Centre for Respiratory Diseases and Meningitis Sentinel Surveillance in South Africa Respiratory Pathogens Report

Report week: 8

Reporting period: 30 December 2024 to 23 February 2025 **Date of data extraction:** 27 February 2025

Data are provisional as on date data extracted. Number of consultations/specimens are reported/analysed by date of consultation/specimen collection. Data cleaning is ongoing and this may result in some changes in subsequent reports. Refer to end of report for methodology and definitions.

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COMMUNICABLE DISEASES

Highlights

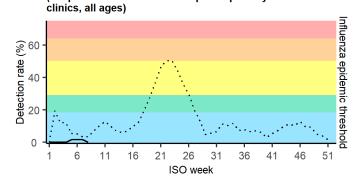
- In week 8 (17 February 2025 to 23 February 2025), we detected 3 (2.7%, 3/113) cases of influenza, 2 (1.8%, 2/113) cases of RSV and 2 (1.8%, 2/113) cases of SARS-CoV-2.
- Although the detection rate of RSV is below the seasonal threshold, the detection rate of RSV in in-patient surveillance is increasing
- In the month of January, we detected 2 (0.6%, 2/337) cases of *Bordetella pertussis*.
- From 30 December 2024 to 23 February 2025, from 824 samples tested we detected 15 (1.8%) cases of influenza, 13 (1.6%) cases of respiratory syncytial virus (RSV), 71 (8.6%) cases of SARS-CoV-2 and 3 (0.5%) cases of *Bordetella pertussis*.

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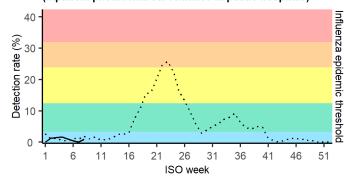
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Influenza & RSV epidemic thresholds

Influenza transmission (Outpatient ILI surveillance in public primary health care



Influenza morbiditidy and mortality (Inpatient pneumonia surveillance in public hospitals)



RSV morbidity and mortality (Inpatient pneumonia surveillance in public hospitals, <5 years)

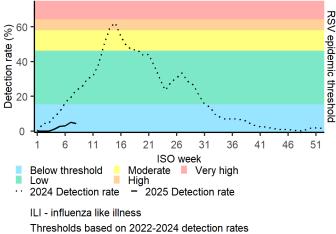
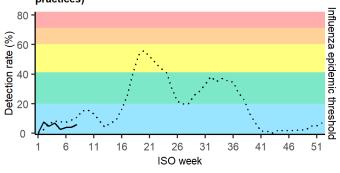


Figure 1: Influenza and respiratory syncytial virus (RSV) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 30 December 2024 to 23 February 2025.



Influenza transmission (Outpatient ILI surveillance in private general practitioner practices)



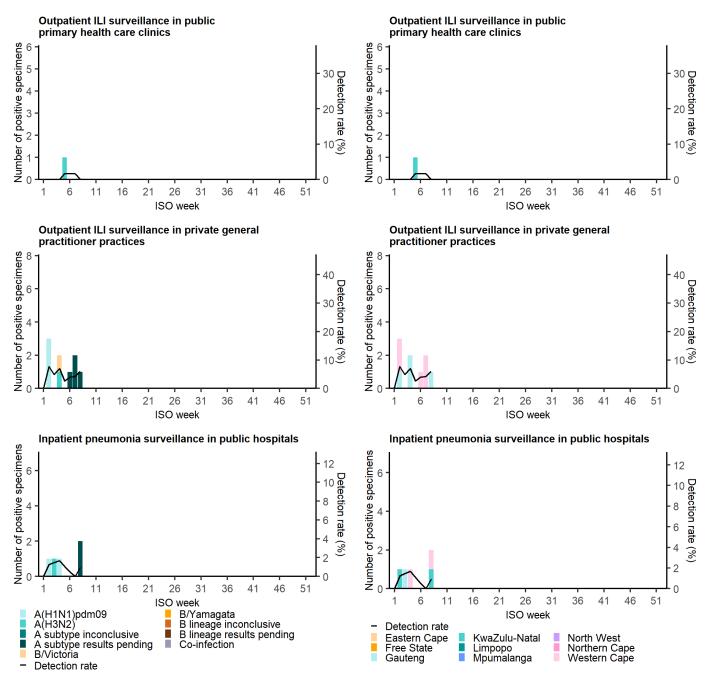
SARS-CoV-2 epidemic thresholds

SARS-CoV-2 transmission SARS-CoV-2 transmission (Outpatient ILI surveillance in public primary health care (Outpatient ILI surveillance in private general practitioner SARS-CoV-2 epidemic threshold clinics, all ages) practices) 80 Detection rate (%) 00 00 09 40 Detection rate (%) 30 20 10 0 0 6 16 21 26 31 36 41 46 51 6 11 16 21 26 31 36 41 46 51 11 ISO week ISO week SARS-CoV-2 morbidity and mortality (Inpatient pneumonia surveillance in public hospitals) SARS-CoV-2 epidemic threshold 40 Detection rate (%) 30 20 0 0 21 51 6 16 26 31 36 41 46 11 ISO week 2025 Detection rate . . 2024 Detection rate Below threshold High Moderate Very high 1 ow ILI - influenza like illness Thresholds based on 2022-2024 detection rates

Figure 2: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 30 December 2024 to 23 February 2025.

SARS-CoV-2 epidemic threshold

Influenza



ILI - influenza like illness, ISO - International Organization for Standardization Detection rate presented as three-week moving average

Figure 3: Number of laboratory-confirmed influenza cases and detection rate by subtype and lineage (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 23 February 2025.

 Table 1: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 23 February 2025.

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending	Co- infection	Total influenza	Total specimens
Edendale Gateway (KZ)	0	1	0	0	0	0	0	0	0	1	89
Agincourt (MP)	0	0	0	0	0	0	0	0	0	0	2
Jouberton (NW)	0	0	0	0	0	0	0	0	0	0	23
Eastridge (WC)	0	0	0	0	0	0	0	0	0	0	41
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0	0	9
Total	0	1	0	0	0	0	0	0	0	1	164

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 31st of January 2025 at Matikwana Hospital.

Table 2: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by province in all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 30 December 2024 to 23 February 2025.

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending	Co- infection	Total influenza	Total specimens
Eastern Cape	0	0	0	0	0	0	0	0	0	0	1
Free State	0	0	0	0	0	0	0	0	0	0	0
Gauteng	1	1	0	1	1	0	0	0	0	4	153
Limpopo	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0	0	0
North West	0	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0	0	0	0
Western Cape	2	0	0	3	0	0	0	0	0	5	29
Total	3	1	0	4	1	0	0	0	0	9	183

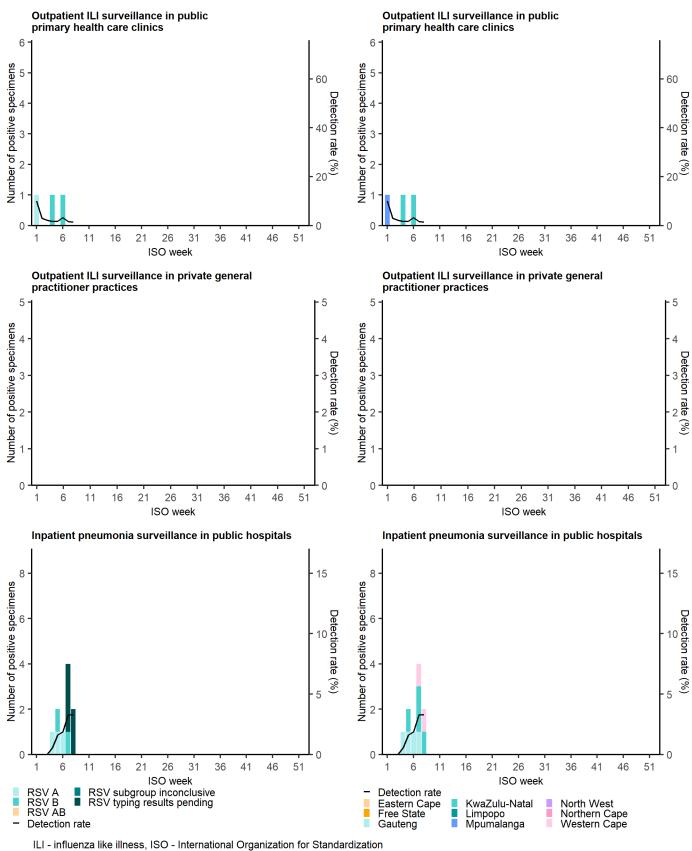
Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 31st of January 2025 at Matikwana Hospital.

Table 3: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by hospital and province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 23 February 2025.

Hospital (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending	Co- infection	Total influenza	Total specimens
Helen Joseph-Rahima Moosa (GP)	0	1	0	0	0	0	0	0	0	1	82
Harry Gwala (KZ)	1	0	0	1	0	0	0	0	0	2	92
Mapulaneng-Matikwana (MP)	0	0	0	0	0	0	0	0	0	0	46
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	28
Klerksdorp-Tshepong (NW)	0	0	0	0	0	0	0	0	0	0	49
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0	0	51
Red Cross (WC)	1	0	0	1	0	0	0	0	0	2	129
Total	2	1	0	2	0	0	0	0	0	5	477

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 31st of January 2025 at Matikwana Hospital.

Respiratory syncytial virus (RSV)



Detection rate presented as three-week moving average

Figure 4: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases and detection rate by type (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 23 February 2025.

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Table 4: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 23 February 2025.

Clinic (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Edendale Gateway (KZ)	0	2	0	0	0	2	89
Agincourt (MP)	1	0	0	0	0	1	2
Jouberton (NW)	0	0	0	0	0	0	23
Eastridge (WC)	0	0	0	0	0	0	41
Mitchell's Plain (WC)	0	0	0	0	0	0	9
Total	1	2	0	0	0	3	164

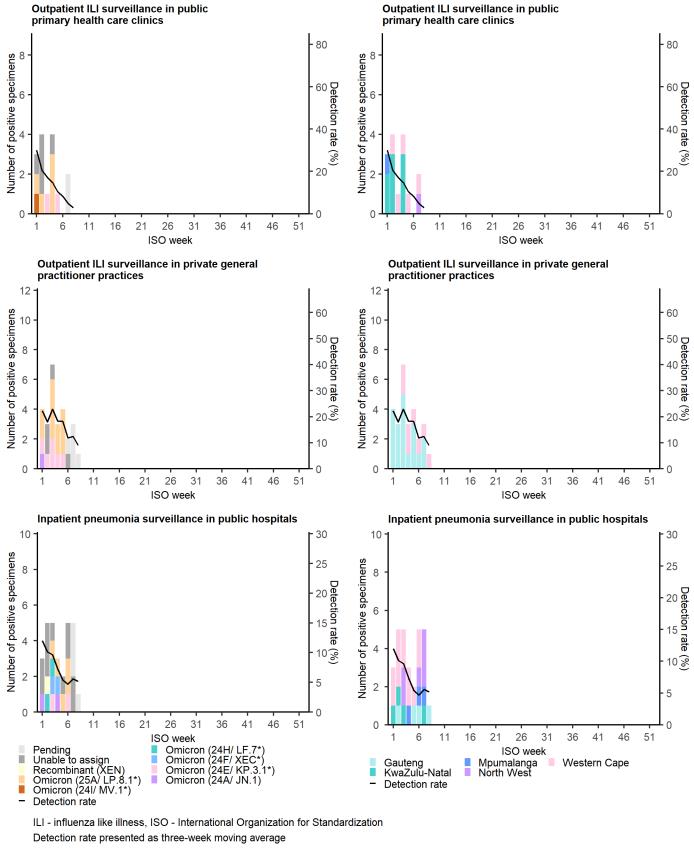
Table 5: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by province in all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 30 December 2024 to 23 February 2025.

Province	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Eastern Cape	0	0	0	0	0	0	1
Free State	0	0	0	0	0	0	0
Gauteng	0	0	0	0	0	0	153
Limpopo	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0
North West	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0
Western Cape	0	0	0	0	0	0	29
Total	0	0	0	0	0	0	183

Table 6: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by hospital and province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 23 February 2025.

Hospital (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Helen Joseph-Rahima Moosa (GP)	3	0	0	0	1	4	82
Harry Gwala (KZ)	0	2	0	0	2	4	92
Mapulaneng-Matikwana (MP)	0	0	0	0	0	0	46
Tintswalo (MP)	0	0	0	0	0	0	28
Klerksdorp-Tshepong (NW)	0	0	0	0	0	0	49
Mitchell's Plain (WC)	0	0	0	0	0	0	51
Red Cross (WC)	0	0	0	0	2	2	129
Total	3	2	0	0	5	10	477

SARS-CoV-2



*Including sub-lineages

Figure 5: Number of laboratory-confirmed SARS-CoV-2 cases and detection rate by variant type (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 23 February 2025.

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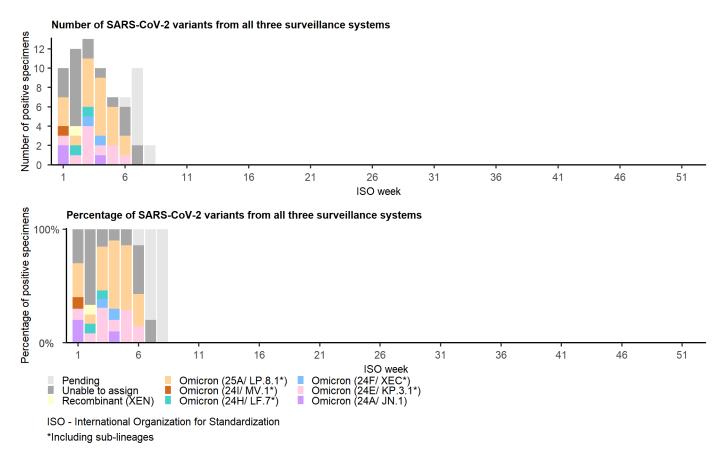


Figure 6: Combined number and percentage of SARS-CoV-2 variants in all ages from three sentinel surveillance systems: outpatient influenza like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 23 February 2025.

 Table 7: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 23 February 2025.

Clinic (Province)	Omicron (24A/ JN.1)	Omicron (24E/ KP.3.1*)	Omicron (24F/ XEC*)	Omicron (24H/ LF.7*)	Omicron (24I/ MV.1*)	Omicron (25A/ LP.8.1*)	Recombinant (XEN)	Pending	Unable to assign	Total SARS-CoV- 2	Total specimens
Edendale Gateway (KZ)	0	0	0	0	1	4	0	0	3	8	89
Agincourt (MP)	0	0	0	0	0	1	0	0	0	1	2
Jouberton (NW)	0	0	0	0	0	0	0	1	0	1	23
Eastridge (WC)	0	2	0	0	0	0	0	1	1	4	41
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	1	1	9
Total	0	2	0	0	1	5	0	2	5	15	164

*Including sub-lineages

Table 8: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by province in all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 30 December 2024 to 23 February 2025.

Province	Omicron (24A/ JN.1)	Omicron (24E/ KP.3.1*)	Omicron (24F/ XEC*)	Omicron (24H/ LF.7*)	Omicron (24I/ MV.1*)	Omicron (25A/ LP.8.1*)	Recombinant (XEN)	Pending	Unable to assign	Total SARS- CoV-2	Total specimens
Eastern Cape	0	0	0	0	0	0	0	0	0	0	1
Free State	0	0	0	0	0	0	0	0	0	0	0
Gauteng	1	4	0	0	0	8	0	2	4	19	153
Limpopo	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0	0	0
North West	0	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0	0	0	0
Western Cape	0	2	0	0	0	3	0	3	0	8	29
Total	1	6	0	0	0	11	0	5	4	27	183

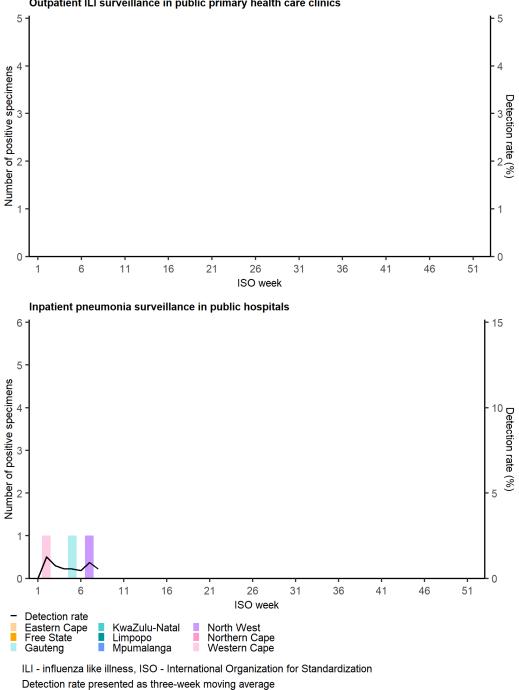
*Including sub-lineages

 Table 9: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by hospital and province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 23 February 2025.

Hospital (Province)	Omicron (24A/ JN.1)	Omicron (24E/ KP.3.1*)	Omicron (24F/ XEC*)	Omicron (24H/ LF.7*)	Omicron (24I/ C MV.1*)	Dmicron (25A/ LP.8.1*)	Recombinant (XEN)	Pending	Unable to assign	Total SARS-CoV- 2	Total specimens
Helen Joseph-Rahima Moosa (GP)	0	0	0	0	0	1	0	1	2	4	82
Harry Gwala (KZ)	0	0	1	0	0	0	0	1	2	4	92
Mapulaneng- Matikwana (MP)	0	0	0	0	0	1	0	0	0	1	46
Tintswalo (MP)	0	0	0	0	0	0	0	0	2	2	28
Klerksdorp-Tshepong (NW)	0	1	0	0	0	0	0	2	3	6	49
Mitchell's Plain (WC)	1	1	0	0	0	0	0	0	2	4	51
Red Cross (WC)	1	0	1	2	0	3	1	0	0	8	129
Total	2	2	2	2	0	5	1	4	11	29	477

*Including sub-lineages

Bordetella pertussis



Outpatient ILI surveillance in public primary health care clinics

Figure 7: Number of laboratory-confirmed Bordetella pertussis cases and detection rate by province in all ages, sentinel surveillance, South Africa, 30 December 2024 to 23 February 2025.

Table 10: Number of laboratory-confirmed *Bordetella pertussis* cases and total number of samples tested by province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 23 February 2025.

Province	Positive	Pending testing	Total specimens
KwaZulu-Natal	0	1	89
Mpumalanga	0	0	2
North West	0	0	23
Western Cape	0	13	50
Total	0	14	164

Table 11: Number of laboratory-confirmed *Bordetella pertussis* cases and total number of samples tested by province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 23 February 2025.

Province	Positive	Pending testing	Total specimens
Gauteng	1	6	82
KwaZulu-Natal	0	1	92
Mpumalanga	0	6	74
North West	1	0	49
Western Cape	1	20	180
Total	3	33	477

Methods

Table 12: Programme descriptions for sentinel surveillance in South Africa

Programme	Influenza-like illness (ILI)	Viral Watch	National Syndromic Surveillance for Pneumonia
Description	Outpatient ILI surveillance in public primary health care clinics	Outpatient ILI surveillance in private general practitioner	Inpatient pneumonia surveillance in public hospitals
Charles	2012	practices	2000
Start year	2012	1984 EC, FS, GP, LP, MP, NC,	2009
Provinces	KZ, NW, WC, MP.	NW, WC.	EC, GP, KZ, MP, NW, WC.
Type of site	Primary health care clinics.	General practitioners.	Public hospitals.
Case definition	duration for children <1 year), without a more likely diagnosis	ILI: An acute respiratory illness with a temperature (≥38°C) and cough, & onset ≤10 days.	(≥38) or history of fever AND cough AND symptoms of any duratic Suspected pertussis: Any person with an acute cough illness lastin
Specimens collected	Mid-turbinate nasal swabs.	Throat and/or nasal swabs or Nasopharyngeal swabs.	Mid-turbinate nasal swabs.
Main pathogens tested	Influenza virus, RSV, SARS-CoV-2, B. pertussis.	Influenza virus, RSV, SARS-CoV-2.	Influenza virus, RSV, SARS-CoV-2, B. pertussis.
Testing Methods	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV- 2/FluA/FluB/RSV PCR kit. B. pertussis: Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture.	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/ FluA/FluB/RSV PCR kit.	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV- 2/FluA/FluB/RSV PCR kit. B. pertussis: Multiplex real-time PCR (Ta et al., J Clin Microbiol 2011) and culture.
 LI M N N Si Si U Ei av d 	vailable from: http://CRAN.R-project.org/web/package=men /e used the "original method" included in the package to det stablished from available years of historical data to calculate ollows: Below seasonal threshold, low activity, moderate acti ke illness (ILI in primary health care clinics) are used as an inc urveillance are used as an indicator of influenza-associated m he three week moving average of the detection rate remains SV, thresholds from outpatient influenza like illness (ILI in pri isease transmission in the community and thresholds from p	in progress e to poor sequence qua ng the Moving Epidemi n) designed to calculate termine the start of the thresholds of activity. ivity, high activity, very dicator of disease trans norbidity and mortality above or below the se imary health care clinic neumonia surveillance	lity OR low viral load (Ct≥35) c Method (MEM), a sequential analysis using the R Language, e the duration, start and end of the annual influenza epidemic. e season. MEM uses the 40th, 90th and 97.5th percentiles Thresholds of activity for influenza and RSV are defined as high activity. For influenza, thresholds from outpatient influen mission in the community and thresholds from pneumonia . For influenza the start and end of the season is defined as on asonal threshold for two consecutive weeks, respectively. For
cl Si tł	nildren <5 years from inpatient pneumonia surveillance in pu ARS-CoV-2 thresholds were calculated using the mean stand	iblic hospitals remains ard deviation (MSD) me the selected historical	above or below 15% for two consecutive weeks, respectively. ethod, where the seasonal threshold level is determined using years and severity levels are based on the mean plus one, thre

Laboratory testing for influenza, RSV, SARS-CoV-2 and B. pertussis:

Influenza A and B viruses, RSV and SARS-CoV-2 were tested using a commercial multiplex RT-PCR assay (Allpex SARS-CoV-2/FluA/FluB/RSV PCR kit, Seegene Inc., Seoul, South Korea). A specimen was considered positive for influenza A, B or RSV if the PCR cycle threshold (Ct) was <40 for the respective target, and considered positive for SARS-CoV-2 when the Ct was <40 for ≥1 of the S, N or RdRp gene targets. *B.pertussis* was tested using a previously described RT-PCR method (Tatti KM, et al. Journal of Clinical Microbiology. 2011;49(12):4059-4066). A specimen was considered positive when the IS481 and/or ptxS1 gene targets

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are detected with a Ct <45.

Further characterization of influenza, RSV, and SARS-CoV-2:

Influenza A and B positive specimens were subtyped using the US Centres for Disease Control and Prevention (CDC) RT-PCR protocol and reagents (International Reagent Resource (IRR) [Available from: https://www.internationalreagentresource.org/). RSV positive specimens were subgrouped using an in-house assay (Pretorius M, et al. Journal of Infectious Diseases. 2012(1537-6613)). SARS-CoV-2 positive specimens were sequenced using the Illumina COVIDSeq protocol (Illumina, CA, USA).

SARS-CoV-2 whole-genome sequencing and genome assembly for SARS-CoV-2 genomic surveillance:

RNA extraction: RNA was extracted either manually or automatically in batches, using the QIAamp viral RNA mini kit (QIAGEN, CA, USA) or the Chemagic 360 using the CMG-1049 kit (PerkinElmer, MA, USA). A modification was done on the manual extractions by adding 280 μ l per sample, in order to increase yields. 300 μ l of each sample was used for automated magnetic bead-based extraction using the Chemagic 360. RNA was eluted in 60 μ l of the elution buffer. Isolated RNA was stored at -80 °C prior to use.

PCR and library preparation:

Sequencing was performed using the Illumina COVIDSeq protocol (Illumina Inc., CA, USA) or nCoV-2019 ARTIC network sequencing protocol v3 (https://artic.network/ncov-2019). These are amplicon-based next-generation sequencing approaches. Briefly, for the nCoV-2019 ARTIC network sequencing protocol, the first strand synthesis was carried out on extracted RNA samples using random hexamer primers from the SuperScript IV reverse transcriptase synthesis kit (Life Technologies, CA, USA) or LunaScript RT SuperMix Kit (New England Biolabs (NEB), MA, USA). The synthesized cDNA was amplified using multiplex polymerase chain reactions (PCRs) using ARTIC nCoV-2019 v3 primers. For the COVIDSeq protocol, the first strand synthesis was carried out using random hexamer primers from Illumina and the synthesized cDNA underwent two separate multiplex PCR reactions. For Illumina sequencing using the nCoV-2019 ARTIC network sequencing protocol, the pooled PCR products underwent bead-based tagmentation using the Nextera Flex DNA library preparation kit (Illumina Inc., CA, USA). The adapter-tagged amplicons were cleaned up using AmpureXP purification beads (Beckman Coulter, High Wycombe, UK) and amplified using one round of PCR. The PCRs were indexed using the Nextera CD indexes (Illumina Inc., CA, USA) according to the manufacturer's instructions. For COVIDSeq sequencing protocol, pooled PCR amplified products were processed for tagmentation and adapter ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and clean-up was performed as per protocols provided by the manufacturer (Illumina Inc., CA, USA). Pooled samples from both COVIDSeq protocol and nCoV-2019 ARTIC network protocol were quantified using Qubit 3.0 or 4.0 fluorometer (Invitrogen Inc., MA, USA) using the Qubit dsDNA High Sensitivity assay according to manufacturer's instructions. The fragment sizes were analyzed using TapeStation 4200 (Invitrogen Inc., MA, USA). The pooled libraries were further normalized to 4nM concentration and 25 µl of each normalized pool containing unique index adapter sets were combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCL (pH7), respectively. 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeg 500/550 HighOutput Kit v2 and run on the Illumina NextSeg 550 instrument (Illumina Inc., CA, USA)

Assembly, processing and quality control of genomic sequences:

Raw reads from Illumina sequencing were assembled using the Exatype NGS SARS-CoV-2 pipeline v1.6.1, (https://sars-cov-2.exatype.com/). The resulting consensus sequence was further manually polished by considering and correcting indels in homopolymer regions that break the open reading frame (probably sequencing errors) using Aliview v1.27, (http://ormbunkar.se/aliview/) (Larsson, 2014). Mutations resulting in mid-gene stop codons and frameshifts were reverted to wild type. All assemblies determined to have acceptable quality (defined as having at least 1 000 000 reads and at least 40 % 10 X coverage) were deposited on GISAID (https://www.gisaid.org/) (Elbe & Buckland-Merrett, 2017; Shu & McCauley, 2017).

Classification of lineage, clade and associated mutations:

Assembled genomes were assigned lineages using the 'Phylogenetic Assignment of Named Global Outbreak Lineages' (PANGOLIN) software suite (https://github.com/hCoV-2019/pangolin) (Rambaut et al., 2020), a tool used for dynamic SARS-CoV-2 lineage classification. The SARS-CoV-2 genomes in our dataset were also classified using the clade classification proposed by NextStrain (https://nextstrain.org/), a tool built for real-time tracking of the pathogen evolution (Hadfield et al., 2018).