Medicine

UNIVERSITY OF Southampton

Whole genome sequencing of carriage and disease isolates of *Streptococcus pneumoniae* serotype 22F reveals lineage specific divergence and niche adaptation

Cleary DW[1,2], Devine VT[1], Jefferies JMC[1,2], Webb JS[2,3,4], Bentley SD[5], Gladstone RA[5], Faust SN[1,3,6], and Clarke SC[1,2,3]

1. Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, UK 2. Institute for Life Sciences, University of Southampton, UK 3. Southampton NIHR Respiratory Biomedical Research Unit, University Hospital Southampton Foundation NHS Trust, 4. Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, UK 5. Pathogen Genomics, Wellcome Trust Sanger Institute, UK, 6. NIHR Southampton Wellcome Trust Clinical Research Facility, University Hospital Southampton Foundation NHS Trust, UK

Introduction

Streptococcus pneumoniae is a Gram positive commensal of the human nasopharynx. A highly recombinogenic bacterium, there are >90 known serotypes as defined by antisera cross-reactivity with the capsular polysaccharide. It is a leading global cause of pneumonia, bacteraemia and meningitis. Invasive pneumococcal disease (IPD) exhibits high levels of mortality and morbidity (1.6 million deaths per year globally), particularly in children under five years of age (O'Brien *et al.*, 2009).

IPD, Carriage and 22F

•Epidemiology has been driven by the introduction of vaccines PCV7 (2006) and PCV13 (2010). Vaccine serotype IPD reduced as a consequence.
•22F highlighted as a increasing serotype in carriage (our unpublished data).
•22F exhibited the largest proportional increase in IPD from 2010 (4.37 %) to 2011 (6.23 %) and in 2012 was the fifth most common serotype observed in IPD (7.4 % of cases, n=963) (ECDC, Surveillance of Invasive Bacterial Diseases in Europe 2012).

Aims

To compare the genomes of pairs of 22F serotypes from two STs (ST433 and ST698) isolated from cases of IPD or carriage.

Methods

Isolates were sequenced using 454™ 8kb and MiSeq 2x250 (V2) paired-end chemistry. Assemblies were done using MIRA 4.0.2 and annotated with PROKKA 1.10. MLST and virulence gene discovery was done using SRST2 v0.1.3. Genome comparisons were undertaken using the sequence comparison tool in RAST and the BLAST Ring Image Generator (BRIG). Core genome SNP phylogeny was constructed using Wombac with resultant trees visualised using FigTree. Breseq was used to identify non-synonomous mutations between the carriage and disease isolates.

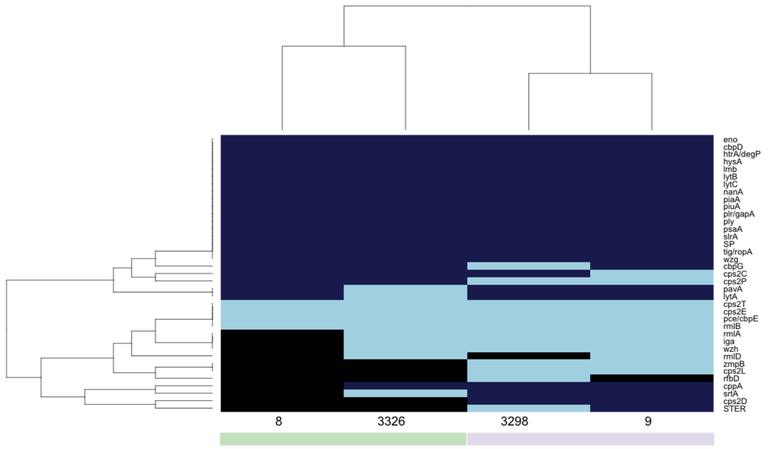

Figure 1: Distribution of common virulence genes found in *S. pneumoniae*. Key: Dark blue – known allele, light blue – novel allele (10 % sequence dissimilarity to known allele), black – not identified.

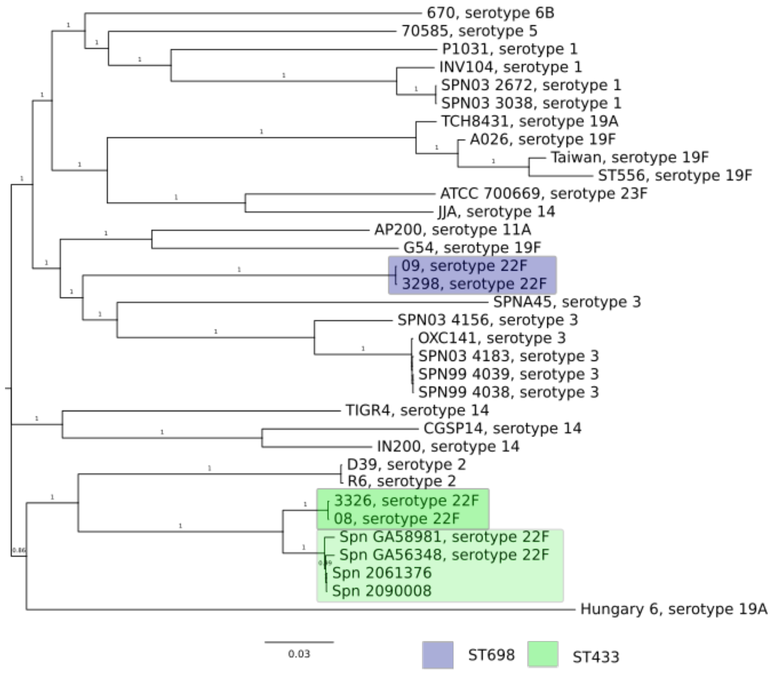

Figure 2: Maximum likelihood neighbor-joining tree showing phylogenetic placement of the serotype 22F isolates of ST698 (purple) and ST433 (green).

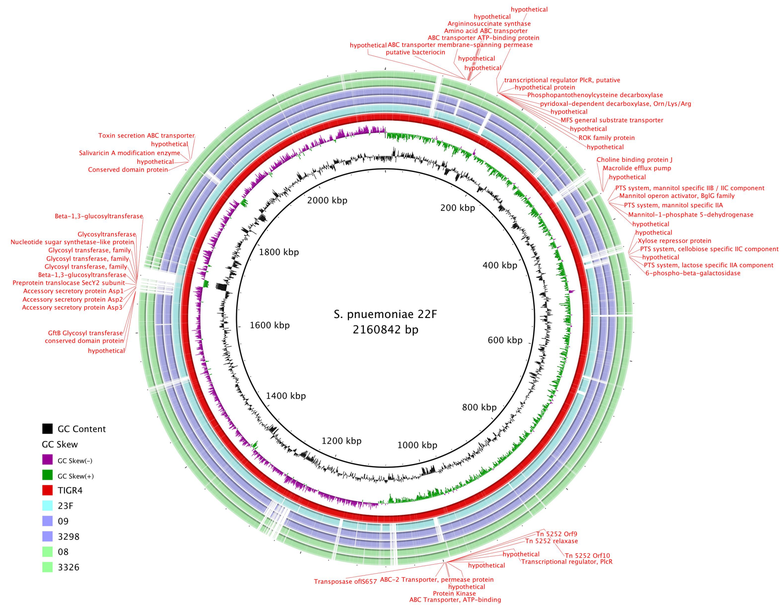

Figure 3: Genome comparisons of four *S. pneumoniae* serotype 22F isolates against TIGR4 and ATCC70669 23F.

Results

Distribution of virulence genes is shown in Fig. 1. Phylogeny is shown in Fig. 2. Overall topology is in agreement with previous findings (Donati *et al.*, 2010). Genome comparisons are shown in Fig. 3. Contrasting gene repertoires included bacteriocins, lantibiotics, and toxin-antitoxin systems. Non-synonymous mutations in *pgdA* (peptidoglycan N-acetylglucosamine deacetylase A) were found in the carriage isolates. Deacetylation of peptidoglycan is known to enable resistance to lysozyme upon invasion. Intact prophage, in addition to numerous additional phage insertions, were also seen in the carriage isolate of ST433 (Cleary *et al.*, *in press*).

Conclusions

IPD remains a significant global challenge. Serotype replacement means it is vital to explore the genome repertoire of emerging serotypes of clinical significance.

References: O'Brien *et al.*, (2009). Lancet 374: 893–902; ECDC, http://ecdc.europa.eu (2012); Donati *et al.*, (2010) Genome Biology 11:R107; Cleary *et al.*, (2016) GBE, *in press*.

d.w.cleary@soton.ac.uk @bacterioskeptic