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Hello! I am Rodrigue Bikangi, I am biologist and I have a Master's degree in Cellular Biology and Physiology specialized in Molecular Biology. I work at the Center of medical research Lambaréné in Gabon where I live and where I supervise the laboratory of immunology and molecular biology.

Here, I will try to share a little bit of my experience on the SARS-CoV-2 pandemic from my specific field, which is mainly the diagnosis of infections, but also the investigation of variants and the spread of mutated strains of SARS-CoV-2.

First, I am going to introduce the technical background. Then, I am going to outline some of the limitations we encountered here, how we were able to analyze the few sequences we were generating, and on which platforms we use to share these sequences and the experience I have gained from helping during this pandemic period.

So, the CERMEL has systematically used the molecular biology's platforms and continues to do so today.

We established our sequencing methodology on RT-PCR.

It started very early in March 2020 with the arrival of the first patient, the first suspected SARS-CoV-2 infection.

Everything was laid out, to implement protocols, a methodology that allowed us to diagnose populations that could be suspected of SARS-CoV-2 infection.

As a result of that, we gradually used Gene-Xpert as well because we have a tuberculosis laboratory that generally uses Gene-Xpert for tuberculosis infections, whether its multiresistant tuberculosis or other types as well.

That was, therefore, an alternative method that we adopted.

Then, we also acquired RDTs, Rapid Diagnostic Tests.

There were a number of them donated by the country's health authority and we used these as well.

These RDTs were used most often for emergency purposes.

Then we tried to use saliva tests.

Specifically with a set and a machine offered to us by a company whose name I won't mention, but which wanted to assist us in the context of the procedures that could be put in place for the diagnosis of SARS-Cov-2.

That's for the diagnosis in itself.

Now, when it comes to sequencing, in March 2021 we used the genomic research here in Gabon, starting in January, but it was in March 2021 that we tried to produce our first sequences.

That was done on the premise of Sanger Sequencing, including targeted sequencing of the mutation regions of SARS-CoV-2.

Then we slowly migrated to the Oxford Nanopore platform to be able to do the whole genome.

We used the MinION in order to do that.

It is worth remembering that we had three types of sequencers here at CERMEL, the 3500 Analyzer, the Illumina MiniSeq, but also the MinION. The problem with the Illumina MiniSeq is that we had major technical problems with it and therefore it could not be used until now.

It is not used for these reasons.

We therefore, systematically use for the sequencing of SARS-CoV variants, for research purposes, the 3500 Analyzer, the targeted Sanger-type sequencer, and the MinION, which allows us to do the whole genome.

So, here is a presentation of the platforms we have used and the tests we have been able to implement, both in terms of diagnosis and sequencing.

The first of many limitations was of human resources. That is to say that there were not enough qualified specialists to be able to implement and establish these different procedures.

It must also be said that the panic created around transmission based on lethality, but also on the nature of cases of reinfection with the virus, made technicians and also some research engineers concerned about being able to perform the PCR tests under the right conditions.

So at the beginning, there were only three of us at the center who could perform these tests.

As time passed, we trained our colleagues and others to try to strengthen the diagnosis team because, as the pandemic spread, the number of infections increased and the infected population grew.

It was, therefore, not sustainable with only three people.

We went from a team of three people to five people, five to eight people, and eight to twelve people.

So, it was a very large team around which there were tasks and activities established.

However, at the beginning, it was really a major challenge.

The other challenge was the lack of supply for inputs, especially consumables.

We were able to issue purchasing orders, but unfortunately these are processed in Europe, and it took a long time for the orders to be delivered.

So, we had to place the order in January or at the beginning of the pandemic, but we could only place an order in April, and it arrived only in October, for instance.

This problem placed us in a situation where we had to adapt to the reagents and consumables used by other laboratories that did not necessarily have the same equipment as us.

Then we had to consider different primers, for example, which match with which type of probe, because the fluorochromes are not systematically the same and so we had to think, we had to adapt, we had to put ourselves in the appropriate context and try to organize our work by adapting our approach, but also by adapting to the kits that had just been introduced.

This was also a very strong limitation because the borders were closed.

With the borders closed, the freight was not the one we were used to and that was before consistent.

We therefore had real problems with the supply of inputs and consumables. I have already said earlier, but this is in the context of sequencing, we had this difficulty in starting sequencing because our machine for the whole genome was not available.

So, the Illumina MiniSeq that we have here had a problem and we couldn't start very early on the search for SARS-CoV variants.

When that's understood, you have to be aware that we did the best we could and we still went ahead and started, as I mentioned earlier, sequencing the mutant forms of SARS-CoV-2 between March and April, and we continued all the way through 2021 in order to have some sequences.

So, how were these sequences meant to be interpreted?

Once the sequences had been generated, they were retrieved.

Once they were retrieved, in terms of targeted sequencing, they were cleaned up.

We used two types of software, BioEdit and Chromas, which are free software and can be easily found on the internet.

These are the programs that enabled us to take the different sequences and to align them.

We aligned them against the Wuhan sequence as a reference.

It was then necessary for us, since the Wuhan strain sequence was known, to look at the mutations on this sequence, on the Spike protein of our produced sequences to see if there were differences with the Wuhan sequence.

So, we had a file that established at which level, in which type of region there were mutations, for example L452 or E484, or others. They are located on the spike protein, and we simply needed to do the alignments based on the original sequence.

Once these alignments were made, we could see on the sequences produced, which ones were similar to the original sequence, which ones could be the targets of known mutations that actually had this replacement or this protein substitution.

Based on that, we could conclude that there was a mutation by looking at two regions, as suggested, where the mutation was actually to be found.

That's how we were able to detect the alpha variant first and then the kappa variant.

We carried on with the delta variant, but it became more and more difficult because the mutations became increasingly strong, but also increasingly large over the whole area of the spike protein.

Then, fortunately, we also had the Oxford Nanoprobe technology with the MinION.

So, we used the MinION for whole genome sequencing.

It was much easier because there are scripts, everything is done, so that when the sequence is generated, it immediately displays the mutation or the variant on the generated sequence.

If we actually had issues, we could just go to NextClade

and insert another sequence.

It could show us the lineage of the sequence and to which mutation this sequence could belong.

So overall, that's actually how we worked to analyze our different sequences.

It must be said that we do not have a very strong background and training in sequence analysis and mutational analysis.

We are working on it, and we obviously hope that we will succeed.

However, that's how we detected, interpreted and analyzed the sequences that we were able to generate not only from Sanger Sequencing, but also from the whole genome through MinION in a very simple way.

Once these sequences were produced, there were explicit instructions from the health authority to systematically share these sequences and so we used the GISAID platform for which the identifiers had been created.

You can therefore now find the sequences that were produced both at CERMEL, but also in other centers in Gabon on the investigation of SARS-CoV variants, the Alpha sequences, the Kappa sequences and even the Delta and Omicron sequences. They are available and uploaded to the GISAID platform.

This was mandatory in the framework of the implementation of the genomic surveillance protocol in Gabon. What I have learned and what I can share based on my experience with other laboratories or with other researchers or people who would like to start up or who are involved in the same activity is that we must regularly invest in training people, in exchanging technologies, and in capacity strengthening.

We were a little fortunate in Africa because many countries are doing research, but very few have equipment that can provide a clear response when we are in such cases.

These situations can still happen, and maybe they will happen again.

Today, I think we should work especially in the direction of inter-institutional, perhaps interstate or subregional collaborations, to strengthen our local capacities and to contribute a little more in the field of molecular biology, but also of biology.

So, this is a bit of what I can share about my experience.

It must be said that it was not quite easy.

We have worked a lot, but we are managing it little by little. That's what I wanted to share with you.

Thank you again.