

EVIDENCE BRIEF

SARS-CoV-2 Omicron Variant Sub-Lineage BA.3

Published: April 2022

Key Messages

- At this time, BA.3 is one of 73 sub-lineages of the Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) B.1.1.529 (Omicron) variant of concern.¹
- BA.3 was first reported on November 18, 2021, at around the same time as BA.1 and BA.2, all initially in southern Africa.^{2,3}
- BA.3 accounted for 0.02% of the 2,842,071 global Omicron sequences submitted to GISAID from March 7 to April 3, 2022.⁴
- One sample matching the BA.3 sequence was detected so far through representative surveillance in Canada from a sample collected in Toronto,⁵ Ontario on April 1, 2022.
- Limited in-vitro data suggest that BA.3 may evade immunity acquired from infection with B.1 (a major basal lineage prevalent in 2020) and BA.1.^{6,7}
- Limited in-vitro data suggest that BA.3 may evade immunity acquired from vaccination slightly more efficiently than BA.1 and BA.2,^{7,8} and has otherwise comparably reduced monoclonal antibody efficacy as with other Omicron sub-lineages (including against bamlanivimab, casirivimab, imdevimab and sotrovimab).⁹
- The current risk of BA.3 importation in Ontario is moderate given one BA.3 case detected so far in the province and the fact that detection has been reported in at least 64 countries. However, the risk of sustained transmission in Ontario is yet to be defined considering the limited available evidence on BA.3 to inform the risk assessment, the low incidence of BA.3 worldwide, and the lack of major mutational differences to currently circulating Omicron sub-lineages. The overall risk assessment may change as new evidence emerges.

Issue and Research Question

- Seventy-three sub-lineages under the parent SARS-CoV-2 B.1.1.529 (Omicron) variant of concern have been identified globally to date, with the most prevalent sub-lineages being B.1.1.529.1 (BA.1), B.1.1.529.1.1 (BA.1.1), B.1.1.529.2 (BA.2), and B.1.1.529.3 (BA.3). More sub-lineages are likely to develop over time as the SARS-CoV-2 virus continues to evolve.

- The emergence of SARS-CoV-2 variants is generally a result of mutations that occur over time as the virus replicates in host cells. As with many RNA viruses, SARS-CoV-2 is prone to frequent mutations. The ongoing high rates of SARS-CoV-2 community transmission in the context of Omicron’s emergence have led to concerns for novel variants emerging with different transmissibility, virulence, or immune evasion potential from prior infection and/or vaccination.¹⁰
- This evidence brief summarizes available information and evidence on the Omicron variant sub-lineage BA.3 relevant to the risk of transmission in Ontario.

Methods

From January 17, 2021, to April 20, 2022, Public Health Ontario (PHO) Library Services conducted daily searches of primary and preprint literature using the MEDLINE database (search strategies available upon request). In addition, PHO performed grey literature searches daily using news feeds in the Shared Library Services Partnership. English-language peer-reviewed and non-peer-reviewed (preprint) records that described COVID-19 variants were included.

Main Findings

Genomic Features

- The spike protein of BA.3, the main protein responsible for host cell invasion and vaccine-derived immunity, carries 34 mutations compared to the reference SARS-CoV-2 Wuhan-Hu-1 genome:^{2,11-15}
 - 21 mutations are shared with all Omicron sub-lineages: G143D, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K.
 - 11 mutations are shared with BA.1 and BA.1.1: A67V, H69del, V70del, T95I, V143del, Y144del, Y145del, N211I, L212V, V213R, and G446S.
 - 2 mutations are shared with BA.2: S371F and D405N.
 - No spike mutation is uniquely found in BA.3 compared to other Omicron sub-lineages.
- Beyond the spike protein, BA.3 carries the following mutations:
 - 6 structural protein mutations shared with all Omicron sub-lineages: T9I (envelope), P13L, ERS31del, RG203KR (nucleocapsid), Q19E, and A63T (membrane).
 - In addition, BA.3 has 1 structural protein mutation shared with BA.2: S413R (nucleocapsid).
 - 7 non-structural protein mutations shared with all Omicron sub-lineages: NSP4 T492I, NSP5 P132H, NSP6 S106del, NSP6 G107del, NSP6 F108del, NSP12 323L, and NSP14 I42V.
 - In addition, BA.3 has 4 non-structural protein mutations shared with BA.2: NSP1 S135R, NSP3 G489S, NSP3a T223I, and NSP4 T327I.
 - 4 non-structural protein mutations unique to BA.3: NSP3a T22V, NSP6 A88V, nucleotide substitutions C832T, and nucleotide substitution C11235T.

- The impact pertaining to the lack of unique spike mutation, 4 unique non-spike mutations, and relative synergy of mutations otherwise shared with BA.1, BA.1.1, and/or BA.2, has not yet been fully elucidated. However, mutations common to all Omicron sub-lineages have been hypothesized to functionally increase infectivity and reduce pathogenicity as compared to non-Omicron lineages.^{3,16}

Epidemiology

- As of April 18, 2022, 2,875 sequences of the BA.3 sub-lineage of the Omicron variant of concern have been reported in at least 64 countries in all six WHO regions. Most of the BA.3 sequences have been detected in the United Kingdom (n=648), Poland (n = 452), India (n=427), Germany (n=373), Israel (n=180), South Africa (n = 166), the United States (n = 157) at cumulative prevalence $\leq 1\%$ in these countries.¹⁷
 - In the European Union, BA.3 is designated as a variant under monitoring in view of the low incidence and absence of significant impact on the epidemiology of COVID-19. Variants under monitoring are those that could have properties similar to those of variants of concern but supporting evidence is either weak or not yet assessed by the European Centre for Disease Prevention and Control.¹⁸
 - As of April 8, 2022, only one BA.3 case was identified in Canada through the Ontario COVID-19 Genomics Network (OCGN) representative whole genome sequencing surveillance program.⁵ The sample was collected on April 1, 2022, in Toronto, Ontario. No other BA.3 case has been reported in Canada otherwise to date.¹⁷

Infectivity and Transmissibility

- In a pre-print *in-silico* study of spike mutation profiles, Kumar et al. suggested that BA.3 and BA.2 have slightly higher binding affinity to the human angiotensin converting enzyme-2 (hACE-2) receptor than wild-type, BA.1, and BA.1.1 lineages, suggestive of potentially increased tropism and infectivity.¹¹
- The absence of some key spike mutations found in BA.1 and BA.2 has been a source of speculation by Desingu et al. as one potential reason behind the relatively lower number of BA.3 cases reported globally so far.²
- Beyond the similar period of introduction in the global population yet outcompeted distribution in BA.3 cases compared to BA.1, BA.1.1, and BA.2, no in-vivo or in-vitro data are otherwise available to inform if BA.3 is more transmissible than the other Omicron sub-lineages.

Pathogenicity and Severity

- According to the same pre-print in-silico study from Kumar et al., the spike protein of BA.3 and BA.2 were also computationally estimated to have slightly more hydrophobic residues than wild-type, BA.1, and BA.1.1 lineages, suggestive of potentially increased pathogenicity.¹¹
- No in-vivo or in-vitro data are otherwise available to inform if BA.3 causes more severe infections compared to other Omicron sub-lineages.

Immune Evasion

VACCINATION

- Arora et al. reported that BA.3 may evade immunity from vaccination with Pfizer-BioNTech Comirnaty COVID-19 vaccine relatively more efficiently than B.1 (a major basal lineage from 2020 identical to wild-type with the exception of one mutation at D614G) and BA.2, but with comparable efficiency as BA.1. Using neutralization assays and a pseudovirus engineered to express either the BA.1, BA.2, BA.3 or B.1 spike protein, reduced neutralization efficiency by sera from vaccinated individuals compared to B.1 was observed:⁷
 - Sera from double-vaccinated individuals (n=10):
 - BA.3: reduced 17.1-fold compared B.1; P = 0.0020
 - BA.2: reduced 8.7-fold compared to B.1; P = 0.0020
 - BA.1: reduced 17.0-fold compared to B.1; P = 0.0020
 - Sera from triple-vaccinated individuals (n=9):
 - BA.3: reduced 2.4-fold compared to B.1; P = 0.0039
 - BA.2: reduced 1.9-fold compared to B.1; P = 0.012
 - BA.1: reduced 2.5-fold compared to B.1; P = 0.0020
 - Sera from triple-vaccinated individuals (n=10) with breakthrough COVID-19 infection during October 2021-January 2022 in Germany (when Delta was predominant):
 - BA.3: reduced 11.8-fold compared to B.1; P = 0.0039
 - BA.2: reduced 11.1-fold compared to B.1; P = 0.0039
 - BA.1: reduced 9.1-fold compared to B.1; P = 0.0020
- Kurhade et al. reported in a pre-print study that BA.3 may evade **immunity from vaccination** relatively more efficiently than BA.1 and BA.2. Using neutralization assays, the authors found that sera (n=22) collected at one month after dose 3 of the Pfizer-BioNTech Comirnaty COVID-19 vaccine were able to neutralize a chimeric SARS-CoV-2 virus engineered to express the BA.1, BA.2, or BA.3 spike protein, but at reduced efficiency of 3.6-, 4.0- and 6.4-fold (P < 0.0001) respectively, compared to neutralization of a wild-type (USA-WA1/2020) strain.⁸

PRIOR INFECTION

- Arora et al. (see above) reported that BA.3 may evade immunity from prior infection that occurred in Germany between February to May, 2020 and December 2020 to February 2021 (prior to the introduction of variants of interest) relatively more efficiently than B.1 and BA.2, but with comparable efficiency as BA.1. Reduced neutralization efficiency against BA.3 by convalescent sera (n=10) compared to B.1 was observed using a pseudovirus engineered to express the spike protein of interest:⁷
 - BA.3: reduced 37.1-fold compared to B.1; P = 0.0020

- BA.2: reduced 9.2-fold compared to B.1; P = 0.0020
- BA.1: reduced 32.7-fold compared to B.1; P = 0.0020
- Using neutralization assays, Zou et al. found in a pre-print study that sera collected from vaccine-naïve individuals (n=20) at 8 to 62 days post diagnosis of BA.1 infection were able to neutralize a chimeric SARS-CoV-2 virus engineered to express the BA.2 or BA.3 spike protein, as well as a wild-type (USA/WA1-2020) strain, but at reduced efficiency by 4.2-, 4.4- and 28.4-fold (P < 0.0001) respectively, compared to neutralization of a chimeric SARS-CoV-2 virus expressing the BA.1 spike protein.⁶

MONOCLONAL ANTIBODIES

- Using neutralization assays, Ai et al. tested the resistance of chimeric vesicular stomatitis viruses expressing either the wild-type (with additional D614G mutation), BA.1, BA.1.1, BA.2, or BA.3 spike proteins toward 10 spike-targeting monoclonal antibodies. They reported in their pre-print study severely impaired neutralization activity of imdevimab, casirivimab, bamlanivimab, etesevimab, tixagevimab, regdanvimab and amubarvimab against all four Omicron spike proteins compared with the wild-type spike protein. Only bebtelovimab remained potent across Omicron spike proteins while sotrovimab and cilgavimab have lost their neutralizing activity substantially against BA.3 (24.5- and 12.9-fold reduced compared to wild-type spike protein). By comparison, BA.1 and BA.1.1 were relatively more neutralized by sotrovimab and less neutralized by cilgavimab compared to BA.3, whereas BA.2 was relatively more neutralized by both sotrovimab and cilgavimab compared to BA.3.⁹

Testing and Surveillance

- Based on its mutation profile and the reported cases detected worldwide, routine molecular testing is likely able to detect BA.3 similarly to the other main Omicron sub-lineages.
- Considering the common mutations shared between BA.1, BA.1.1, BA.2, and BA.3, it is anticipated that most antigen tests would perform similarly among these sub-lineages, although further in-vitro and/or in-vivo testing would be required to confirm this hypothesis.
- The routine whole genome sequencing surveillance activities conducted through the Ontario COVID-19 Genomics Network (OCGN) would be able to detect the BA.3, similarly to the other main Omicron sub-lineages. This is the method that was used to confirm the first case of BA.3 identified in Canada.
- Although variant of concern polymerase chain reaction (PCR) screening by S gene target failure (SGTF) is no longer routinely conducted in the province, the BA.3 sub-lineage would share a similar pattern as BA.1 and BA.1.1 (presence of SGTF) due to the H69del/V70del mutations, which could be used to distinguish these sub-lineages from the currently predominant BA.2 sub-lineage (absence of SGTF).

Ontario Risk Assessment

The current risk of BA.3 sub-lineage transmission in Ontario is moderate with a high degree of uncertainty. The prevalence of BA.3 can rise with conditions that lead to spread of the SARS-CoV-2 virus in the population including suboptimal vaccine (and booster) coverage, waning immunity, and the ability of the Omicron variant of concern to escape immunity from natural infection and/or vaccination, increased transmissibility, reduced transmission competition from other circulating strains, and lack of

preventive measures and layers of protection to reduce transmission. The overall risk assessment may change as evidence emerges (see [Table 1](#)).

Table 1. Risk Assessment for Omicron Variant Sub-Lineage BA.3

Issue	Risk Level	Degree of Uncertainty
Importation in Ontario	Moderate	High
Increased Transmissibility	Insufficient information	High
Increased Disease Severity	Insufficient information	High
Re-infection	Insufficient information	High
Reduced Vaccine Effectiveness/Breakthrough Infections	Insufficient information	High
Impacts on Molecular and Antigen Testing	Low	Moderate
Impacts on Variant Surveillance	Low	Moderate

Implications for Practice

- The risk of mutation occurrence in the SARS-CoV-2 virus increases with ongoing transmission, and the risk of new sub-lineages emerging remains very high given the high transmissibility and immune evasion of the currently dominant Omicron variant, the current high levels of Omicron transmission in the province and globally, and the lifting of public health measures across Canada and globally.
- A comprehensive surveillance strategy including genomic sequencing for prompt identification of new variants that facilitates timely public health interventions to reduce their local introduction and spread is important to mitigate the emergence and spread of new variants.
- Due to limitations of individual public health measures (i.e., vaccination, masking, measures to reduce contacts), an approach that layers various measures should be used to mitigate community spread, including:
 - Achieving high, equitable vaccination (including primary series and booster doses) coverage globally as quickly as possible. Vaccination is a key public health tool, especially in lowering the risks of COVID-19-related hospitalizations and deaths.¹⁹ However, as COVID-19 vaccination and previous SARS-CoV-2 infection do not provide sterilizing immunity, a COVID-19 strategy that relies entirely on vaccination and previous infection will not contain transmissions in the context of variants that lower vaccine effectiveness.
 - Staying home when sick and when potentially infectious to others; wearing a well-fitting mask in indoor public settings; maintaining good ventilation in indoor spaces; practicing physical distancing, reducing contacts and avoiding crowded, closed indoor spaces; and practicing respiratory etiquette and washing hands should continue to be promoted for all.

References

1. O'Toole Á, Scher E, Rambaut A. VOC reports: lineage list. Oxford: SARS-CoV-2 lineages; 2022 [cited 2022 Apr 20]. Available from: https://cov-lineages.org/lineage_list.html
2. Desingu PA, Nagarajan K, Dhama K. Emergence of Omicron third lineage BA.3 and its importance. *J Med Virol*. 2022;94(5):1808-10. Available from: <https://doi.org/10.1002/jmv.27601>
3. Viana R, Moyo S, Amoako DG, Tegally H, Scheepers C, Althaus CL, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature*. 2022;603(7902):679-86. Available from: <https://doi.org/10.1038/s41586-022-04411-y>
4. World Health Organization. COVID-19 weekly epidemiological update: edition 86: 5 April 2022 [Internet]. Geneva: World Health Organization; 2022 [cited 2022 Apr 18]. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20220405-weekly-epi-update-86.pdf?sfvrsn=3f01a460_4&download=true
5. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Weekly epidemiological summary: SARS-CoV-2 whole genome sequencing in Ontario, April 19, 2022 [Internet]. Toronto, ON: Queen's Printer for Ontario; 2022 [cited 2022 Apr 25]. Available from: https://www.publichealthontario.ca/-/media/Documents/nCoV/epi/covid-19-sars-cov2-whole-genome-sequencing-epi-summary.pdf?sc_lang=en
6. Zou J, Kurhade C, Xia H, Liu M, Xie X, Ren P, et al. Cross-neutralization of Omicron BA.1 against BA.2 and BA.3 SARS-CoV-2. *bioRxiv* 486409 [Preprint]. 2022 Mar 31 [cited 2022 Apr 7]. Available from: <https://doi.org/10.1101/2022.03.30.486409>
7. Arora P, Zhang L, Rocha C, Sidarovich A, Kempf A, Schulz S, et al. Comparable neutralisation evasion of SARS-CoV-2 omicron subvariants BA.1, BA.2, and BA.3. *Lancet Infect Dis*. 2022 Apr 12 [Epub ahead of print]. Available from: [https://doi.org/10.1016/S1473-3099\(22\)00224-9](https://doi.org/10.1016/S1473-3099(22)00224-9)
8. Kurhade C, Zou J, Xia H, Cai H, Yang Q, Cutler M, et al. Neutralization of Omicron BA.1, BA.2, and BA.3 SARS-CoV-2 by 3 doses of BNT162b2 vaccine. *bioRxiv* 485633 [Preprint]. 2022 Mar 28 [cited 2022 Apr 7]. Available from: <https://doi.org/10.1101/2022.03.24.485633>
9. Ai J, Wang X, He X, Zhao X, Zhang Y, Jiang Y, et al. Antibody resistance of SARS-CoV-2 Omicron BA.1, BA.1.1, BA.2 and BA.3 sub-lineages. *bioRxiv* 487489 [Preprint]. 2022 Apr 7 [cited 2022 Apr 8]. Available from: <https://doi.org/10.1101/2022.04.07.487489>
10. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Conditions for the emergence of new SARS-Cov-2 variants [Internet]. Toronto, ON: Queen's Printer for Ontario; 2022 [cited 2022 Apr 20]. Available from: https://www.publichealthontario.ca/-/media/Documents/nCoV/voc/2022/03/covid-19-conditions-emergence-new-variants.pdf?sc_lang=en
11. Kumar S, Karuppanan K, Subramaniam G. Omicron (BA.1) and sub-variants (BA.1, BA.2 and BA.3) of SARS-CoV-2 spike infectivity and pathogenicity: a comparative sequence and structural-based computational assessment. *bioRxiv* 480029 [Preprint]. 2022 Feb 11 [cited 2022 Apr 18]. Available from: <https://doi.org/10.1101/2022.02.11.480029>

12. Abbas Q, Kusakin A, Sharrouf K, Jyakhwo S, Komissarov AS. Follow-up investigation and detailed mutational characterization of the SARS-CoV-2 Omicron variant lineages (BA.1, BA.2, BA.3 and BA.1.1). bioRxiv 481941 [Preprint]. 2022 Feb 26 [cited 2022 Apr 7]. Available from: <https://doi.org/10.1101/2022.02.25.481941>
13. European Centre for Disease Prevention and Control. Assessment of the further spread and potential impact of the SARS-CoV-2 Omicron variant of concern in the EU/EEA, 19th update [Internet]. Stockholm: European Centre for Disease Prevention and Control; 2022 [cited 2022 Apr 18]. Available from: <https://www.ecdc.europa.eu/sites/default/files/documents/RRA-19-update-27-jan-2022.pdf>
14. Communicable Diseases Genomics Network. PHLN/CDGN laboratory case definition: SARS-CoV-2 variant of concern (VOC) Omicron [Internet]. Melbourne: Communicable Diseases Genomics Network; 2021 [cited 2022 Apr 18]. Available from: <https://static1.squarespace.com/static/5e4f5b7ee8b790561bbb65e4/t/61c39eec284ead034cd90776/1640210157313/FINAL+-+CDGN+laboratory+case+definition+SARS-CoV-2+variant+of+concern+VOC+Omicron+-+20+December+2021.pdf>
15. cov-lineages; O'Toole Á, Hinrichs A, Rambaut A. pango-designation [Internet]. 2021 [cited 2022 Apr 18]. GitHub. Third sublineage in B.1.1.529 (Omicron-related) #367. Available from: <https://github.com/cov-lineages/pango-designation/issues/367>
16. Jung C, Kmiec D, Koepke L, Zech F, Jacob T, Sparrer Konstantin MJ, et al. Omicron: what makes the latest SARS-CoV-2 variant of concern so concerning? J Virol. 2022;96(6):e02077-21. Available from: <https://doi.org/10.1128/jvi.02077-21>
17. Latif AA, Mullen JL, Alkuzweny M, Tsueng G, Cano M, Haag E, et al; Center for Viral Systems Biology. outbreak.info: BA.3 lineage report [Internet]. La Jolla, CA: SuLab; 2022 [cited 2022 Apr 20]. Available from: <https://outbreak.info/situation-reports?pango=BA.3>
18. European Centre for Disease Prevention and Control. Communicable disease threats report: CDTR week 14, 3-9 April 2022 [Internet]. Stockholm: European Centre for Disease Prevention and Control; 2022 [cited 2022 Apr 18]. Available from: <https://www.ecdc.europa.eu/sites/default/files/documents/Communicable-disease-threats-report-9-april-2022-public.pdf>
19. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Enhanced epidemiological summary: confirmed cases of COVID-19 following vaccination in Ontario: December 14, 2020 to April 10, 2022 [Internet]. Toronto, ON: Queen's Printer for Ontario; 2022 [cited 2022 Apr 25]. Available from: https://www.publichealthontario.ca/-/media/Documents/nCoV/epi/covid-19-epi-confirmed-cases-post-vaccination.pdf?sc_lang=en

Citation

Ontario Agency for Health Protection and Promotion (Public Health Ontario). SARS-CoV-2 Omicron variant sub-lineage BA.3. Toronto, ON: Queen's Printer for Ontario; 2022.

Disclaimer

This document was developed by Public Health Ontario (PHO). PHO provides scientific and technical advice to Ontario's government, public health organizations and health care providers. PHO's work is guided by the current best available evidence at the time of publication. The application and use of this document is the responsibility of the user. PHO assumes no liability resulting from any such application or use. This document may be reproduced without permission for non-commercial purposes only and provided that appropriate credit is given to PHO. No changes and/or modifications may be made to this document without express written permission from PHO.

Public Health Ontario

Public Health Ontario is an agency of the Government of Ontario dedicated to protecting and promoting the health of all Ontarians and reducing inequities in health. Public Health Ontario links public health practitioners, front-line health workers and researchers to the best scientific intelligence and knowledge from around the world.

For more information about PHO, visit publichealthontario.ca.

©Queen's Printer for Ontario, 2022

Ontario 