Title

Regional clustering of influenza A viruses carrying PA I38T in Japan: matched-control HA clustering using GISAID sequences, 2022–2025

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Abstract

Background: Baloxavir marboxil (BXM) resistance in influenza A virus (IAV) is most often linked to a substitution at polymerase acidic (PA) residue 38, especially I38T. Whether PA I38T viruses circulate as local transmission clusters in Japan remains uncertain.

Methods: We analyzed 2,577 IAV sequences from GISAID with strain-name years 2022–2025. The longest ATG-initiated PA open reading frame (ORF; ≥650 aa) was translated and residue 38 was classified as I, T (I38T), non-I/T, or ambiguous. For each I38T virus, matched haemagglutinin (HA) controls were defined by identical subtype, prefecture (city strings normalized), and year. HA coding sequences (1,698 nt) were compared using p-distance excluding ambiguous sites. Average-linkage hierarchical clustering was performed within each matched stratum using a p-distance threshold ≤0.005, and neighbor-joining HA trees were generated for visualization.

Results: A valid PA ORF was obtained for 2,568/2,577 entries. At PA38, I was observed in 2,518 (98.05%), T (I38T) in 25 (0.97%), non-I/T in 7 (0.27%; M=2, N=2, V=2, S=1), and ambiguous calls in 18 (0.70%). Annual I38T frequencies (denominator including non-I/T and ambiguous calls) were 4/121 (3.31%) in 2022, 18/932 (1.93%) in 2023, 2/815 (0.25%) in 2024, and 1/700 (0.14%) in 2025. In matched-control HA analyses, 16/25 (64%) I38T viruses belonged to local HA clusters containing ≥2 I38T viruses (seven subtype–prefecture–year strata), whereas 9/25 (36%) were dispersed among controls or formed singleton clusters.

Conclusions: When all PA38 calls are counted, PA I38T remains infrequent (<1%) but is frequently embedded within tightly related local HA lineages, supporting limited yet measurable regional spread of BXM resistance-associated IAVs. Continued integrated PA/HA genomic surveillance is warranted.

Keywords: influenza A; baloxavir; PA-I38T; antiviral resistance; GISAID; clustering