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BRIEF REPORT

The Emergence and Impact of the $M1_{UK}$ Lineage on Invasive Group A Streptococcus Disease in Aotearoa New Zealand

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$M1_{UK}$ is associated with current surges in invasive infection globally, partly due to increased production of superantigen streptococcal pyrogenic exotoxin A. We show that $M1_{UK}$ is now the dominant invasive *emm*1 lineage in Aotearoa New Zealand and is genomically related to community infections, suggesting that measures that effectively prevent group A *Streptococcus* pharyngitis in children could reduce invasive disease.

Keywords. *emm*1; group A streptococcus; invasive group A streptococcal disease; $M1_{UK}$.

A toxigenic variant of *Streptococcus pyogenes* serotype M1 (*emm*1) is responsible for the recent unprecedented notifications of scarlet fever and invasive group A streptococcal (iGAS) infection in the United Kingdom [1, 2]. Since its emergence in 2008, this variant (known as $M1_{UK}$) has become the dominant *emm*1 lineage in many countries, reportedly expanding and associated with disease surges throughout the northern hemisphere and Australia [1–3].

$M1_{UK}$ is differentiated from earlier global *emm*1 lineages ($M1_{global}$) based on the presence of 27 chromosomal single-nucleotide polymorphisms (SNPs). Having evolved via intermediate sublineages, $M1_{UK}$ is characterized by its increased production of the streptococcal pyrogenic exotoxin A (SpeA) superantigen [1]. Notably, the immune response to enhanced SpeA expression by $M1_{UK}$ has not been described.

This retrospective genomic analysis describes the prevalence of $M1_{UK}$ in invasive *emm*1 strains over the past decade in Aotearoa New Zealand (NZ), which has a high incidence of iGAS in comparison with countries that have a similar human development index [4]. We provide a novel description of the SpeA antibody response in children with $M1_{UK}$ and $M1_{global}$ pharyngitis and compare the prevalence of $M1_{UK}$ in the community vs invasive disease.

METHODS

Bacterial Strains

The Institute of Environmental Science and Research's Invasive Pathogens Laboratory received 417 group A *Streptococcus* (GAS) isolates collected from patients between January 2013 and November 2023 that were assigned *emm*1 based on standard *emm*-typing procedures (https://www.cdc.gov/strep-lab/php/group-a-strep/emm-typing.html). Isolates were referred from diagnostic laboratories around the country for passive (nonnotifiable) surveillance and included if received by 6 December 2023. All *emm*1 isolates were analyzed to determine the proportion that belong to the $M1_{UK}$ lineage.

Genomic Analysis

Isolates were determined to be $M1_{UK}$ by a combination of allele-specific polymerase chain reaction (AS-PCR) and whole genome sequencing. Heat-killed suspensions from the earliest *emm*1 isolates (2013–2014, n = 88) were screened for *rofA*, *gldA*, and *pstB* SNPs characteristic of the $M1_{UK}$ lineage by a recently reported AS-PCR [5]. Genotypically characterized strains were used as controls (Supplementary Data 1). This method confirmed the genotype of 34 of 88 isolates. Isolates with non–wild type AS-PCR results or that failed to amplify (54/88) were sequenced with the remainder of the *emm*1 isolates not subjected to AS-PCR (2015–November 2023, 328/329; Supplementary Data 1). Genomic DNA was extracted, and libraries were prepared by the plexWell 96 Library Preparation Kit (SeqWell) and sequenced on an Illumina NextSeq with 150–base pair paired-end chemistry.

Genomes were assembled with SKESA (https://github.com/ncbi/SKESA), and *emm* type was confirmed in silico (https://github.com/MDU-PHL/emmtyper). Genome assemblies were subjected to in silico PCR that detected wild type or SNP alleles in the *rofA*, *gldA*, and *pstB* genes, as in the AS-PCR, to differentiate

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the $M1_{global}$, $M1_{UK}$, and $M1_{intermediate}$ lineages [5]. Phylogenetic analyses were performed on the 377 NZ invasive *emm*1 genomes and compared with 539 publicly available global genomes from 2004 to 2020 [3], including 4 historical NZ isolates (Supplementary Data 2). Additionally, 59 community *emm*1 genomes were included that had been obtained from a study of school-aged children conducted from 2018 to 2019 in Auckland [6]. Overall 15,087 SNPs relative to $M1_{global}$ MGAS5005 were used to construct maximum likelihood phylogeny via IQ-TREE2 version 2.0.6 (https://github.com/iqtree/iqtree2) with model selection and 2000 ultrafast bootstrapping. Alignment was generated by snippy version 4.6.0 (https://github.com/tseemann/snippy) and recombinant corrected with gubbins version 3.2.2 [7]. Phylogeny was visualized by Microreact (https://microreact.org/).

SpeA Serology

Sera were obtained from the community GAS infection study described previously (ethics approval 17/NTA/262 [8]). Convalescent samples from children with GAS-positive pharyngitis caused by an *emm*1 strain (n = 36) [6] or non-*emm1* strains (n = 15 infected with *emm*53 or *emm*81) were tested for anti-SpeA antibodies, as were matched healthy children with negative throat swab and no evidence of a recent GAS infection (n = 23). Convalescent samples were collected at least 2 weeks after the initial positive swab to ensure seroconversion. Antibodies were quantified by an immunoassay in which SpeA, expressed and purified as described [9], was covalently coupled to MagPlex microspheres (beads) via the xMAP Antibody Coupling Kit (Luminex; Thermo Fisher) following published protocols [10]. Sera were diluted 1:1000, and net median fluorescence intensity was determined by subtracting background values from no-serum control wells.

RESULTS

$M1_{UK}$ Is the Dominant Invasive *emm*1 Lineage

Passive surveillance data show that the $M1_{UK}$ lineage was present in NZ in 2013 but as a low proportion (<2%) of all invasive *emm*1 isolates that year (Figure 1*A*). The proportion of $M1_{UK}$ isolates increased from 2015, outcompeting the $M1_{global}$ lineage from 2019, and is now the dominant iGAS *emm*1 lineage in NZ. Between 2020 and 2022, coinciding with the COVID-19 pandemic, fewer iGAS isolates were referred annually to the Institute of Environmental Science and Research, and the proportion of *emm*1 isolates decreased (Supplementary Figure 1). In 2023 *emm*1 proportions returned to prepandemic levels, with $M1_{UK}$ being the dominant *emm*1 lineage (88%). Furthermore, the proportion of $M1_{UK}$ increased to 12.6% of all invasive isolates in 2023 to November, up from 0.2% 10 years earlier.

Phylogenetic analysis of NZ isolates as compared with global context isolates shows that there have been multiple introductions of the $M1_{UK}$ lineage into NZ over the last decade (Figure 1*B*). The largest NZ $M1_{UK}$ clade likely has phylogenetic origins in the United Kingdom, and a smaller, more recently expanding subclade is related to isolates from Queensland, Australia.

Community and Invasive $M1_{UK}$

In 2018 and 2019 combined, 16 of 36 (44%) invasive *emm*1 isolates referred from the Auckland region belonged to the $M1_{UK}$ lineage; the remainder belonged to the $M1_{global}$ and $M1_{intermediate}$ lineages (Supplementary Data 2). During a similar period, 20 of 59 (34%) Auckland community *emm*1 isolates collected from children with pharyngitis were $M1_{UK}$, which was not significantly different (χ^2 test, $P > .05$). Phylogenetic analysis of this 2018–2019 Auckland subset did not show clustering by infection type, suggesting that community and invasive isolates are genomically related (Figure 1*B*).

Antibody Response to SpeA in Children With Pharyngitis

The level of anti-SpeA antibodies was significantly higher in children with culture-positive GAS pharyngitis caused by $M1_{UK}$ or $M1_{global}$ as compared with healthy controls ($P < .001$). However, there was no significant difference ($P > .01$) in anti-SpeA antibody levels in children infected with non-*emm*1 strains as compared with healthy controls or those with $M1_{UK}$ or $M1_{global}$ infections (Figure 1*C*).

DISCUSSION

$M1_{UK}$ has outcompeted its global counterpart and is now the dominant *emm*1 lineage associated with invasive disease in many countries [2, 3]. This study shows that NZ has a similar pattern, where $M1_{UK}$ is now the dominant invasive *emm*1 lineage referred to the national reference laboratory for passive surveillance. Phylogenetic analyses indicate that multiple $M1_{UK}$ introductions have occurred over the last decade with evidence of subsequent local clonal expansion. This echoes the multiple international introductions and subsequent establishment of specific GAS sequence clusters observed in the Auckland community [6] and highlights the influence of globally expanding GAS lineages on the NZ population structure. The high level of genomic similarity between circulating community $M1_{UK}$ strains and those referred for surveillance in this study indicates that the community is a source of invasive infection in NZ. This supports recent evidence from the United Kingdom that community transmission of $M1_{UK}$ pharyngeal strains can lead to invasive infection [2].

$M1_{UK}$ is associated with increased SpeA expression in vitro [1], although it is unclear how this correlates with immune responses following clinical infection. For children with pharyngitis, the antibody response to SpeA was not significantly different between $M1_{UK}$ and $M1_{global}$ infections in this study, suggesting that any increase in SpeA expression is not associated with a concurrent increase in antigen-specific antibodies. However, it remains

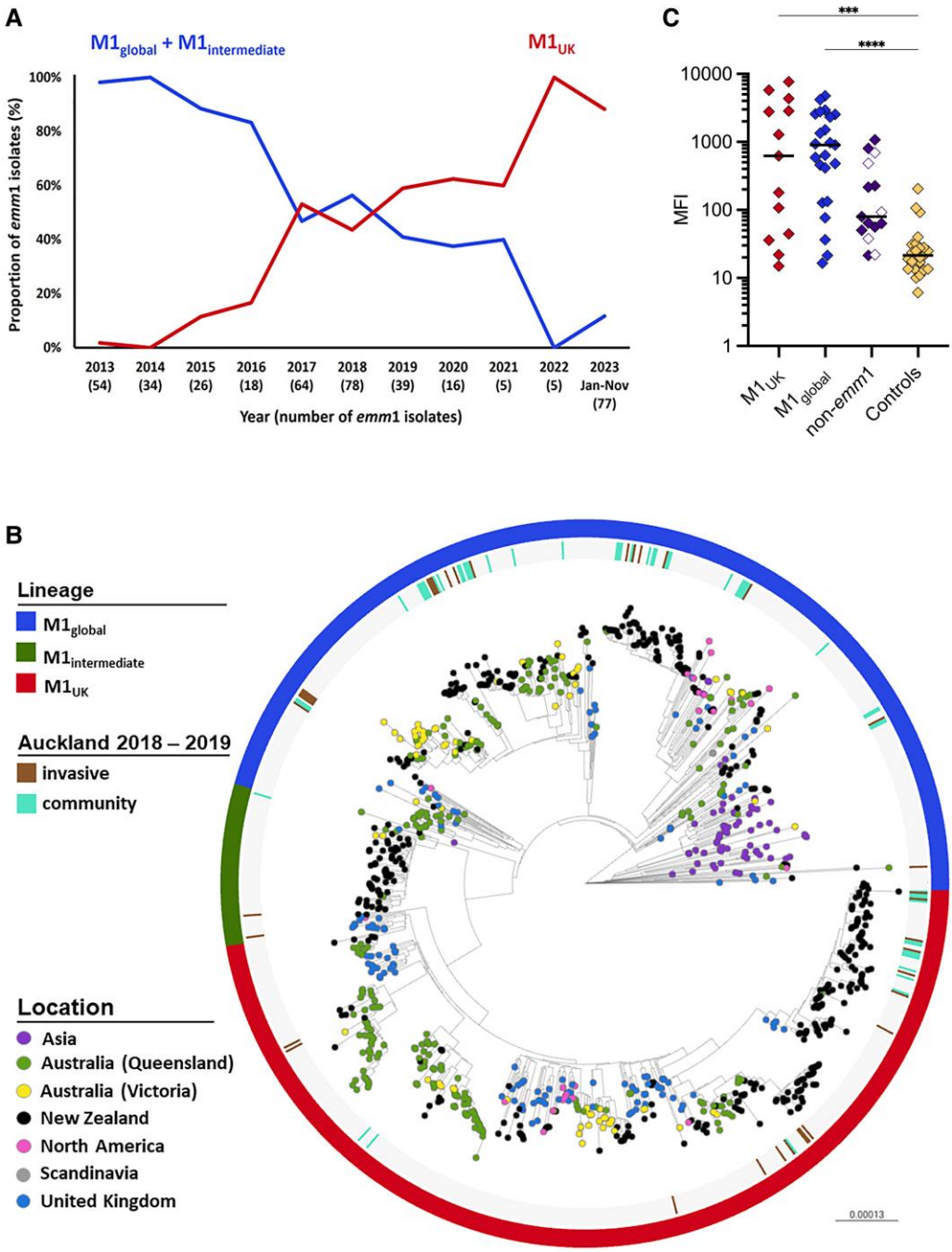

Figure 1. *A*, Proportion of *emm*1 lineages: $M1_{global}$ and $M1_{intermediate}$ combined (blue) and $M1_{UK}$ (red) as a proportion of all passive surveillance invasive *emm*1 isolates from New Zealand collected from patients by year (January 2013–November 2023). All $M1_{intermediate}$ strains in this study are the $M1_{13SNPs}$ sublineage (Supplementary Data 1). *B*, Maximum likelihood phylogeny of 377 New Zealand invasive group A streptococcal isolates, 539 global context isolates [3], and 59 community *emm*1 isolates from Auckland school-aged children [6]. Terminal nodes are colored by location, and the outer ring indicates *emm*1 lineage. The inner ring shows invasive and community isolates from the Auckland 2018–2019 subset. Scale bar: number of substitutions per site. *C*, Anti-SpeA antibody responses in sera from children with pharyngitis ($M1_{UK}$, n = 13; $M1_{global}$, n = 23; non-*emm*1, n = 15) vs healthy controls (n = 23). The non-*emm*1 group combines data from children with *emm*81 (open diamonds) and *emm*53 (closed diamonds) pharyngitis. Horizontal lines show median values. Statistical significance was assessed by the Kruskal-Wallis test with Dunn test for multiple comparisons. ***$P < .001$. ****$P < .0001$. MFI, mean fluorescence intensity.

possible that differences in the magnitude of anti-SpeA antibodies may be present in $M1_{UK}$ invasive disease, where SpeA production might be expected to play a more central role in pathogenesis. Of note, some children infected with non-*emm*1 strains had elevated SpeA antibodies, which may be a result of cross-reactivity between SpeA and other superantigens expressed by the infecting strain types (*emm*53 and *emm*81 [6]) or prior infections with SpeA-producing strains.

Concurrent with the COVID-19 pandemic, this study shows a decrease in the proportion of referred *emm*1 iGAS isolates. This is in keeping with the hypothesis that public health measures, including the use of masks, decreased transmission of strains typically associated with pharyngitis, such as *emm*1, rather than those more typically associated with skin infections [11]. The increase in *emm*1 iGAS isolates referred in 2023 may reflect a return to prepandemic numbers. However, given the surges in invasive disease and scarlet fever driven by $M1_{UK}$ overseas [1, 2], ongoing national surveillance of this variant remains warranted.

In summary, genomic surveillance has traced the emergence of $M1_{UK}$ in NZ, including multiple international introductions, followed by local clonal expansion. The genomic similarity between community and invasive strains suggests that measures that effectively prevent GAS pharyngitis in schoolchildren, such as timely diagnosis, antibiotic treatment, and future vaccines, may also reduce iGAS infections.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Data availability. Reads for isolates sequenced for this study are available in the NCBI Sequence Read Archive under BioProjects PRJNA1100230 and PRJNA1104651.

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