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Retrospective versus real-time Ebola virus sequencing

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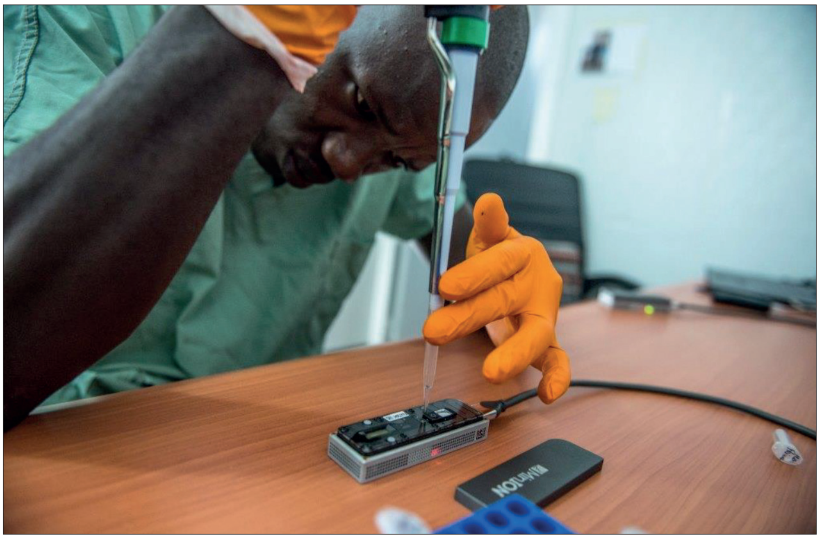
In *The Lancet Infectious Diseases*, Placide Mbala-Kingebeni and colleagues[1] report the effect of retrospective viral genome analysis of the 2018 Équateur Province Ebola virus disease (EVD) outbreak in the Democratic Republic of the Congo. They describe the outputs of an international response, working with local scientists, using in-country, whole-genome sequencing. The 2018 Democratic Republic of the Congo EVD outbreak was the ninth recorded in the country. 54 cases were reported with 33 fatalities, resulting in a case fatality rate of approximately 60%, which is in line with that of the much larger 2013–16 west African outbreak.

Mbala-Kingebeni and colleagues describe how they sequenced 16 Ebola virus (EBOV) genomes to uncover the presence of a novel strain that they named "Tumba". The genome evaluation was done with an Illumina iSeq100 (Illumina Technologies, San Diego, CA, USA), although an initial short read of an early diagnostic sample was done with the highly field-adaptable Oxford Nanopore Technologies' MinION device. Importantly, the sequencing and analysis was done in the capital of the Democratic Republic of the Congo, Kinshasa.

In-silico analysis and antibody binding assays revealed nucleotide variations that could affect the epitope binding site of a key component of the experimental therapeutic monoclonal antibody cocktail ZMapp. This further highlights the dangers of using monospecific monoclonal antibody therapeutics to treat viral infections. A successful phase 3 trial of the recombinant viral-based vaccine VSV-ZEBOV[2] was done during the 2013–16 west African EBOV outbreak. The authors' in silico analysis of the glycoprotein sequence of the "Tumba" strain suggested only a minor potential effect on the immune profile of the target antigen, which is based on the 1995 Kikwit outbreak strain of EBOV. The authors also evaluated the effects of genome sequence variations on the performance of several EBOV molecular diagnostics, concluding that they would not have a negative effect on their performance.

Although the retrospective sequencing of the diagnostic samples started only 3 weeks before the outbreak was declared over, Mbala-Kingebeni and colleagues propose a protocol for the rapid assessment of pathogen sequence on the efficacy of current medical countermeasures, which could have an effect on future incidents.

The 2013–16 west African outbreak was associated with more than 28 000 cases of EVD, which resulted in more than 11 000 fatalities.[3] Permission from local government to ship samples to Europe and North America enabled early mass sequence analysis by the European mobile laboratory (EMLab)[4] and others,[5] which revealed important factors about the timing of the initial spillover event, mutation rate, suitability of available medical countermeasures, and the transmission dynamics during the first year of the outbreak. A subsequent review of approximately 2000 sequences from the outbreak provided greater insight into contributions of geographical, political, economic, and social factors on transmission throughout the region.[6]



EMLab

When we presented our retrospective sequence analysis of 180 EBOV genomes from the first year of the west African outbreak[4] to Guinean and WHO officials in Conakry, Guinea, in March 2015, we and response leaders quickly realised the true value of sequencing data to support epidemiology. We were asked to provide a real-time sequencing service with turn-around times of less than 48 h. Fortuitously, the EMLab was able to request and secure the assistance of MinION and phylogenetics experts to provide a robust sequencing and molecular epidemiology service[7] to local response authorities and WHO, with turnaround times of as little as 18 h, with an average of 48 h. Combined with other regional sequencing services, the resulting analysis revealed an array of transmission chains, including a porous border between Sierra Leone and Guinea. Molecular epidemiology provided insights into many sources of infections that classic field epidemiology was unable to identify. Perhaps the most impressive application of real-time sequencing was illustrated by the investigation of an EBOV flare-up in a previously declared disease-free region of forested Guinea in March, 2016.[8] The diagnostic sample from a female patient who had died was sequenced by the EMLab MinION unit, revealing a close alignment with a sample from a male survivor from the same region, who was tested in 2014, and fortunately included in an early sequencing study.[4] Sequencing a semen sample from the survivor showed that he was the source of the infection. The realisation that survivors could reactivate the outbreak led to a programme of vaccination of contacts of survivors in Guinea led by WHO. This investigation also highlighted the importance of mass sequencing of positive diagnostic samples.

An application of a metagenomic MinION protocol to provide real-time sequencing for the 2018 Nigerian Lassa fever outbreak revealed the increased number of cases was due to multiple spillover events from the rodent reservoir, rather than an increase in human-to-human transmission.[9] The application of methods that do not rely on previous knowledge of the target pathogen illustrates potential utility for even unknown pathogens.

Extensive genome sequencing of outbreaks can add significantly to our understanding of pathogen transmission dynamics, which can greatly assist the epidemiological response and target the often scarce resources to curtail outbreaks, and reduce cases and associated fatalities. The eastern region of The Democratic Republic of the Congo is experiencing the second largest EBOV outbreak in history. The outbreak was first reported in August, 2018, and shows no sign of diminishing, which is partly the result of the severe security issues within the affected region.[10] More than 90 000 individuals have been vaccinated with the VSV ZEBOV vaccine. Although sequencing is being done in Kinshasa, real-time services do not appear to be operational in the outbreak zones. It is unfortunate that the extensive experience of providing real-time EBOV sequencing from the west African outbreak was not used from the start, to provide molecular epidemiology assistance for the current outbreak.

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I have received MinION reagents from Oxford Nanopore Technologies in support of the EMLab sequencing unit in Guinea, 2015, and methodology development for metagenomics of Lassa fever MinION sequencing. My family member did a 4-week internship at Oxford Nanopore Technologies in 2018.

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