Molecular characterization of circulating DENV-2 during outbreak in Northern Senegal, Rosso 2018

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Abstract:

Globally 390 millions of people are at risk of dengue infection; over the past 50 years the virus incidence increased thirty-fold. In Senegal, an unprecedented occurrence of outbreaks and sporadic cases was noticed since 2017. In October 2018 an outbreak of DENV-2 was reported in Rosso area in the north of Senegal at the border with Mauritania. Out of the 187 blood specimen samples collected, 27 were positives by qRT-PCR and 8 were serologically positive for DENV IgM. Serotyping using qRT-PCR reveals that isolates were positive for DENV-2. A subset of DENV-2 positives samples was selected and subjected to full genome sequencing followed by phylogenetic analysis. Analysis of 06 nearly completed genome sequences (n= 6) revealed that isolates belong to the cosmopolitan genotype and are closely related to the Mauritanian strains detected between 2017 and 2018 and those detected in many West African countries such as Burkina Faso or Cote d'Ivoire. Our results suggest a transboundary circulation of the DENV-2 cosmopolitan genotype between Senegal and Mauritania and call for a need of coordinated surveillance of arboviruses between these two countries. Interestingly, high level of homology between West African isolates highlights endemicity and call for a set-up of sub-regional viral genomic surveillance which will lead to a better understanding of viral dynamic, transmission and spread across Africa.

Keywords: Dengue, Dengue virus 2, cosmopolitan genotype, Senegal, Mauritania, Molecular characterization, Full genome sequencing

Introduction:

Dengue fever is considered to be one of the most prevalent arboviral infection worldwide [1]. According to the WHO, around 390 millions of people are at risk of dengue infection globally. [2] An infection by dengue virus causes clinical manifestation ranging from flu like illness (Dengue Fever) to life threatening disease (Severe Dengue) [3] with a case fatality rate of 1-5 %. [4–6] The virus mainly transmitted by mosquitoes vectors of the genus Aedes is known to be prevalent in tropical and subtropical regions. [7] Dengue virus (DENV) is an arbovirus belonging to the family flaviviridae, genus flavivirus. [8] The virus exists in four antigenically

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and phylogenetically distinct serotypes namely DENV 1-4 sharing around 65 % of genome similarity at the nucleotide level. [9] An infection with one the up mentioned dengue virus serotype gives a long lasting immunity against this particular serotype. [10] Despite the virus circulation in African since the nineteenth century; his epidemiology in many African countries is less known compared to Asian and American countries.[3] This lack of knowledge about dengue occurrence in Africa is mainly linked to the low awareness against this virus, others prevalent malaria like pathogen and the lack of surveillance and diagnostic tools.
In the recent years, several dengue virus outbreaks were reported in many African countries including Kenya, Burkina Faso, Cote d'Ivoire, Mauritania, Senegal [11–15] highlighting its active circulation.
In Senegal the first report of dengue circulation was made in Bandia area in 1970. [16] Since then, many sporadic cases and outbreak were noticed. [17] Interestingly, an upsurge and yearly occurrence of outbreaks were noticed since 2017 affecting many areas and marked by the co-circulation of many serotypes [17,18]; supporting the hyper-endemicity of DENV. [18] In 2018, an outbreak of DENV was reported in the north of the country at Rosso area located in Saint-Louis region at the border with Mauritania where dengue virus circulation was reported. [14] This study aimed to describe laboratory findings and results from molecular characterization of obtained isolates during the DENV outbreak in Rosso area in 2018.

Material and Methods

Ethical consideration

In this study, we used samples collected as part of approved ongoing surveillance conducted by the Institut Pasteur de Dakar (a World Health Organization Collaborating Centre for Arboviruses and Haemorrhagic Fever Reference and Research). All samples from humans were de-identified before we performed virus characterization and analyses; thus, no patient information can be reported.

Outbreak notification and patients enrolment

In Senegal since 2011 a national wide Syndromic Sentinel Surveillance Network has been implemented namely (4S network), coordinated by the Ministry of Health (MoH) and Institut Pasteur de Dakar (IPD). [19] This network previously dedicated to the surveillance of influenza like illnesses (ILI) was reviewed in 2015 to include arboviruses and others pathogens throughout a syndromic and integrated approaches mainly based on fever. [20] Indeed, An early warning system (EWS) of diseases under surveillance was set up in 2015 that allows the MoH to detect and alert quickly any abnormal health event.

In early 2018 this system allowed the detection of simultaneous dengue outbreaks in Fatick and Touba [21] overlapping with the period of Touba's Grand Magal, a religious event gathering more than 1.3M of people. To contain the spread and the apparition of other foci at the same time, all healthcare facilities across Senegal were alerted by the MoH to report suspected DENV cases according to the case definition of fever and 2 of the following symptoms: headache, arthralgia, myalgia, diarrhea, vomiting, or retro-orbital pain.
Following this, upsurge of high number of unexplained febrile illness cases were noticed in Rosso area (16°30′ 46.00″N, 15°48′ 18.00″W) located in the north of Senegal in Saint-Louis

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region (Figure 1). Intriguingly, collected patients' blood samples were negative for malaria Rapid Diagnostic Tests (RDTs) therefore a subset of collected blood samples were (then) sent to the WHOCC for arboviruses and haemorrhagic fever viruses, held by the IPD, to roll out a potential arboviral etiology. At IPD the performed molecular tests yield positive results for Dengue virus (DENV). Following the confirmation of DENV circulation, all patients visiting health care facilities in Saint-louis region with symptoms matching the case definition were enrolled and 5ml of venous blood was collected into a dry tube then shipped to the IPD virology lab for diagnosis.

Figure1: Map showing the location of Rosso (green dot) in Saint-Louis region (area highlighted in dark grey). The arrow represents the link between places where the outbreak occured (Rosso) and the Virology Lab located at the IPD located in Dakar (yellow dot)

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Laboratory molecular and serological assays

At the laboratory, whole blood samples were centrifuged at 4000rpm for 2 min to obtain sera; which were further aliquoted on 2ml tubes and stored at – 80 degrees for biobanking purposes. RNA extraction was performed using Qiagen RNA mini kit (Qiagen, Hilden, Germany) with 140µl of sera as input matrix; RNA was eluted in a final volume of 60µl.
To assess dengue infection, extracted RNA were subjected to pan DENV assay qRT-PCR [22]according to a protocol previously described by Dieng and Colleagues. All samples with a Ct values below 37 were considered as DENV positive. panDENV positive samples were then subjected to serotyping using TibMolBiol (TibmolBio, Berlin, Germany) Modular Dx Dengue typing kit (Cat-No. 40-0700-24), according to previously published protocol [15], which allow simultaneous dengue virus serotyping from 5 µl of RNA input.
To reduce the likelihood of missing some cases by using only molecular detection tools sera (from), patients with history of fevers of more than 5 days were subjected to Enzyme Linked immunosorbent assay for the detection of DENV immunoglobulin M (IgM). [23] To roll out cross reactivity with others flaviviruses, positive IgM samples were confirmed by Plaque reduction neutralization test (PRNT). [24]

Full genome analysis of DENV-2 isolates

Using Nextera DNAflex protocol, full genome sequencing was attempted on a subset of DENV2 qRT-PCR positive sera. Briefly extracted RNA were subjected to cDNA synthesis using Reverta-L RT kit (Amplisens, Russia), according to the manufacturer's instructions. DNA amplifications were performed by using a multiplex PCR primer scheme designed to amplify on two pools the entire coding region of DENV-2 [25]. For each samples, equal concentration of pool 1 and pool 2 amplicons were pooled per sample prior to library preparation using illumine DNA preparation kit according to manufactures recommendations; whole genome sequencing was performed on Illumina Iseq 100 using Illumina Iseq 100 reagents (300 cycles). Consensus sequences were generated by de novo assembling using Genome Detective (https://www.genomedetective.com/app/ accessed on 15 November 2020). To define the genotype of newly assembled DENV-2 we used Genome detective dengue typing tool (https://www.genomedetective.com/app/typingtool/dengue/).

Dataset and Phylogenetic Analysis

In addition to the full genome obtained during this study (n = 6), we downloaded from Genbank representative full genome sequences of DENV-2 cosmopolitan genotype with informations regarding date and country of isolation. Sequences were aligned using MAFFT [26]; manually curated with Geneious prime 2021 (Biomatters, New Zealand). Maximum Likelihood (ML) trees were estimated using IQTREE v.1.5.5 [27] with automatic model selection conducted by ModelFinder using the Bayesian information criterion (BIC) [28] generated phylogenetic trees were visualized using Figtree v.1.4.4 (http://tree.bio.ed.ac.uk).

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Result

From 09 October 2018 to 23 November 2018 a total of 187 sera samples from febrile patients suspected to have DENV infection were collected and shipped to the WHOCC for arboviruses and haemorrhagic fever viruses at the Institut Pasteur de Dakar for laboratory tests. Performed molecular assay using panDENV assay qRT-PCR confirmed the detection of 27 positives cases among enrolled patients. Additionally, 8 patients sera were confirmed to harbouring DENV specific IgM either by ELISA followed by PRNT (Figure S1). Serotyping on DENV positive RNA reveals that the circulating virus belongs to DENV-2 serotype. For molecular characterization the subset of 8 isolates were successfully amplified by the used scheme; this allows to retrieve 06 nearly complete full genome sequences.

Used online genome detective typing tool and/or phylogenetic analysis based on full length viral coding region revealed that isolates obtained during this study belong to the DENV-2 cosmopolitan genotype and cluster with strains sampled from West Africa between 2016 to 2019 (Figure 2A and Figure 2B). Interestingly, based on full genome phylogenetic tree, our isolates (highlighted in Purple) were closely related to those detected during the dengue outbreak in Mauritania in 2017 – 2018 (Figure 2 B). Senegalese and Mauritanian strains share up to 99.8 % of nucleotide pairwise identity which correspond to around 99.9 % amino acid pairwise identity (Figure 3); compared to MT980921 all Senegalese isolates sampled in 2018 contains 3 unique amino acid changes located at position 1183 (V changed to an M), at position 1405 (R changed to K) and at position 2266 (P changed to T) (Figure S2). Interestingly, group of strains isolated in West-Africa (Figure 2B) share over 99.6% of nucleotide pairwise identity.

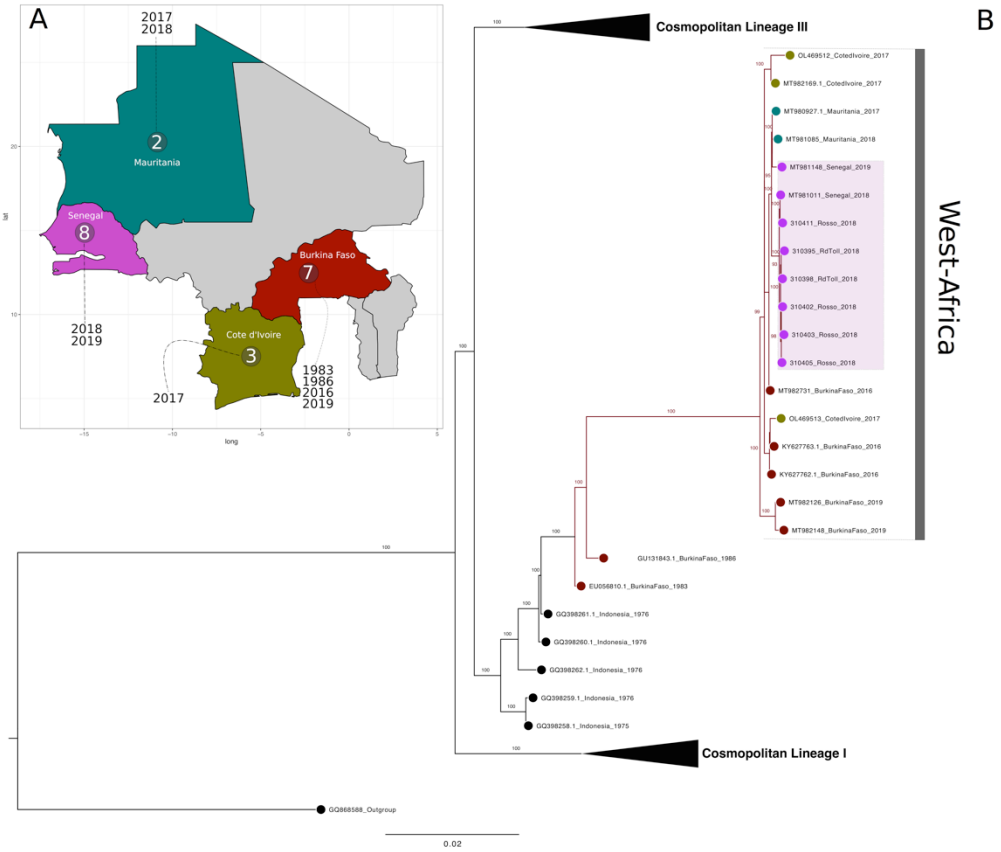

Figure 2: Phylogenetic tree based on full length genome sequences of DENV-2 collected during the Rosso outbreak in 2018. The tree was drawn using representative sequences of

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DENV-2 cosmopolitan genotype available on Genbank (Panel B). The map shows the geographic distribution of African countries where full genome sequences of DENV-2 are available; for each country the number of used isolates on the Panel B are represented the number written in white colour inside the circle (Panel A).
Tips of sequences collected from African countries are coloured according to the same colour scheme on used Panel A.

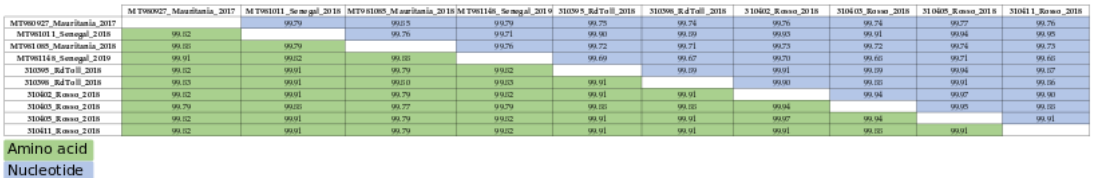

Figure 3: Pairwise nucleotide sequences identity between Senegalese and Mauritanian strains.

Discussion:

In Senegal all dengue virus serotypes were reported. [18,29] For a long time the landscape of dengue virus occurrence was marked (for years) by the circulation of sylvatic DENV-2 in southern part of the country. [30,31] In 2009 a major shift occured with the first urban dengue epidemic with 196 confirmed cases caused by the DENV-3 affecting big cities such as Dakar, Louga and Thiès. [32] Since 2017 a Multifoci and multiserotypes circulation of DENV has been noticed [18]. From 2017 to now only serotype 1 and serotype 3 were principally incriminated serotypes during reported major outbreaks; the serotype 2 was only associated with sparse sporadic cases. To our knowledge this study is the first one to report laboratory findings and full genome sequences from dengue outbreak at the northern part of Senegal. Among collected samples, 27 were dengue positives by qRT-PCR, serotyping of confirmed cases reveals that they belong to the serotype 2; molecular characterization based on full-length genome analysis (n = 06) revealed that isolates belong the cosmopolitan genotype of DENV-2. This particular genotype was previously reported in Louga area in 2017 from 04 patients out of 131 positives cases [17], in Thiès area in 2018 from 04 out of 17 generated full genome sequences [17]. Both detections highlight a widespread but a sparse occurrence of a non sylvatic DENV-2 strain across the country. But nevertheless, this particular DENV-2 strain has never been before the main serotype during any of the previously reported outbreaks till now.

The cosmopolitan genotype is known to be the most widely distributed Dengue 2 around the world. [33] Phylogenetic analysis reveals that our isolates form a well-supported cluster (100 % bootstrap) (Figure 2B) with strains detected in many West African countries between 2016 and 2019 with a higher level of pairwise nucleotide sequence identity (99.6 %). Many west African countries such as Burkina Faso in 2016 and Cote d'Ivoire in 2017, Senegal in 2017 reported circulation of DENV-2 at the same time (frame) supporting a probable regional circulation of the same strain. [12,17,34] Dieng and colleagues reported an importation of DENV-2 strain closely related to those circulating to Burkina Faso from Cote d'Ivoire to Senegal in 2017. [13]

Interestingly, sequences of isolates obtained during this study were more closely related to strains circulating in Mauritania between 2017 and 2018 [14] and share high nucleotides and amino acid pairwise sequences identity of 99.8 % and 99.9 % respectively. Both findings support that Mauritanian and Senegalese strains share a recent common ancestor and support a likelihood of transboundary circulation of the virus between both countries. Analysis of translated polyprotein reveals that Senegalese strains possess unique amino acid changes all located on non-structural proteins; mutations on this gene are known to impact virus parameters associated with spread and dispersion as well as fitness. [35] Rosso share border with the southern part of Mauritania where *Aedes spp* mosquitoes are known to be present since late 1960. [36] Despite the presence of DENV vector, dengue fever epidemic had never been reported in Mauritania prior to 2014 [14] but in contrast Senegal is hyperendemic for DENV. [18]

On another hand Rosso is known to be a commercial hub with occurrence of intense trading activities between Senegal and Mauritania making the borders porous, facilitating vectors and virus dispersal.

The combination of these factors increases the likelihood of transboundary DENV transmission between Senegal and Mauritania, which share 742 km of borders.

In contrast to Mauritania, Senegal possesses an effective syndromic surveillance system (4S Network) since 2015 allowing an early warning against unhabitual health events. Findings from this study stresses the need to reinforce surveillance system at the borders and to implement coordinated outbreak investigations in case of epidemic occurrence near to shared borders between both countries.
To better understand DENV spread, dispersal and dynamic in Africa, particularly in West-Africa, improved genomic surveillance is urgently needed.

Conclusion

In summary, this work describes the result of molecular characterization DENV-2 causing an outbreak in Rosso area (Senegal) at the Mauritanian borders. Obtained 06 whole genome sequences were contextualized to a global dataset containing few available sequences from African countries. Performed phylogenetic analysis revealed that strains collected in Rosso are more related to those detected in Mauritania between 2017 and 2018 but still closely related to isolates circulating in many West-African countries between 2016 and 2019.
This work, in combination to previous reports, highlights that DENV is becoming a major public health problem in West-Africa where the same strain is seem to be endemic
According to the scarcity of genomic data from African DENV isolates it is crucial for better understanding of DENV 1-4 dynamic and its spreading in Africa particularly in West-Africa to set a pan African genomic surveillance of DENV similar to those implemented during ongoing SARS-COV 2 pandemic.

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Author Contributions:
Conceptualization, I.D., A.A.S., O.F. (Ousmane Faye) and O.F. (Oumar Faye); data curation, I.D., M.N ; formal analysis, I.D. ; funding acquisition, O.F. (Ousmane Faye) and A.A.S.; methodology, I.D., M.N., M.H.D.N., S.S. (Safietou Sankhé) ; resources, A.A.S., O.F. (Ousmane Faye) and O.F. (Oumar Faye); software, I.D., A.G. ; supervision A.A.S., O.F. (Oumar Faye) ; validation, O.F. (Oumar Faye); visualization, I.D ; writing—original draft, I.D.; writing—review and editing, I.D., M.M.D., M.H.D.N., A.A.S., L.M.N., O.F. (Ousmane Faye), S.S. (Safietou Sankhé), S.N.S. (Samba Sagn), A.G., A.B., C.L., G.F., and O.F. (Oumar Faye). All authors have read and agreed to the published version of the manuscript.

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