Detection of Epidemic Scarlet Fever Group A Streptococcus in Australia

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Sentinel hospital surveillance was instituted in Australia to detect the presence of pandemic group A Streptococcus strains causing scarlet fever. Genomic and phylogenetic analyses indicated the presence of an Australian GAS emm12 scarlet fever isolate related to United Kingdom outbreak strains. National surveillance to monitor this pandemic is recommended.

Keywords. Streptococcus pyogenes; scarlet fever; outbreak; superantigen.

Epidemic scarlet fever caused by the gram-positive bacterial pathogen Streptococcus pyogenes (group A Streptococcus [GAS]) resulted in significant childhood disease in the 19th and early 20th centuries, gradually abating in parallel with improved living conditions, enhanced standards of care, and the advent of antibiotics. By the beginning of this millennium, scarlet fever was considered a disease of negligible concern [1]. Yet, epidemic scarlet fever has been reported in Hong Kong [2, 3] and mainland China [4, 5] since 2011, and in the United Kingdom (UK) since 2014 [6–8] (Supplementary Figure 1). GAS isolates from these outbreaks have been multiclonal, encompassing several GAS emm types including emm1, emm12 (UK, Hong Kong, mainland China), emm3, and emm4 (UK), and frequently encoding superantigens SpeA, SpeC, and SSA. Scarlet fever is a notifiable disease in Hong Kong, mainland China, and the UK. However, many national public health systems, including that of Australia, do not require scarlet fever case notification, making global tracking of epidemic strains intrinsically difficult. In 2016 we established a sentinel hospital surveillance (described in the Supplementary Information) to monitor the importation of GAS isolates causing epidemic scarlet fever into Australia. GAS isolates from 8 confirmed cases of scarlet fever (between 2016 and 2017) were selected for further analysis (Supplementary Figure 2; Supplementary Table 1; Supplementary Information).

The GAS emm sequence type was determined and isolates were screened by polymerase chain reaction (PCR) for the presence of genes encoding superantigens ssa, spec, speA, and the erythromycin resistance gene ermB (recently associated with scarlet fever isolates from Hong Kong and mainland China) (described in the Supplementary Information). This preliminary PCR analysis identified the atypical presence of the ssa gene [9] in an Australian emm12 GAS isolate (designated SP1336) causing scarlet fever (Supplementary Figure 2C; Supplementary Table 1; Supplementary Information).

The complete genome sequence of SP1336 was determined using a multiplexing approach incorporating sequence data derived from short-read (Illumina), long-read (Pacific Bioscience and Oxford Nanopore), and PCR-derived capillary sequencing. This approach (described in the Supplementary Information) resolved ambiguity within the SP1336 genome due to the presence of multiple prophages that affected the provisional genome assembly. After several rounds of assembly validation, we determined that SP1336 contained a 1 878 827-bp circular genome (Figure 1; GenBank accession number CP031738).

Phylogenetic analysis of the 248 emm12 genomes relative to the benchmark Hong Kong scarlet fever isolate HKU16 [2] revealed general geographical segregation of scarlet fever isolates from Hong Kong, mainland China, and the UK as reported previously (Figure 1C) [4]. SP1336 shared a close evolutionary relationship (17 core genome single-nucleotide polymorphisms) to a subclade of isolates from the UK that included clinical scarlet fever isolates. This clade did not share a recent evolutionary history with 2 other Australian emm12 strains isolated from non–scarlet fever cases in the 1990s, indicating that they have evolved independently. Instead, these data suggest an evolutionary relationship between scarlet fever cases in the UK and this scarlet fever isolate from Australia.

One of the defining clinical features of scarlet fever is the cutaneous rash believed to be driven by potent immunostimulatory toxins such as the GAS streptococcal pyrogenic exotoxins.
Figure 1. Population genomics of the Australia scarlet fever strain SP1336. A, Genome ring of the SP1336 emm12 group A Streptococcus (GAS) genome showing (from inner ring): GC skew, GC plot, position and name of prophage elements (red blocks), and location and orientation of coding sequences. B, Genomic organization of ΦSP1336.3 and ΦSP1336.1 relative to their closest genetic relatives; ΦHKU.ssa (from an emm12 Hong Kong scarlet fever strain HKU360 [3]) and ΦMGAS10750 (from an emm14 pharyngitis isolate from the United States [10]), respectively. Nucleotide sequence diversity is scaled from 100% (black) to 80% (yellow). C, Midpoint-rooted maximum-likelihood phylogenetic tree of 248 emm12 GAS strains built on 2633 polymorphic sites within the nonrecombinogenic core genome relative to the HKU16 reference isolate [2]. Clinical association and country of isolation are displayed next to the tree in addition to the relative distribution of selected virulence genes, and antimicrobial resistance genes and ssa-carrying prophages (ΦHKU.vir [2], ΦHKU.ssa [3], and ΦSP1336.3) within the emm12 population (black shading refers to gene carriage). Phylogenetic branch relating to the Australian scarlet fever isolate SP1336 is colored in green and indicated by an arrow for visual aid. Abbreviation: SNP, single-nucleotide polymorphism.
The emergence of major scarlet fever clades in China has been linked to the acquisition of SSA-, SpeC-, and Spd1-encoding prophages [3]. Analysis of the prophage content of the SP1336 genome revealed 4 prophage elements designated ΦSP1336.1 (harboring the exotoxin SpeC and the DNase, Spd1), ΦSP1336.2 (toxins SpeH and SpeI), ΦSP1336.3 (superantigen SSA), and ΦSP1336.4 (strepodornase, SdaD2) (Figure 1A). This emm12 prophage profile is similar to that found in the ongoing scarlet fever outbreaks in the UK, Hong Kong, and mainland China [3, 4]. Comparative analyses of the ΦSP1336.3 and other ssa-harboring prophages from Hong Kong and mainland China showed that ΦSP1336.3 is approximately 95% identical at the nucleotide level to the ssa prophage ΦHKU.ssa from the Hong Kong scarlet fever strain HKU360 (Figure 1B) [3]. ΦSP1336.3 also shared a high degree of synteny with ΦSP1336.1 (~90%), itself approximately 99% nucleotide identity to prophage ΦMGAS10750.1 from a pharyngitis patient in the United States (Figure 1B) [10]. No multidrug resistance genes or transposable elements were identified in the SP1336 isolate, commensurate with the evolutionary-related emm12 UK lineage.

Scarlet fever cases have risen in multiple countries since 2011 (Supplementary Figure 1) [2–8]. Rather than subsiding, case numbers of scarlet fever have again significantly increased in recent years both in Hong Kong (Supplementary Figure 1A) and the UK [11]. The burden of this outbreak on healthcare services is substantial with hospital admissions from scarlet fever increasing 3-fold from baseline at the peak of the outbreak [8]. We have become increasingly concerned about the spread of scarlet fever–inducing GAS to countries where this disease is not notifiable, and thus instigated our own localized notification system in Australia. Here we report the detection of an emm12 GAS strain in Australia that harbors scarlet fever–associated phage-like elements [3, 4] and shares a recent common ancestor to a cluster of scarlet fever isolates from the UK. The presented data highlight the dynamic nature of toxin-harboring prophages within polylysogenic GAS genomes. Furthermore, the maintenance of distinct virulence gene sets (SSA, SpeC, Spd1, SpeH, SpeI) suggests a positive selection for particular toxin combinations within the current scarlet fever pandemic.

Outbreaks of scarlet fever in the UK, Hong Kong, and mainland China have been characterized as multiclonal, encompassing multiple emm types [2–4, 6–8]. While northern Asian and UK emm12 scarlet fever isolates are distantly related, these geographically discrete strains have evolved independently into distinct clades [3, 4], excluding the worldwide spread of a single emm12 scarlet fever clone. GAS superantigens SpeA, SpeC, and Ssa have been associated with scarlet fever isolates in several studies, but this association is not universal [2–4, 6]. The underlying cause of disease resurgence remains unknown. Immune status changes in the human population resulting in increased susceptibility to infection, introduction of genetic elements encoding superantigens into the GAS population, coinfection with an unknown agent that predisposes the host to scarlet fever, and environmental change have all been proposed as possible triggers for the observed resurgence in disease [12]. While a single dominant strain fails to explain the recent international upsurge in scarlet fever, the distant identification of a strain associated with the UK outbreak may herald similar outbreaks elsewhere. In national health systems where scarlet fever is not notifiable, sentinel hospital surveillance of the type used in this study to rapidly identify and monitor the dissemination of GAS isolates causing epidemic scarlet fever is warranted. Such surveillance would underpin public health interventions aimed at limiting further propagation.

Supplementary Data
Supplementary materials are available at Clinical 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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