Emerging Invasive Group A *Streptococcus* $M1_{UK}$ Lineage Detected by Allele-Specific PCR, England, 2020[1]

Xiangyun Zhi, Ho Kwong Li, Hanqi Li, Zuzanna Loboda, Samson Charles, Ana Vieira, Kristin Huse, Elita Jauneikaite, Lucy Reeves, Kai Yi Mok, Juliana Coelho, Theresa Lamagni, Shiranee Sriskandan

Increasing reports of invasive *Streptococcus pyogenes* infections mandate surveillance for toxigenic lineage $M1_{UK}$. An allele-specific PCR was developed to distinguish $M1_{UK}$ from other *emm*1 strains. The $M1_{UK}$ lineage represented 91% of invasive *emm*1 isolates in England in 2020. Allele-specific PCR will permit surveillance for $M1_{UK}$ without need for genome sequencing.

Upsurges in invasive group A *Streptococcus* (GAS) infections have been widely reported in England and elsewhere (*1*), emphasizing the need to examine the relationship between circulating *S. pyogenes* that cause pharyngitis and scarlet fever and cases of invasive disease. Although many factors, such as exposure history, underlying conditions, viral co-infection, and genetic susceptibility, might increase susceptibility to *S. pyogenes* infection, strain-specific virulence might also be crucial.

In England, where both scarlet fever and invasive *S. pyogenes* infections are notifiable, pronounced upsurges in scarlet fever were recorded over an 8-year period (*2*,*3*) but subsided during the COVID-19 pandemic. During the 2015–16 season, a notable increase in invasive infections was observed that had not been evident previously (*4*). Both scarlet fever and invasive infections were associated with the emergence of $M1_{UK}$, a new sublineage of *emm*1 *S. pyogenes* (*4*) that appeared to outcompete the highly successful, contemporary epidemic *emm*1 $M1_{global}$ strain, which emerged and spread globally during the 1980s (*5*,*6*). Despite an unchanged phage repertoire, $M1_{UK}$ strains produce more superantigenic scarlet fever toxin SpeA (streptococcal pyrogenic exotoxin A) than contemporary $M1_{global}$ *S. pyogenes* strains (*4*).

*emm*1 *S. pyogenes* strains are highly virulent (*5*) and disproportionately associated with invasive infections; any increase in the prevalence of *emm*1 strains in persons with pharyngitis or scarlet fever is, therefore, a public health concern. Known distribution of $M1_{UK}$ is largely limited to those countries undertaking and reporting genome sequencing (Figure 1). $M1_{UK}$ has been identified in other countries in Europe, from a single isolate in Denmark (*4*) to dominant status in the Netherlands (*7*). The lineage has also been reported in North America; the Public Health Agency of Canada reported that 17/178 (10%) of *emm*1 isolates from 2016 were $M1_{UK}$ (*8*). This finding contrasts with a reported $M1_{UK}$ frequency of just 0%–2.8% of *emm*1 isolates in the United States, according to the Active Bacterial Core surveillance system of the US Centers for Disease Control and Prevention; however, the low US frequency was associated with severe infections (*9*). Of note, most reports used genomic data that were >5 years old, so a reappraisal of prevalence is needed. A recent study in Australia using data through 2020 indicated expansion of $M1_{UK}$ in Queensland and Victoria (*10*). The authors identified acquisition of an additional phage encoding superantigen genes *ssa* and *spec* and a single-nucleotide polymorphism (SNP) implicated in SpeA upregulation in the $M1_{UK}$ lineage. Multicountry increases in GAS infections (*1*) since pandemic restrictions were lifted underscore the importance of increasing global

Author affiliations: Imperial College London, London, UK (X. Zhi, H.K. Li, H. Li, Z. Loboda, S. Charles, A. Vieira, K. Huse, E. Jauneikaite, L. Reeves, K.Y. Mok, S. Sriskandan); United Kingdom Health Security Agency, London (J. Coelho, T. Lamagni)

DOI: https://doi.org/10.3201/eid2905.221887

[1]Data from this study were presented at the 21st Lancefield International Symposium on Streptococci and Streptococcal Diseases; Stockholm, Sweden; June 7–10, 2022.

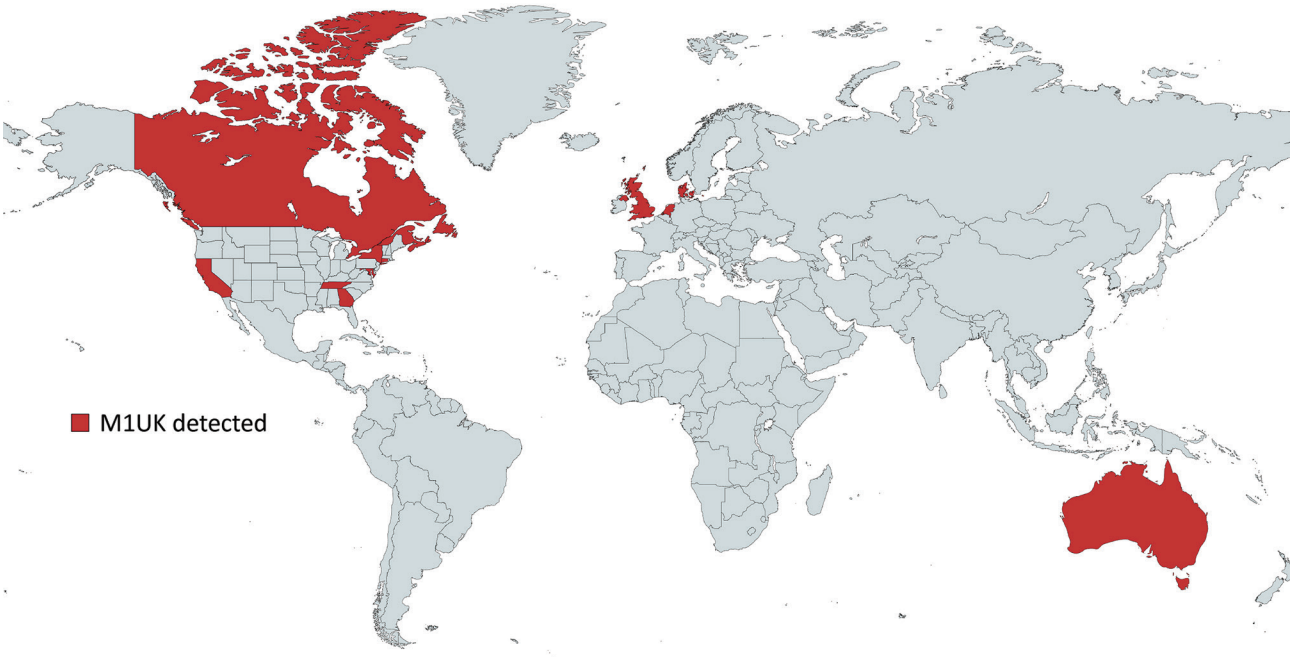

Figure 1. Countries and US states with reported $M1_{UK}$ *Streptococcus pyogenes* cases. Map created by using MapChart (https://www.mapchart.net) as part of a study of emerging invasive group A *Streptococcus* $M1_{UK}$ lineage detected by allele-specific PCR, England, 2020.

surveillance of lineages that have potentially enhanced fitness, such as $M1_{UK}$.

The Study

Genetic distinction between $M1_{UK}$ and $M1_{global}$ strains is possible by using whole-genome sequencing to detect the 27 SNPs that characterize the $M1_{UK}$ lineage (*4*), but sequencing technology is not available in all countries. We designed an allele-specific PCR (AS-PCR) method to detect $M1_{UK}$-specific SNPs in the *rofA*, *gldA*, and *pstB* genes. We chose amplification targets to separate $M1_{UK}$ and $M1_{global}$ strains but also to identify strains from less common intermediate sublineages that had only 13 or 23 of the 27 $M1_{UK}$-specific SNPs (*4*). We optimized PCR conditions for each pair of amplicons by using DNA from control strains for each lineage (Table; Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/5/22-1887-App1.pdf). Collecting bacterial samples from patients was part of routine clinical care; collecting surplus samples after anonymizing patient information was approved by the West London National Research Ethics Committee (approval no. 06/Q0406/20).

To evaluate allele-specific PCR, we tested whether the *rofA* and *pstB* primers correctly identified lineages of 27 newly genome-sequenced noninvasive *emm*1

Table. PCR primers and conditions used to differentiate $M1_{global}$ and $M1_{UK}$ *Streptococcus pyogenes* lineages in study of emerging invasive group A *Streptococcus* $M1_{UK}$ lineage detected by allele-specific PCR, England, 2020*

Target gene	Primer type†	Sequences‡	PCR cycle conditions	Product, bp
rofA	WT sequence	TGTTAATTGCTTGGTTAAATCA	30 cycles of 95°C for 3 min, 45 s; 59.2°C for 30 s; 72°C for 1 min (final cycle: 5 min)	278
	Forward-SNP	5′-TGTTAATTGCTTGGTTAAAG**t**A-'3		
	Forward-WT	5′-TGTTAATTGCTTGGTTAAAG**C**A-'3		
	Reverse	5′-GCTCATCTCCTAACGGATTCTT-'3		
gldA	WT sequence	AGATGGGTTAGCAACATGG	30 cycles of 95°C for 3 min, 45 s; 61.8°C for 30 s;72°C for 1 min (final cycle: 5 min)	292
	Forward-SNP	5′-AGATGGGTTAGCAACA**a**G-'3		
	Forward-WT	5′-AGATGGGTTAGCAACA**G**G-'3		
	Reverse	5′-GAATAGCACCTGTCAGCG-'3		
pstB	WT sequence	GATAAATCAATCTTAGACCA	30 cycles of 95°C for 3 min, 45 s; 50°C for 30 s; 72°C for 1 min (final cycle: 5 min)	287
	Forward-SNP	5′-GATAAATCAATCTTAGA**T**A-'3		
	Forward-WT	5′-GATAAATCAATCTTAGA**T**CA-'3		
	Reverse	5′-CGTGAGGCTTGCTGCATTGAG-'3		

*SNP, single-nucleotide polymorphism; WT, wild-type.
†Forward primers were designed to detect either the targeted SNP ($M1_{UK}$) or WT ($M1_{global}$) sequences.
‡Lowercase bold letters in primer sequences denote the base complementary to the targeted SNP in the $M1_{UK}$ sequence. Underlined uppercase letters indicate an additional mismatched base introduced into primer sequences to increase primer specificity.

S. pyogenes strains isolated during 2017–18 and collected by the infection bioresource at Imperial College. We artificially enriched the isolates for $M1_{global}$ strains to ensure adequate numbers of each lineage: 8/27 isolates were $M1_{global}$, and 19/27 were $M1_{UK}$. PCR amplification of *rofA* and *pstB* alleles from those isolates assigned 100% of strains to the correct lineage previously identified by sequencing (Appendix Table 1).

To evaluate the ability of AS-PCR to identify *emm*1 isolates from $M1_{global}$, $M1_{UK}$, and intermediate sublineages (*4*), we tested 16 strains from 2013–2016 that comprised 4 isolates each of $M1_{global}$, $M1_{13snps}$, $M1_{23snps}$, and $M1_{UK}$ lineages (Appendix Table 2). SNPs were correctly detected in the *rofA* gene from all $M1_{13snps}$, $M1_{23snps}$, and $M1_{UK}$ isolates (Appendix Table 3). SNPs were also correctly detected in *gldA* from all $M1_{23snps}$ and $M1_{UK}$ isolates but not $M1_{global}$ or $M1_{13snps}$ isolates. Finally, SNPs in *pstB* were only identified in $M1_{UK}$ isolates. Thus, in all cases, SNP profiles determined by AS-PCR were consistent with strain-specific genome sequences.

In England, submission of all isolates from invasive infection is requested by the UK Health Security Agency reference laboratory for *emm* genotyping. *emm*1 isolates are routinely the dominant genotype among invasive sterile-site isolates, typically representing 20%–30% of invasive infections. During 2020, when incidence of common respiratory infections was reduced by COVID-19–related public health interventions, *emm*1 *S. pyogenes* frequency varied each month from 0%–24% of all invasive infections and decreased toward the end of the year. We subjected all 305 invasive *emm*1 *S. pyogenes* isolates from 2020 that were available for this study to AS-PCR (Appendix Table 4). AS-PCR identified $M1_{UK}$-specific SNPs in *rofA*, *gldA*, and *pstB* in 278/305 (91.1%) of isolates, which were, therefore, assigned to the $M1_{UK}$ lineage. No SNPs were detected in the remaining 27 isolates, which were assigned to $M1_{global}$; no intermediate lineage *emm*1 strains were identified in isolates collected during 2020 by using AS-PCR.

We performed Western blot analysis of 10 $M1_{UK}$ isolates identified by AS-PCR. We confirmed that SpeA production was similar to $M1_{UK}$ strains tested previously; however, we did not quantify SpeA production.

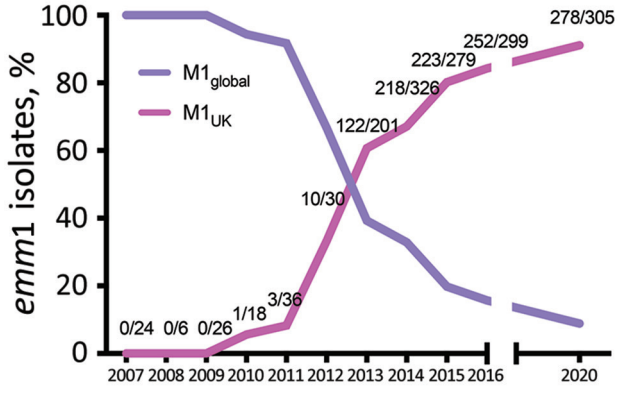

Figure 2. Prevalence of $M1_{UK}$ and $M1_{global}$ *Streptococcus pyogenes* lineages over time in study of emerging invasive group A *Streptococcus* $M1_{UK}$ lineage detected by allele-specific PCR, England, 2020. We determined percentages of *emm*1 isolates in England that belonged to $M1_{UK}$ or $M1_{global}$ lineages by using all available *emm*1 *S. pyogenes* genome sequences for 2007–2016 (*4*) and all available invasive isolates from 2020 that we tested by allele-specific PCR. Numbers on graph indicate number of isolates assigned as $M1_{UK}$/total number sequenced for each year. Graph was adapted and updated from data previously described (*4*).

Conclusions

The longevity of emergent *S. pyogenes* lineages in a population is difficult to predict. Although an $emm89_{emergent}$ acapsular lineage has disseminated globally (*11*), an emergent *emm*3 SpeC-producing lineage, associated with upsurges in scarlet fever and invasive infections, ceased to be detectable within a few years (*12*). Taken together with previously reported genome-sequenced *emm*1 isolates (Figure 2), AS-PCR results indicated that the $M1_{UK}$ lineage continued to expand among invasive *S. pyogenes* isolates from 2016 to the end of 2020 in England.

Increased invasive GAS activity in several countries (*1*) indicates a need for ongoing surveillance of novel lineages, given the potential public health effects. AS-PCR provides a readily available method to detect $M1_{UK}$ that is straightforward and, for screening purposes only, can be simplified by using only *rofA* primers to identify $M1_{UK}$ or associated sublineages. A limitation of our study is that the assay requires validation in reference laboratory settings. AS-PCR does not replace genome sequencing as the preferred method for surveillance of highly pathogenic bacteria, but sequencing is not widely available and is expensive.

*emm*1 strains have accounted for >50% of invasive infections in children in England during the 2022–23 season (*13*). Our results indicate that the $M1_{UK}$ lineage remained dominant in England and expanded to the end of 2020, and contact tracing in 2018 demonstrated a high frequency of secondary acquisition of $M1_{UK}$ in school outbreak settings (*14*). Given the recognized association between *emm*1 *S. pyogenes* and fatal outcome of invasive infections (*15*), enhanced surveillance for the $M1_{UK}$ sublineage is warranted. We conclude that AS-PCR is a readily available method to determine whether *emm*1 *S. pyogenes* isolates belong to the $M1_{UK}$ clade without need for genome sequencing and will improve surveillance of invasive GAS strains.

Acknowledgments

We thank the National Institute for Health Research (NIHR) Imperial Biomedical Research Centre (BRC); NIHR Health Protection Research Unit (HPRU) in Healthcare-associated Infection and Antimicrobial Resistance at Imperial College London; Medical Research Council Centre for Molecular Bacteriology and Infection; scientific and biomedical staff from the NIHR BRC Colebrook laboratory, NIHR HPRU, NIHR BRC Imperial Genomics Facility, and Northwest London Pathology/Imperial College Healthcare Trust Diagnostic laboratory for contributing to the collection, biobanking, typing, and sequencing of bacterial isolates; and staff at the UK Health Security Agency reference laboratory who genotyped *emm*-invasive isolates used in this study.

This study was funded by UK Research and Innovation Medical Research Council (MR/P022669/1) and NIHR Imperial Biomedical Research Centre.

About the Author

Dr. Zhi is a postdoctoral research associate at Imperial College London. Her research focuses on pathogenesis and vaccine prevention of *Streptococcus pyogenes* disease.

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Address for correspondence: Shiranee Sriskandan, Section of Adult Infectious Diseases, Department of Infectious Disease, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London, W12 0NN, UK; email: s.sriskandan@imperial.ac.uk