longuM	94299	1.94%
Prevotella melaninogenica	93160	1.91%
Dysosmobacter welbionis	89796	1.84%
Prevotella intermedia	89723	1.84%
Faecalibacterium prausnitzii	74165	1.52%
Bacteroides uniformis	68538	1.41%
Intestinimonas Butyriciproducens	68394	1.40%
Bifidobacterium bifidum	62769	1.29%
Veillonella parvula	45998	0.94%
Bifidobacterium pseudocatenulatum	38444	0.79%
Campylobacter jejuni*	29508	0.61%
Bifidobacterium catenulatum	27401	0.56%
Prevotella multiformis	24272	0.50%
Prevotella denticola	23245	0.48%

not suggest any causative agent and hence no specific treatment. As a result, there was no improvement in diarrhea. Analysis of sequence data revealed a total of 275450 reads. The total reads that passed trimming and quality checks were 188403. Reads which mapped to *Campylobacter* and humans were 751 and 11 respectively (this means there was very little contamination). The list of references identified by KMA [8], which consisted of references with a significant number of aligned reads according to KMA's default settings, was comprised mostly of commonly occurring gut microbes such as *Escherichia coli* and a series of Bacteroidetes, Actinobacteria, and Proteobacteria [9].8 Interestingly, a *Campylobacter jenuni* was also identified with 751 reads aligning to the same reference. A number of different other bacterial species were revealed, but only two have previously been identified as causative agents of diarrhea in humans, namely *Escherichia coli* and *Campylobacter jejuni* (Table 1). There is only limited knowledge on the abundances of potential pathogens in clinical samples, but in a previous study found that *E. coli* had to be present in very high numbers and preferably with identified virulence factors to be considered the causative agents [10], whereas even a very low abundance (>0.1%) of *C. jejuni* could be considered causal [4]. The identified plasmids and virulence genes were of no considerable importance [11]0, and there was no obvious reason to believe that the commonly occurring microbes found in the sample should be causing the patient's disease. The screening of antimicrobial resistance did not yield any genes that could be causing problems with specific treatments [12]. The *C. jejuni* reads only constituted 0.000296% of the total number of reads. However, in a previous study, it has been established that this is above the threshold for *Campylobacter-positive* cases [4].

2.2. Treatment and patient outcome

On February 15th following the clinical examination, the patient has prescribed zinc 20mg Od, ORs 50ml for a very motion, intravenous (IV) ceftriaxone 500mg bd for three days, 10ml hemovit syrup every 8 h for two weeks, and 20mg furosemide followed by blood transfusion 180mls every 4 h for one day. The patient was also given IV fluid dextrose normal saline alternating with Ringer lactate.

On the 16th the child was also given 200mg of albendazole per oral but diarrhea did not improve. IV fluids were stopped due to orbital edema.

Following sequencing, the patient was prescribed Erythromycin 125g for two weeks, followed by hemovit, ORS, and Ped zinc.

On the 17th of February, the patient showed some improvement, with vomiting and passing of loose stool only once on that day. No fever or edema was observed. However, the patient was retained at the hospital for three more days for the management of anemia and provided with nutritional counseling while continuing with the antibiotics. With further improvement, the patient was eventually discharged on February 21, 2022.

3. Discussion

To the best of our knowledge, this is the first description of the use of a completely field-deployable sequencing set-up, including DNA purification, sequencing, and bioinformatics analyses being used to perform metagenomic investigations on a real-time patient sample for clinical diagnostics. We were able to investigate a fecal sample from an under-five child with acute diarrhea and identify the causative agent as *C. jejuni*. Although *C. jejuni* infections are self-limiting, in the situation of immune-compromised patients' antibiotic therapy is indicated either erythromycin or ciprofloxacin are drugs of choice [13]. The traditional route of diagnosis is time-consuming and may miss the true cause of the disease, predisposing the patient to the wrong antimicrobials, which can lead to antimicrobial resistance [14]. Availability of rapid and cost-effective diagnostic tests might improve the situation [14].

In the presented case, routine diagnosis failed to determine the course, but within the same day, we could use a bento-lab ONT sequencing, and laptop-based bioinformatics to reveal the course and resistant patterns, which aided in instigating the appropriate medication. The Machame hospital has only limited facilities for diagnostic microbiology, and in general, proper diagnosis of *Campylobacter* is difficult and with a large number of false negatives. However, this kind of scenario is just one among many that are happening in most of our health settings in Tanzania. Current diagnostics techniques used in our hospital settings are far from being optimum, thus bringing high throughput technologies in patient care will improve diagnosis in our settings. This project is working with 6 referral hospitals within the country, where nanopore sequencing technology has been deployed on-site to bring the technology where there is a need.

4. Conclusion

We have demonstrated the feasibility of combining methodologies for rapid sample preparation, ONT sequencing, and simple-to-use freely available software for bioinformatics to rapidly identify the likely causative agent in a patient leading directly to changed treatment and a positive outcome at a site with limited diagnostic facilities. This show that already now this might be useful, especially in low and middle-income countries (LMICs), and has the potential to drastically improve treatments, especially in low-income settings.

5. Ethical consideration

Ethical issues were sorted and ethical approval was obtained through the National Institute of Medical Research (NIMR) certificate number NIMR/HQ/R.8a/Vol. IX/3859. Written informed consent was obtained from the guardian of the patient for the publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal at

the request.

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article. </p>

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17439.

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