

COVID-19 Outbreak, Senegal, 2020

Ndongo Dia,¹ Ndeye Aïssatou Lakh,¹ Moussa Moïse Diagne, Khardiata Diallo Mbaye, Fabien Taieb, Ndeye Maguette Fall, Mamadou Alioune Barry, Daye Ka, Amary Fall, Viviane Marie Pierre Cisse Diallo, Oumar Faye, Mamadou Malado Jallow, Idrissa Dieng, Mamadou Ndiaye, Mamadou Diop, Abdoulaye Bousso, Cheikh Ioucoubar, Marie Khemesse Ngom Ndiaye, Christophe Peyreffite, Louise Fortes, Amadou Alpha Sall, Ousmane Faye,¹ Moussa Seydi¹

Author affiliations: Institut Pasteur, Dakar, Senegal (N. Dia, M.M. Diagne, F. Taieb, M.A. Barry, A. Fall, Oumar Faye, M.M. Jallow, I. Dieng, M. Diop, C. Ioucoubar, C. Peyreffite, A.A. Sall, Ousmane Faye, M. Seydi); Service des Maladies infectieuses de l'hôpital Fann, Dakar (N.A. Lakh, K.D. Mbaye, N.M. Fall, D. Ka, V.M.P.C. Diallo, L. Fortes); Ministère de la Santé et de l'Action Sociale (MSAS), Dakar (M. Ndiaye, A. Bousso, M.K.N. Ndiaye)

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The spread of severe acute respiratory syndrome coronavirus 2 began later in Africa than in Asia and Europe. Senegal confirmed its first case of coronavirus disease on March 2, 2020. By March 4, a total of 4 cases had been confirmed, all in patients who traveled from Europe.

The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was delayed in Africa and Latin America. The earliest recorded case of coronavirus disease (COVID-19) in Africa was identified in Egypt 7 weeks after the beginning of the outbreak (1). On February 28, 2020, Nigeria declared the first confirmed case in sub-Saharan Africa (2). On March 2, Senegal confirmed an imported case, then 2 additional imported cases the next day, and a fourth on March 4.

In Senegal, the Ministry of Health coordinated all standard operating procedures (SOPs) for the detection, notification, case management, and transport of persons with suspected COVID-19 cases from entry points (e.g., airport, harbor), health-care centers, or locality to the referral service, using the initial WHO case definition (3). A nasopharyngeal swab specimen was collected from any symptomatic suspected case-patient or person in contact with confirmed case-patients for SARS-CoV-2-

specific real-time RT-PCR testing at the Institut Pasteur Dakar (IPD) (Appendix, <https://wwwnc.cdc.gov/EID/article/26/11/20-2615-App1.pdf>). Samples were accompanied by a standardized investigation form collecting demographical information, clinical details, and history of exposure (contact with a confirmed case or history of travel).

In the case of a positive diagnosis of SARS-CoV-2 infection, an active surveillance of contacts or co-exposed persons was initiated immediately around the index case. The nasopharyngeal swabs of positive patients were used for the next-generation sequencing.

Senegal experienced its first COVID-19 suspected case on February 26. During February 26–March 4, a total of 26 suspected case-patients (14 female and 12 male) were tested for a possible SARS-CoV-2 infection. Patient age range was 3–80 years (mean 35.16 years; median 33 years). Of the 26 suspected case-patients, 2 male and 2 female were confirmed as SARS-CoV-2 infected; they were 34 (patient 1), 82 (patient 2), 68 (patient 3), and 33 (patient 4) years of age. Because all were probably infected outside of the country, they were reported as imported cases. They all arrived by airplane, 3 from France and 1 from England. One case-patient had traveled manifesting symptoms undetected by the crew members. Patients 2 and 3, a married couple, traveled together; both had diabetes and hypertension, and both experienced mild clinical symptoms. All 4 patients were admitted to the Isolation and treatment Center (ITC) established by the Ministry of Health (MoH) in Dakar, Senegal. They all were afebrile the first day of hospitalization; they required mild supportive care but not oxygen therapy. In the adopted protocol, discharge of a patient from ITC required 2 consecutive negative tests for SARS-CoV-2 taken 48 hours apart. Patient 1 was discharged after 4 days, and patient 4 after 7 days, whereas patients 2 and 3 stayed for 16 days. Indeed, the viral shedding lasted longer with patients 2 and 3, the oldest. Patients 1 and 4 represented a moderate risk for dissemination of the disease, but patients 2 and 3 represented a high risk for diffusion. Investigations of contact cases and swabbing of high-risk contact cases have not to date identified any secondary cases.

We successfully obtained the complete genome sequences from the 4 SARS-CoV-2-positive patients' samples. The 4 complete genomes were nearly identical across the whole genome; sequence identity was >99%. Outside of the stretch of 44 undetermined nucleotides (19360–19403) in the genome of the strain from patient 1, only 1 nucleotide difference was mapped in open reading frame 8 of patient 4's virus isolate genome, at position 28259 with a T→C

¹These authors contributed equally to this article.

synonymous substitution in virus isolate genomes from patients 1, 2, and 3. All genome sequences have been deposited in GISAID database (<https://www.gisaid.org>; accession nos. EPI_ISL_418206–9).

Phylogenetic analyses revealed that SARS-CoV-2 strains from Senegal clustered with strains from diverse origins (Europe, Asia, Latin America, and Africa).

Of note, they were close to the hCoV-19 Netherlands Haarlem 1363688 2020 EPI_ISL 413572 and hCoV-19 Taiwan NTU03 2020 EPI_ISL 413592 strains. The strains from Senegal clustered together, as shown by the phylogenetic branch with a high bootstrap value of 99% (Figure). All strains from Senegal belong to the ORF8-L isoform.

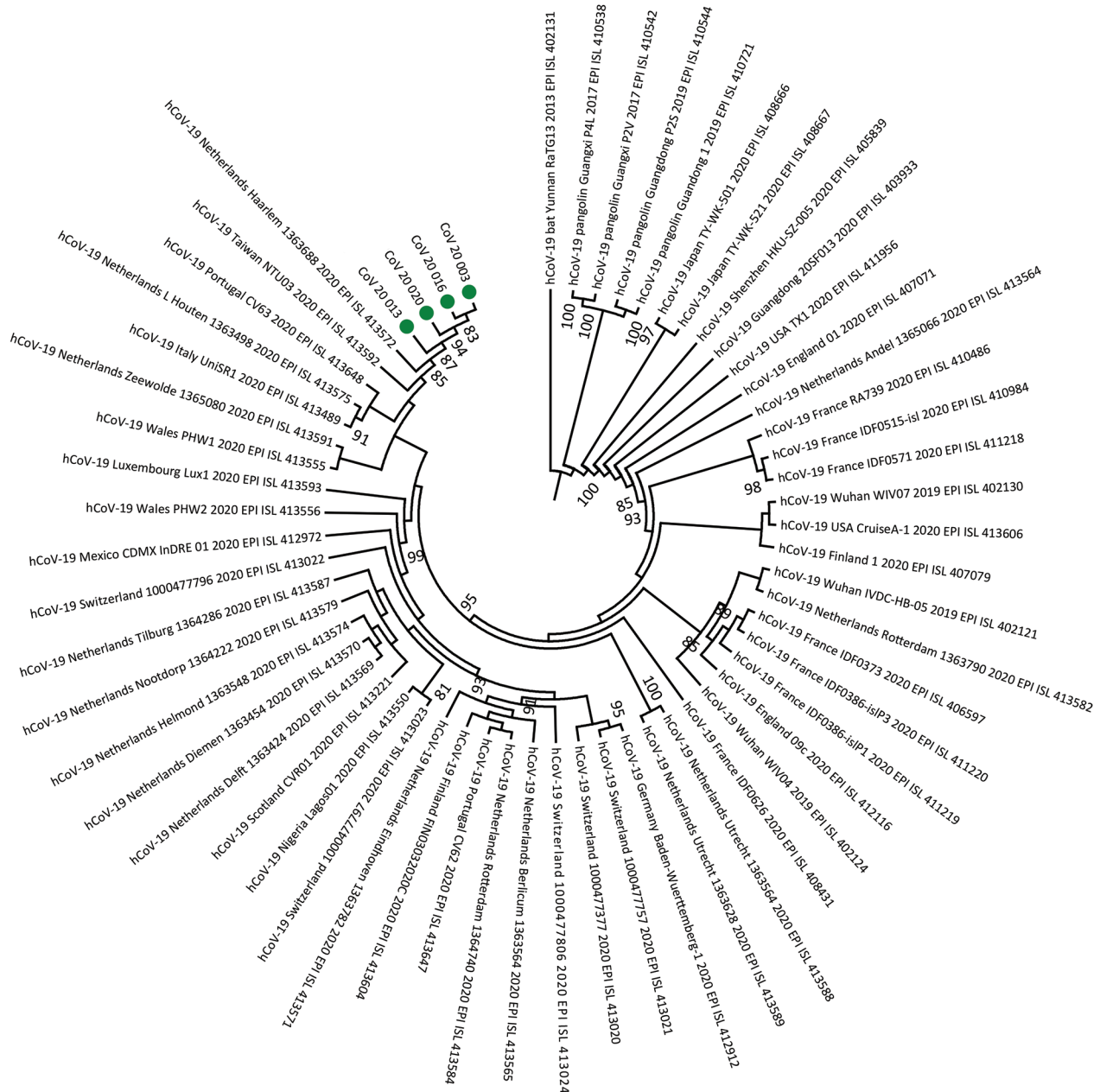


Figure. Phylogeny of 4 severe acute respiratory syndrome coronavirus 2 strains isolated from Senegal (green dots). Whole-genome nucleotide sequences were compared with 56 other genome sequences from the coronavirus disease pandemic retrieved from GenBank and GISAID (<https://www.gisaid.org>) databases. Sequences were aligned with MAFFT (<https://mafft.cbrc.jp/alignment/server>). We generated the phylogenetic tree by the maximum-likelihood method under the HKY85-gamma nucleotide substitution model using IQ-TREE (<http://www.cibiv.at/software/iqtree>). We assessed robustness of tree topology with 1,000 replicates; bootstrap values >75% are shown on the branches of the consensus trees. Phylogenetic analyses revealed that strains from Senegal clustered with strains from diverse origins (Europe, Asia, Latin America, and Africa). CoV, coronavirus; hCoV, human coronavirus.

The diagnosis of these cases showed the surveillance system of Senegal's capacity to quickly detect, isolate, and investigate those cases to take adequate control measures. Our findings indicate that the earliest cases in Senegal or sub-Saharan Africa were imported from Europe, implying that the particularly high volume of direct flights from Europe was a key factor in the spread of the virus in West Africa. However, we cannot exclude the possibility that a few COVID-19 cases were missed at that time in Senegal, including paucisymptomatic or asymptomatic cases (4,5). Our study emphasizes the imperative need for efficient epidemiologic investigations to identify the cases and characterize the transmission modes to prevent, control, and stop the spread of COVID-19.

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About the Author

Dr. Dia is a virologist and head of the Reference Center for influenza and other respiratory viruses at Pasteur Institute Dakar. His primary research interests are the genetic and antigenic dynamics of influenza viruses in Senegal.

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Address for correspondence: Ndongo Dia, Institut Pasteur, Virology, 36 Avenue Pasteur, Dakar 220, Senegal; email: ndia@pasteur.sn

Burkholderia pseudomallei in Soil, US Virgin Islands, 2019

Nathan E. Stone, Carina M. Hall, A. Springer Browne, Jason W. Sahl, Shelby M. Hutton, Ella Santana-Propper, Kimberly R. Celona, Irene Guendel, Cosme J. Harrison, Jay E. Gee, Mindy G. Elrod, Joseph D. Busch, Alex R. Hoffmaster, Esther M. Ellis, David M. Wagner

Author affiliations: Northern Arizona University, Flagstaff, Arizona, USA (N.E. Stone, C.M. Hall, J.W. Sahl, S.M. Hutton, E. Santana-Propper, K.R. Celona, J.D. Busch, D.M. Wagner); US Virgin Islands Department of Health, Charlotte Amalie, US Virgin Islands, USA (A.S. Browne, I. Guendel, C.J. Harrison, E.M. Ellis); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (A.S. Browne, J.E. Gee, M.G. Elrod, A.R. Hoffmaster)

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The distribution of *Burkholderia pseudomallei* in the Caribbean is poorly understood. We isolated *B. pseudomallei* from US Virgin Islands soil. The soil isolate was genetically similar to other isolates from the Caribbean, suggesting that *B. pseudomallei* might have been introduced to the islands multiple times through severe weather events.

Burkholderia pseudomallei is a gram-negative soil-dwelling bacterium and the causative agent of melioidosis (1). *B. pseudomallei* is endemic to tropical regions around the world (1), but its environmental distribution in the Caribbean remains poorly understood. Although it is rare but ecologically established in Puerto Rico (2,3), it has not been isolated from the environment in the neighboring US Virgin Islands (USVI). After the 2017 Caribbean hurricane season, melioidosis developed in 3 persons in the USVI (4), 2 in St. Thomas and 1 in St. John. We aimed to determine whether, as this cluster suggests, *B. pseudomallei* might be endemic to the USVI.

We collected 480 soil and 100 freshwater samples from 29 sites (24 terrestrial and 5 freshwater) on the 3 main USVI islands (i.e., St. Thomas, St. John, and St. Croix) during January 20–April 17, 2019. We selected study sites to maximize geographic distribution across the islands and epidemiologic connection to melioidosis cases in humans (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/26/11/19-1577-App1.pdf>). These efforts followed consensus guidelines for environmental sampling of *B. pseudomallei* (5) and

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Appendix

Material and Methods

COVID-19 Senegalese Surveillance System

In Senegal, the Ministry of Health (MoH) coordinated all standard operating procedures (SOP) for the detection, notification, case management, and transport of suspected coronavirus disease (COVID-19) cases following the initial case definition from the World Health Organization (*1*). MoH established an alert committee with emergency hotline. In accordance with the surveillance protocol, physicians suspecting a COVID-19 case must immediately contact the alert committee in charge to evaluate whether the patient is a possible case (for symptomatic and contact cases). Once the suspicion is confirmed, the patient is then isolated for 14 days at the Fann district university hospital, and biological samples are collected and shipped to the referral laboratory, Institut Pasteur Dakar (IPD) fulfilling the biosafety and biosecurity regulations for Senegal.

A nasopharyngeal swab specimen was collected from symptomatic suspected case or person in contact with confirmed cases and placed in universal viral transport medium (Becton Dickinson, <https://www.bd.com>), stored at 4–8° C, and transported to IPD within 24 hours of collection for testing. Once in IPD Respiratory Virus National Reference Center Laboratory, specimens were processed immediately for SAR-SCoV-2–specific real-time RT-PCR detection, or kept at +4° C until the next morning if delivered after 8 p.m. At least 2 aliquots of each sample were also stored at –80° C for biobanking or additional analysis. Samples were accompanied by a standardized investigation form collecting demographical information, clinical details, and history of exposure (history of travel or contact with a confirmed case) filled by the clinicians.

In the case of a positive diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, an active surveillance of contacts or co-exposed persons was initiated immediately around the index case.

SARS-CoV-2 Diagnosis

The initial extraction steps were carried out in a BSL-3 laboratory. Total RNA was extracted from 200 μL of nasopharyngeal swab using a QIAmp Viral RNA Mini Kit (QIAGEN, <https://qiagen.com>) according to the manufacturer's specifications. RNAs were eluted with 60 μL nuclease-free water and immediately used for testing. An inactivation step was added: shortly after addition of the lysis buffer, the mixture was heated at 65°C during 30 min for viral inactivation.

For the SARS-CoV-2 diagnostic, 4 protocols were used: the Berlin protocol (2) along with the TIBMolBiol kits (<https://www.tib-molbiol.com>), which targeted the *E*, *N* and *RdRP* genes; the HKU protocol (3), targeting the *N* and *RdRP* genes; and the protocol proposed by the Unité des Virus Emergents in Marseille (distributed by European Virus Archive Global) targeting the *E* gene. All protocols were validated using the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, <https://www.thermofisher.com>) in a final volume of 20 μL including 5 μL of extracted RNA. The cycling conditions for all protocols were as follows: 50°C for 15 min and 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

According to IPD algorithm, at this very early stage of the outbreak, a sample was tested simultaneously with the TIBMolBiol targets (*E* and *RdRP*) and the Marseille target (*E* gene). The Berlin protocol (*E*, *N*, and *RdRP*) or HKU protocols were used for additional confirmation of SARS-CoV-2 positive samples if necessary.

Genome Sequencing and Phylogeny

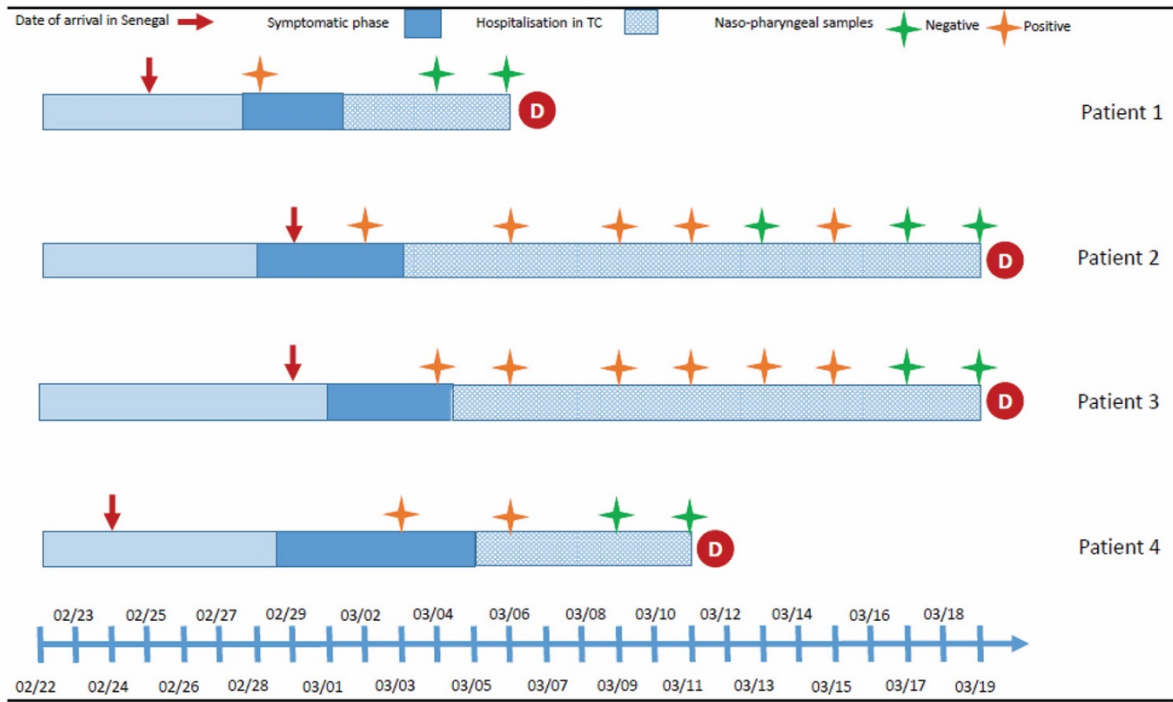
The primary nasopharyngeal swab samples of the first 4 positive patients were directly used for next-generation sequencing. Briefly, for each sample, 11 μL of RNA extracts were reverse transcribed using the IV RT kit (Invitrogen) and the amplicons were obtained using tiled, multiplexed primers designed by ARTIC network (<https://artic.network/ncov-2019>). The sequencing library preparation was carried out using the DNA Flex Library kit (Illumina Technologies, <https://www.illumina.com>) and Nextera DNA UD indexes (Illumina). The concentrations of libraries were measured using a Qubit dsDNA High Sensitivity kit on a Qubit 3.0 fluorometer (ThermoFisher). Then, a pool of 60 pm was loaded into an Iseq 100 system (Illumina) for sequencing. Each sample was processed in duplicate to increase the success of

getting a complete genome sequence and to confirm the quality output. Dry lab analysis was then performed using IPD's local servers. After removing host (human) reads, assembly per alignment using Bowtie2 version 2.0.6 software (<https://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.0.6>) was done in order to construct contigs. The corresponding fasta files were merged using Emboss merger (BLAST, www.ncbi.nlm.nih.gov).

Nucleotide sequences of Senegalese SARS-CoV-2 strains and genome sequences from the current COVID-19 pandemic retrieved from NCBI (<http://www.ncbi.nlm.nih.gov/genbank>) and GISAID (<https://www.gisaid.org>) databases (as of March 14, 2020) were used for phylogenetic analysis. Sequences were aligned with MAFFT. The phylogenetic tree was generated using the maximum-likelihood (ML) method under the HKY85-gamma nucleotide substitution model using IQ-TREE. Robustness of tree topology was assessed with 1,000 replicates.

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Appendix Figure. Schematic description of the course of the 4 COVID-19 cases in Senegal. For each patient, detailed information for their arrival in Senegal and clinical trajectory of the disease are shown, including symptomatology, hospitalization, timeline of results from nasopharyngeal swabs through the course of the disease, and discharge.