Invasive Group A *Streptococcus* Hypervirulent $M1_{UK}$ Clone, Canada, 2018–2023

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To determine invasive group A *Streptococcus* trends in Canada, we characterized *emm*1 isolates collected during 2018–2023. The percentage of hypervirulent $M1_{UK}$ lineage isolates increased significantly, from 22.1% in 2018 to 60.2% in 2023. Genomic analysis identified geographically and temporally associated clusters and genes associated with virulent bacteriophage acquisition.

The hypervirulent $M1_{UK}$ lineage of group A *Streptococcus* (GAS), originally identified in the United Kingdom in 2019, has been associated with increased notifications of scarlet fever and invasive GAS (iGAS) infections (*1*). The $M1_{UK}$ lineage is characterized by increased production of *speA* (streptococcal pyrogenic exotoxin A) and is differentiated from the $M1_{global}$ lineage by 27 key single-nucleotide variants (SNVs) (*1*). Initial characterization of a subset of *emm*1 isolates collected in Canada during 2016–2019 identified 10% of isolates as the $M1_{UK}$ lineage (*2*).

Beginning in 2022, several health organizations, including the World Health Organization and the Pan American Health Organization, reported increased cases of pediatric iGAS in numerous member countries, above seasonal expectations (*3*,*4*). Many countries in Europe, including Belgium, Netherlands, and the United Kingdom (*5*–*7*), have associated increased iGAS disease in the 2022–23 season with *emm*1, particularly the $M1_{UK}$ variant. In light of the worldwide increased iGAS disease activity and the association of those increases with *emm*1, we sought to describe the trends in *emm*1 and $M1_{UK}$ in Canada during 2018–2023.

The Study

We identified 2,582 isolates of iGAS *emm*1 collected during 2018–2023 as part of the passive, laboratory-based surveillance system for iGAS in Canada (*8*). Of those, we sequenced 2,315 isolates by using Illumina NextSeq technology (https://www.illumina.com); the remainder were received as line-listed typing data only. We identified $M1_{UK}$ isolates by mapping whole-genome sequencing reads to reference strain MGAS5005 and identifying 27 characteristic SNVs, as previously described (*1*). We performed core SNV phylogenetic analysis by using the SNVPhyl pipeline (*9*) and identified genomic clusters by using ClusterPicker with default settings (*10*). We assessed presence of

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DOI: https://doi.org/10.3201/eid3011.241068

antimicrobial resistance, toxin, and virulence genes by using the WADE pipeline (https://github.com/phac-nml/wade), the public virulence factor database (http://www.mgc.ac.cn/VFs), and custom database queries. The $M1_{UK}$ genomic data reported in our study have been deposited in the National Center for Biotechnology Information Sequence Read Archive (BioProject PRJNA1137869).

We assessed trends in lineage distribution for statistical significance by using the Cochran-Armitage test for trend and differences between lineages by using the 2-tailed Fisher exact test ($\alpha = 0.05$). We aggregated data regionally into the Western (British Columbia, Alberta), Prairie (Saskatchewan, Manitoba), Central (Ontario, Québec), Eastern (New Brunswick, Nova Scotia, Prince Edward Island, Newfoundland and Labrador), and Northern (Yukon, Northwest Territories, Nunavut) regions of Canada.

In 2018, *emm*1 accounted for 17.1% of iGAS isolates collected in Canada, after which the proportion of *emm*1 decreased significantly, to a low of 0.5% in 2021 ($p<0.0001$), followed by a sharp increase to 24.5% in 2023 ($p<0.0001$) (Figure 1, panel A). Overall, during 2018–2023, a total of 46.2% of the 2,315 sequenced *emm*1 isolates were the $M1_{UK}$ lineage. The proportion of $M1_{UK}$ isolates increased from 22.1% (110/497) in 2018 to 60.2% (711/1,182) in 2023 ($p<0.0001$) (Figure 1, panel B). In 2023, the proportion of $M1_{UK}$ was highest in the Prairie region (66.7%), followed by the Central (62.5%), Western (58.9%) and Eastern regions (35.3%); no $M1_{UK}$ was collected in the Northern region. The only common (n>20) *emm*1 subtype associated exclusively with the $M1_{UK}$ lineage was *emm*1.147; subtypes *emm*1.146 and *emm*1.25 were exclusively associated with the $M1_{global}$ genotype. Subtypes *emm*1.0 and *emm*1.3 were associated with both $M1_{global}$ and $M1_{UK}$ genotypes (40.8% of *emm*1.0 and 95.0% of *emm*1.3 were $M1_{UK}$).

Few antimicrobial resistance determinants were identified within the $M1_{global}$ or $M1_{UK}$ cohorts (Table).

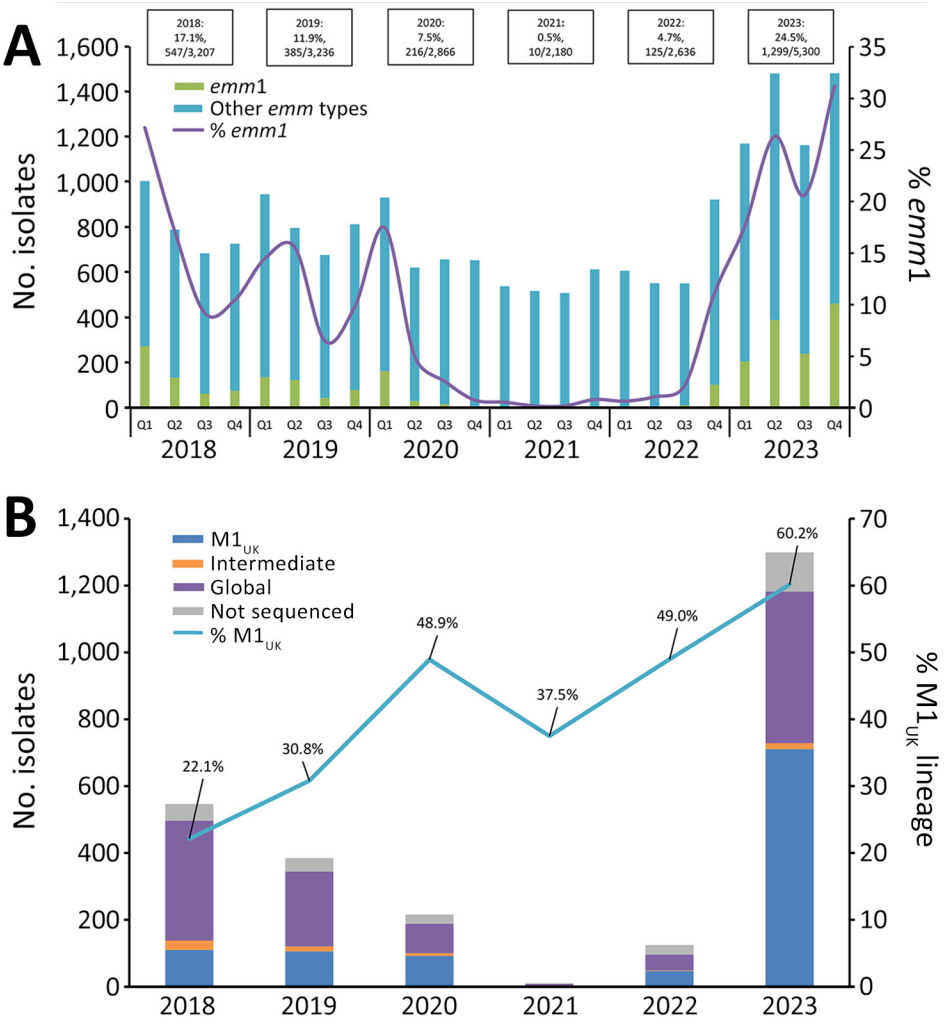

Figure 1. Expansion of invasive group A *Streptococcus emm*1 and the $M1_{UK}$ lineage in Canada, 2018–2023. A) Number of *emm*1 isolates collected, by quarter. B) Percentage of $M1_{UK}$ isolates among *emm*1 isolates collected. Q1, January–March; Q2, April–June; Q3, July–September; Q4, October–December. Annual proportions of *emm*1 are listed above the bars. Intermediate indicates an isolate with a partial $M1_{UK}$ genotype; not sequenced indicates an isolate that was submitted to the National Microbiology Laboratory as line-listed typing data only.

Table. Characteristics of $M1_{UK}$ lineage and other invasive group A *Streptococcus emm*1 isolates collected in Canada, 2018–2023

Isolate feature*	Lineage, no. (%) isolates		p value‡
	$M1_{UK}$, n = 1,069	Other *emm*1, n = 1,246†	
Antimicrobial susceptibility			
Penicillin	100	100	1.000
Erythromycin	99.5 (1,064)	99.2 (1,236)	0.4375
Clindamycin	99.8 (1,067)	99.5 (1,240)	0.2997
Chloramphenicol	100	100	1.000
Levofloxacin	99.9 (1,068)	99.8 (1,243)	0.6291
Tetracycline	99.6 (1,065)	99.1 (1,235)	0.1928
Toxin gene presence			
speA	98.4 (1,052)	97.4 (1,214)	0.1124
speC	43.8 (468)	8.3 (103)	<0.0001
speG	100 (1,069)	99.9 (1,245)	1.000
speH	0	0	1.000
speI	0	0	1.000
speJ	99.5 (1,064)	99.4 (1,238)	0.7817
speK	0.1 (1)	0	0.4618
speL	0	0	1.000
speM	0	0	1.000
smeZ	98.4 (1,052)	98.9 (1,232)	0.3674
ssa	35.5 (379)	4.4 (55)	<0.0001
Virulence gene presence			
Phage-associated DNase, *spd1*	43.9 (469)	8.3 (103)	<0.0001
Phage-associated hyaluronidase, *hylP*	37.8 (404)	3.9 (49)	<0.0001
Gene combinations			
speC + *ssa* + *spd1*	35.4 (378)	8.3 (55)	<0.0001
speC + *spd1*	8.3 (89)	3.9 (48)	<0.0001

*All isolate features (antimicrobial susceptibilities, toxin, and virulence gene presence) were inferred from whole-genome sequences.
†Other *emm*1 with whole-genome sequencing data available.
‡Two-tailed; p<0.05 considered significant.

Compared with other *emm*1 isolates, the $M1_{UK}$ variant demonstrated significantly higher presence of genes *speC* (streptococcal pyrogenic exotoxin C) and *ssa* (streptococcal superantigen), as well as virulence factors *spd1* (phage-associated DNase) and *hylP* (phage-associated hyaluronidase).

Phylogenetic analysis of all *emm*1 isolates identified clear separation of the $M1_{global}$ and $M1_{UK}$ lineages (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/30/11/24-1068-App1.pdf); the isolates within the $M1_{UK}$ cluster differed from those in the $M1_{global}$ cluster by an average of 46 (range 22–80) SNVs. Within the $M1_{UK}$ cluster, isolates differed by an average of 17.6 (range 0–41) SNVs, and there was more variability within isolates of the $M1_{global}$ lineage (average 32.6 [range 0–82] SNVs difference).

ClusterPicker identified 11 large clusters within the $M1_{UK}$ cohort, each cluster containing 10–280 isolates (Figure 2; Appendix Table). In general, the highest proportion of each cluster was collected in 2023, which is consistent with the surge of iGAS disease cases that year. Exceptions include clusters 4 and 11, which included isolates predominantly collected before the *emm*1 decrease that coincided with the COVID-19 pandemic. Clusters 1, 5–7, and 10 were identified exclusively after the COVID-19 pandemic period, and clusters 2 and 8 persisted across the study period. Clusters were generally associated with geographic region: clusters 1, 2, 5, 7, and 10 were strongly associated with the Central region, and clusters 3, 6, 8, and 9 were strongly associated with associated with the Western region. Cluster 4 was mostly found in the Eastern region; Cluster 11 was predominantly from the Prairie region. Within the Central region, approximately two thirds of the total isolates collected during the study period were part of either cluster 1 or cluster 2. Cluster 3 was most common for isolates collected from the Western (40.7%) and Prairie (25.0%) regions, and cluster 4 was most common in the Eastern region (53.8%).

More than 99% of isolates within $M1_{UK}$ cluster 1 possessed *speC*, *ssa*, *spd,1*, and *hylP* (Appendix Table). Within cluster 3, a total of 78.5% of isolates possessed *speC* and *spd1*; presence of *ssa* and *hylP* was sporadic. Those 4 genes were sporadically present within clusters 2 and 7.

Conclusions

Our study highlights expansion of the $M1_{UK}$ GAS lineage in Canada. Initial genomic characterization of *emm*1 isolates in Canada identified only 10% $M1_{UK}$ in a subset of *emm*1 isolates collected during 2016–2019 (*2*); by 2023, $M1_{UK}$ comprised 60.2% of *emm*1 isolates. The proportion of $M1_{UK}$ in Canada in 2023 is much higher than that most recently published from the United States (11%), although considerably lower

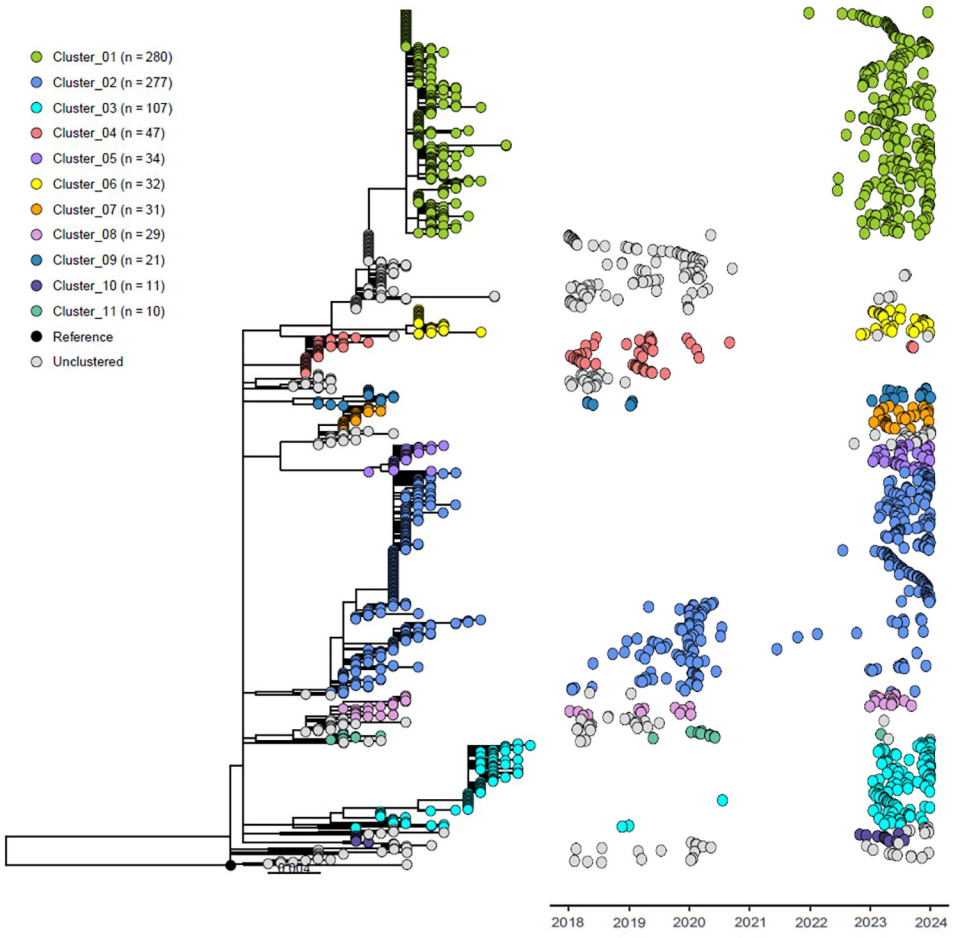

Figure 2. Maximum-likelihood core single-nucleotide variant phylogeny for 1,069 invasive group A *Streptococcus* $M1_{UK}$ lineage isolates collected in Canada, 2018–2023. Eleven large clusters are shown, each containing 10–280 isolates.

than that reported by recent studies from Belgium (78%) and the United Kingdom (95.7%) (*5*,*7*,*11*). Our study findings are consistent with findings of Vieira et al., who noted that the $M1_{UK}$ lineage showed less genomic diversity than the $M1_{global}$ lineage (*7*). Of note, we did not identify any isolates of the novel $M1_{DK}$ lineage, which was originally identified in Denmark and was responsible for 30% of iGAS cases in Denmark in winter 2022–23 (*12*).

Our study identified a large proportion of $M1_{UK}$ isolates with the bacteriophage-encoded DNase *spd1* and *speC*/*ssa* superantigens. The presence of those 3 genes suggests acquisition of a virulent prophage related to ΦHKU488.vir, which has been associated with outbreaks of *emm*12 scarlet fever in Asia (*13*,*14*). However, the lack of antimicrobial resistance determinants in Canadian *emm*1 isolates so far indicates limited transfer of the integrative conjugative elements that have been responsible for macrolide, lincosamide, and tetracycline resistance in GAS outbreaks in Asia (*14*). Although $M1_{UK}$ with this phage were present in Canada before the COVID-19 pandemic, their presence substantially expanded in 2023, particularly in central Canada (Figure 2, cluster 1). $M1_{UK}$ isolates within cluster 3 were associated with *speC* and *spd1* only; that combination is associated with a different prophage, ΦSP370.1 (*15*). Those isolates were more common in western Canada beginning in 2023, suggesting a different path of virulence gene acquisition compared with that of ΦHKU488.vir. Monitoring the spread of the variants of $M1_{UK}$, particularly for development of antimicrobial resistance, will remain critical. Our study underscores the value of linking laboratory data to epidemiologic variables to enhance our knowledge of how GAS variants affect clinical manifestations, outcomes, and risk groups.

Acknowledgments

We thank Angela Yuen, Rachel Hink, and Anastasia Anistratov from the Streptococcus and Sexually Transmitted Infections Section at the National Microbiology Laboratory for their laboratory technical assistance and the staff of provincial and territorial public health laboratories in Canada for participating in the national laboratory surveillance program.

This project was supported by internal funding from the Public Health Agency of Canada.

The authors have no conflicts of interest to declare. A.R.G. and I.M. contributed to study design; all authors contributed to data collection; and A.R.G. performed data analysis, produced visualizations, and wrote the manuscript. All authors reviewed and edited the manuscript.

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