Detection of epidemic scarlet fever group A *Streptococcus* in Australia

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Abstract

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 UK outbreak strains. National surveillance to monitor this pandemic is recommended.

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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]. From 2011, epidemic scarlet fever has been reported in Hong Kong [2, 3], mainland China [4, 5] and in the United Kingdom since 2014 [6-8] (Figure S1). 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 Supplementary Information) to monitor the importation of GAS isolates causing epidemic scarlet fever into Australia. GAS isolates from eight confirmed cases of scarlet fever (between 2016 and 2017) were selected for further analysis (Figure S2, Supplementary Table 1; Supplementary Information). The GAS *emm* sequence type was determined and isolates were screened by 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 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 (Figure S2C, Supplementary Table 1, Supplementary Information).

The complete genome sequence of SP1336 was determined using a multi-platform 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 Supplementary Information) resolved ambiguity within the SP1336 genome due to the presence of multiple related prophages that affected the provisional genome assembly. After several rounds of assembly validation, we determined that SP1336 contained a 1,878,827 base pair circular genome (Figure 1, Genbank accession number CP031738).

Phylogenetic analysis of the 248 *emm*12 genomes relative to benchmark Hong Kong scarlet fever isolate HKU16 [2] revealed general geographical segregation of scarlet fever isolates from Hong Kong and mainland China, and the UK as reported previously (Figure 1C) [4]. SP1336 shared a close evolutionary relationship (17 core genome SNPs) to a sub-clade of isolates from the UK that included clinical scarlet fever isolates. This clade did not share a recent evolutionary history with two other Australian *emm*12 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 pyogenic exotoxins. The emergence of major scarlet fever clades in China has been linked to the acquisition of SSA, SpeC and Spd1 encoded prophages [3]. Analysis of the prophage content of the SP1336 genome revealed four 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 (streptodornase, SdaD2) (Figure 1A). This *emm*12 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 ~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 ~99% nucleotide identity to prophage ΦMGAS10750.1 from a US pharyngitis patient (Figure 1B) [10]. No multidrug resistance genes or transposable elements were identified in the SP1336 isolate, commensurate with the evolutionary-related *emm*12 UK lineage.

Scarlet fever cases have risen in multiple countries since 2011 (Figure S1) [2-8]. Rather than subsiding, case numbers of scarlet fever have again significantly increased in recent years both in Hong Kong (Figure S1A) and the UK [11]. The burden of this outbreak on healthcare services is substantial with hospital admissions from scarlet fever increasing threefold 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-harbouring prophages within poly-lysogenic 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, co-infection 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.

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Conflict of interest statement

The authors declare no conflicts of interest.

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Figure legends

Figure 1. Population genomics of the Australia scarlet fever strain SP1336. (A) Genome ring of the SP1336 *emm*12 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 *emm*12 Hong Kong scarlet fever strain HKU360 [3]) and ΦMGAS10750 (from an *emm*4 pharyngitis isolate from the USA [10]) respectively. Nucleotide sequence diversity is scaled from 100% (black) to 80% (yellow). (C) Midpoint-rooted maximum-likelihood phylogenetic tree of 248 *emm*12 GAS strains built on 2,633 polymorphic sites within the non-recombinogenic core genome relative to the HKU16 reference isolate [2]. Clinical association and country of isolation is 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 *emm*12 population (black shading refers to gene carriage). Phylogenetic branch relating to the Australian scarlet fever isolate SP1336 is coloured in green and indicated by an arrow for visual aid.