Short Communication

Strengthening laboratory surveillance of viral pathogens: Experiences and lessons learned building next-generation sequencing capacity in Ghana

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A B S T R A C T

Objective: To demonstrate the feasibility of applying next-generation sequencing (NGS) in medium-resource reference laboratories in Africa to enhance global disease surveillance.
Methods: A training program was developed to support implementation of NGS at Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. The program was divided into two training stages, first at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, followed by on-site training at NMIMR for a larger cohort of scientists.
Results: Self-assessment scores for topics covered during the NGS training program were higher post-training relative to pre-training. During the NGS Training II session at NMIMR, six enterovirus isolates from acute flaccid paralysis cases in Ghana were successfully sequenced by trainees, including two echovirus 6, two echovirus 11 and one echovirus 13. Another genome was an uncommon type (EV-B84), which has not been reported in Africa since its initial discovery from a Côte d'Ivoire specimen in 2003.
Conclusions: The success at NMIMR provides an example of how to approach transferring of NGS methods to international laboratories. There is great opportunity for collaboration between institutes that have genomics expertise to ensure effectiveness and long-term success of global NGS capacity building programs.

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Introduction

Next-generation sequencing (NGS) technologies are rapidly becoming commonplace in public health laboratories. Whole-genome characterization using NGS provides increased resolution for determining pathogen transmission pathways and can be employed in instances where the etiological agent is unknown. The Virology Department at Noguchi Memorial Institute for Medical Research (NMIMR) in Ghana serves as a regional reference laboratory for polio, influenza, and rotavirus, and is currently building NGS capacity for investigating priority pathogens and diseases. To support NMIMR with their NGS initiative, the US Centers for Disease Control and Prevention (CDC) designed a structured NGS training program, provided training and reagents for NMIMR staff, and collaborated to sequence specimens collected as part of poliovirus surveillance activities in Ghana.

Methods

During April 2017, an initial consultation was held at NMIMR to survey laboratory equipment and identify training needs. Discussions focused on transfer of protocols applicable for the Illumina MiSeq system, since the Virology Department had already installed this platform prior to the consultation. Also, the flexibility and throughput of the platform makes it amenable to targeted and

metagenomic investigations of viral pathogens. Based on this meeting, a two-phase NGS training program was devised which utilized methods previously developed for training of US state public health labs by the Division of Viral Disease, CDC (Figure 1). Phase I of the program consisted of a 10-week period to acquire training materials and finalize travel logistics, leading to a four-week NGS training at CDC for two scientists. In Phase II, a four-week preparation period was used to acquire remaining NGS starter supplies and supporting equipment needed at NMIMR, and to identify viral specimens appropriate for sequencing during the on-site NGS training. The two-week training at NMIMR (NGS Training II) for 8 scientists was facilitated by a trainer from CDC, and the scientists from NMIMR who attended the NGS Training I session. Trainees from the NGS Training II session were asked to complete pre- and post-training self-assessments, evaluating their knowledge in key laboratory and bioinformatics competencies on a 1 to 4 scale (from 1 having no familiarity, to 4 being an expert on the competency).

During the NGS Training II session, non-polio enterovirus (EV) isolates from six acute flaccid paralysis (AFP) cases in Ghana were sequenced. Details regarding the laboratory methods, sequencing and analysis performed can be found in the Supplement.
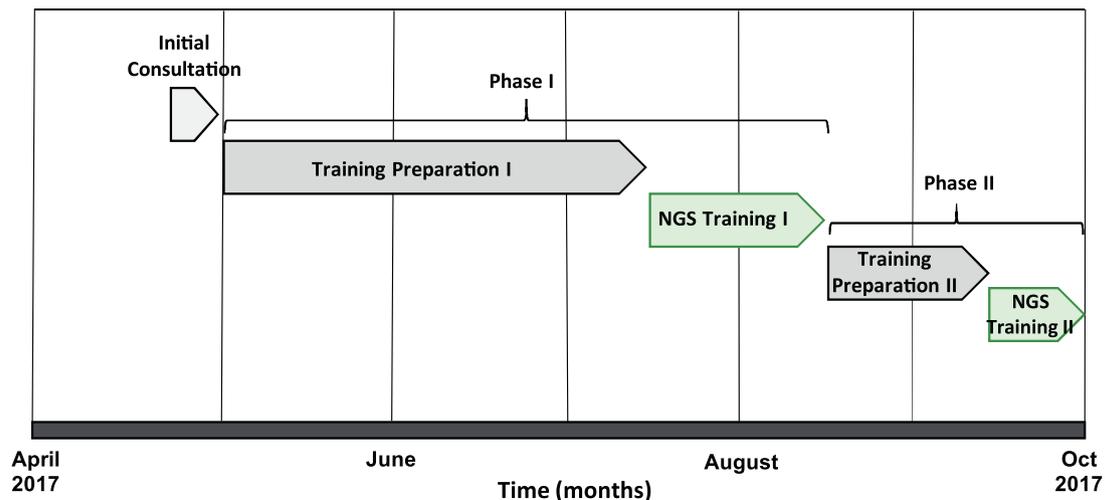
Results

Compared to pre-assessment levels, trainees reported improved understanding in all competencies covered in the training (Figure S1), with the greatest improvements reported in NGS library preparation, calculations for sample dilution and pooling, and reference mapping (2.5-, 2.25- and 2.08-point increase in average reported scores, respectively). In the pre-assessment survey, many of the trainees indicated that they had some previous experience in laboratory procedures that overlap with NGS preparation, including nucleic acid extraction, reverse transcription and PCR, as well as BLAST analysis. This explains why average pre-assessment levels tended to be higher (>2) in these categories.

The EV isolates sequenced during the training consisted of four different types: an enterovirus B84 genome (**MH005795** EV-B84 GHA:BAR:TES/2017), two echovirus 6 genomes (**MH005791** E6 GHA:CEN:ASE/2017, **MH005794** E6 GHA:CEN:UDW/2017), two echovirus 11 genomes (**MH005790** E11 GHA:UER:PUS/2017, **MH005793** E11 GHA:BAR:TAN/2017), and an echovirus 13 genome (**MH005792** E13 GHA:VOL:KRN/2017) (Figure 2). The E6, E11 and E13 genomes represent the first complete coding sequences from Africa for these EV types (Tables S1–S2), and were most closely related to viruses detected in 2013–2014 from Niger (Fernandez-Garcia et al., 2017) (Figure 2B–D). Despite a greater geographical separation (Figure 2A), the E11 genomes shared greater nucleotide identity than did the two E6 genomes from the Central province (Figure S2). The EV-B84 isolate from Brong-Ahafo was most closely related to the EV-B84 prototype genome isolated from an AFP case in Cote d'Ivoire in 2003 (Oberste et al., 2007) (Figure 2). Since the initial discovery of EV-B84, reports of this virus have been limited to a handful of sequences with a broad geographic distribution (Bessaud et al., 2012; Patil et al., 2015; Zheng et al., 2016).

NGS Training Schedule

Figure 1. Gantt chart depicting the next-generation sequencing (NGS) training schedule for Noguchi Memorial Institute for Medical Research (NMIMR) from late April through September 2017. The duration and a brief description of activities performed at each stage in the training schedule is described in the table below.
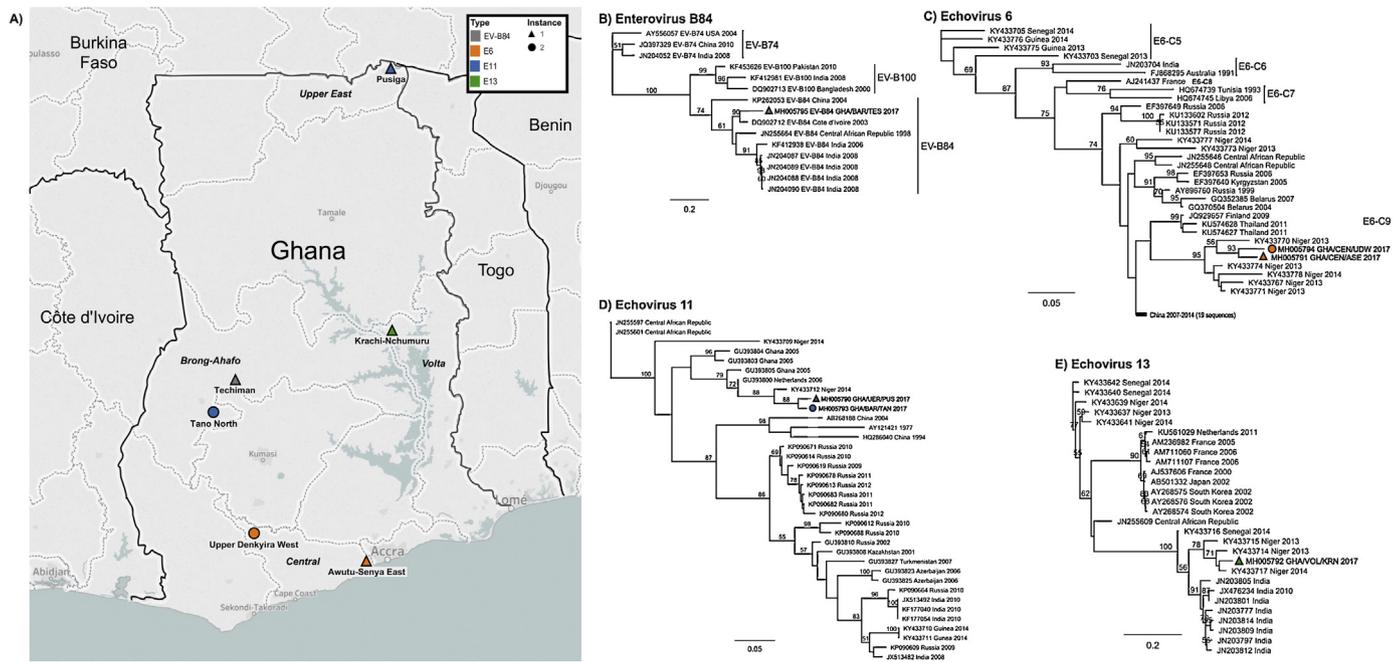
Stage	Duration	Activities
Initial Consultation	1 week	Discussions at NMIMR regarding training needs, equipment/reagent survey
Training Preparation I	10 weeks	Purchase and shipment of training supplies, travel logistics for trainees
NGS Training I	4 weeks	Hands-on intensive training at CDC for two researchers from NMIMR
Training Preparation II	4 weeks	Acquisition of remaining supplies, travel logistics for trainer
NGS Training II	2 weeks	On-site training at NMIMR for eight researchers

Figure 2. (A) Geographic distribution of the six non-polio enterovirus isolates from AFP cases in Ghana. (B–E) Phylogenetic relationship of enterovirus B84 (B), echovirus 6 (C), echovirus 11 (D) and echovirus 13 (E) isolates. Sequences generated in this study are highlighted in bold, with the colored symbol matching the geographical location of the collected specimen in the adjacent map (A).

Discussion

Because of the ability of NGS to rapidly detect and characterize emerging pathogens (Parker and Chen, 2017), implementation of NGS technologies in Ghana and other public health laboratories in Africa is an important step towards meeting International Health Regulations as outlined by the World Health Organization (WHO, 2016). It also provides an opportunity to better leverage existing surveillance programs, particularly for diseases that can be caused by multiple viral pathogens. For example, the high proportion of influenza-negative samples (94%) during a severe acute respiratory and acute febrile illness surveillance study in Ghana (Jones et al., 2016) highlights the need to implement technologies that can detect additional respiratory viruses. The generation of complete EV genomes by NMIMR laboratory personnel during the training demonstrates that NGS methodologies can be applied in new laboratories in a relatively short period of time (*i.e.*, months). The two-phase training program utilized for NMIMR may serve as starting point for designing future programs; further refinement of methods for transferring and evaluating NGS capacity after training is warranted, as well as leveraging collaborations with other institutes involved in global NGS capacity building (Cui et al., 2015). The Africa CDC has recently visited the NGS facility and is in discussions with NMIMR to support the expansion of NGS activities (WK Ampofo, personal communication), as well as plans to utilize NGS as a core facility/resource for Ghana and neighboring countries. These are powerful technologies, and we are confident that with optimal resource planning and careful positioning of these platforms, a sustainable program can be maintained in Ghana serving the western African region.

Ethical approval

Collection of stool specimens from AFP cases were performed as outlined in the Global Polio Laboratory Network procedures, and investigation of viruses collected and tested for AFP surveillance is approved under the CDC Human Subjects Research Office as a public health activity.

Conflict of interests

The authors declare no conflict of interest to report.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.02.008.

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