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Short communication

Genomic investigation of a *Streptococcus pneumoniae* serotype 24F strain causing meningoencephalitis in Hong Kong

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ABSTRACT

Pneumococcal conjugate vaccines (PCVs) successfully decreased the incidence of invasive pneumococcal disease in children. However, many countries have reported serotype replacement and a rebound in diseases from non-vaccine serotypes. Here, we report the genomic investigation of a *Streptococcus pneumoniae* strain M215 that caused severe meningoencephalitis in an infant in 2019. The strain was assigned to serotype 24F using the bioinformatic pipeline SeroBA and pneumococcal type specific anti-sera. The strain was resistant to cotrimoxazole from mutations in both folA and folP genes. It was susceptible to penicillin and other non-β-lactam antibiotics. Phylogenetically, it belongs to Global Pneumococcal Sequence Cluster (GPSC) 6 and multi-locus sequence type 162. A total of 38 virulence genes were detected in the genome of M215. Upon comparison of the profile of virulence genes, GPSC6 but not non-GPSC6 strains of serotype 24F and related serotypes were found to possess the major virulence determinant, pilus islet-1, comprising genes encoding sortases (*srtB, srtC, srtD*), pilus proteins (*rrgA, rrgB* and *rrgC*) and one transcriptional regulator (*rlrA*), which was previously described to be characteristic feature of international clones in the pre-PCV era. In our locality, this represented the first detection of serotype 24F and GPSC6/ST162 causing serious pneumococcal disease. The emergence of the non-vaccine serotype 24F GPSC6/ST162 lineage with molecular feature of high virulence is concerning and emphasizes the need for full characterization of strains causing severe disease.

1. Introduction

Pneumococcal meningitis is the most severe complication of invasive pneumococcal disease (IPD). Although antibiotics are highly effective in killing the bacteria, morbidity and mortality from pneumococcal meningitis remains high because of neuronal damage from the infection and inflammatory process. In 2015, it was estimated that pneumococcal meningitis accounted for 2% of all IPD and 12% of pneumococcal deaths globally (Wahl et al., 2018). Pneumococcal conjugate vaccines (PCVs) have significantly reduced the incidence of IPD and pneumococcal meningitis caused by vaccine serotypes (Garcia et al., 2021; Wahl et al., 2018). However, a relative increase in pneumococcal diseases caused by non-vaccine serotypes has been observed (Hulten, 2018). In countries using the 10-valent or 13-valent PCVs (PCV10/13) for 5–7 years with primary series uptake above 70%, the top serotypes are five (15B/C, 8, 12F, 10A and 2F) included in higher-valency PCVs under evaluation as well as three others (24F, 23B and 23A) not in any known investigational products (Garcia et al., 2021). Nonetheless, significant variations in the leading serotypes were observed in different countries and areas, highlighting the importance of ongoing surveillance (Garcia et al., 2021).

In Hong Kong, PCV13 has been widely used in infant since 2011 with uptake above 97% (Ho et al., 2019). Furthermore, a monitoring system has been introduced since 2015 to actively monitor changes in the IPD incidence and the serotype distribution. Although serotype 3 is targeted by PCV13, the relative prevalence of serotype 3 remained high in our locality with 60% of the IPD in age < 5 years caused by this serotype in recent years (Ho et al., 2019). Other top IPD serotypes included 19A,

Abbreviations: GPSC, Global Pneumococcal Sequence Cluster; IPD, invasive pneumococcal disease; PCVs, pneumococcal conjugate vaccines; RD, region of diversity.

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15B/C and 15A (Ho et al., 2019). In the years after PCV13 use, 0–1 case of pneumococcal meningitis annually in children < 5 years were notified to the Department of Health with 15B/C as the top serotypes. Recently, we encountered a case of severe pneumococcal meningitis in an infant. Using multiplex PCR assays and slide agglutination test, the isolate could not be assigned to any of the serotypes commonly encountered in our locality (Ho et al., 2011, 2019; Iovino et al., 2020). In view of this and the severity of the pneumococcal disease in the patient, the isolate was further characterized by whole genome sequencing in this report.

2. Methods

2.1. Patient description

In 2019, a previously healthy 11 month-old boy was hospitalized with high fever (40 ^{o}C) and vomiting for one day. Several hours after hospitalization, he was noted to have seizures with varying flaccid and hypertonic limbs, and erratic breathing. His vaccination history was up to date according to the recommended immunization schedule, including two doses of PCV13 at 2 month and 4 month of age. Blood tests were significant for leukocytosis (19.3 $\times 10^{9}$/L, 75% neutrophils) and a low sodium level (128 mmol/L, normal range 136–148 mmol/L). Empirical treatment with intravenous vancomycin and cefotaxime was started for suspected meningitis. Patient developed shock with unequal pupils and was intubated for mechanical ventilation in the intensive care unit. Emergency brain imaging revealed cerebral edema and other features consistent with bacterial meningoencephalitis (Supplementary file 1, Fig. S1A and S1B). External ventricular catheter was inserted for drainage of cerebrospinal fluid (CSF) showing glucose 3.7 (normal range 2.2–3.9 mmol/L), protein 0.21 (normal range, 0.12–0.60 g/L) and increased leukocytes (35 cells/μL, 58% neutrophils, 34% lymphocytes, 8% monocytes). Gram stain of the CSF was negative and no growth was obtained from the CSF which was collected after initiation of antibiotics. Further multiplex PCR of the CSF using BIOFIRE FILMARRAY meningitis/encephalitis panel (bioMérieux, France) was positive for *S. pneumoniae*. PCR results for other pathogens in the panel were all negative. On day 2 after hospitalization, blood culture was positive for *S. pneumoniae*. Vancomycin was discontinued after the antimicrobial susceptibility of the isolate is known. Following treatment, patient's condition improved. However, bilateral subdural collections with pressure effect were demonstrated in brain imaging (Supplementary file 1, Fig. S1C) on day 27 after hospitalization. Upon advice from neurosurgery, it was managed conservatively. Cefotaxime was continued based on a diagnosis of pneumococcal meningoencephalitis and bacteremia. Brain imaging was repeated on hospitalization day 34 showing persistent subdural collections suspicious of subdural empyemas, encephalomalacia and atrophy in the left frontal and parietal lobes, and dilatation of the posterior horns of the lateral ventricles (Supplementary file 1, Fig. S1D). The duration of cefotaxime was extended to 6 weeks to cover the possibility of subdural empyema. Patient was discharged on Day 47. Assessment before discharge showed severe neurological sequelae including generalized spasticity, right facial nerve palsy and impaired response to light and sound.

2.2. Microbiological studies

Only one serotype was detected in the blood culture. Culture of the CSF was negative. The *S. pneumoniae* isolate from blood culture was cultured on blood agar plate and was identified by a Bruker MALDI-TOF system, bile solubility and optochin susceptibility. Antimicrobial susceptibility was determined using Etest strips (BioMerieux Inc., USA) (penicillin and cefotaxime) and the CLSI's disc diffusion methods (erythromycin, clindamycin, cotrimoxazole and vancomycin). Pneumococcal factor antisera from the Statens Serum Institut (SSI), Denmark were used for differentiation of types within serogroup 24 at the Public Health Laboratory Services Branch of the Centre for Health Protection, Department of Health.

2.3. Whole genome sequencing and bioinformatics

An isolate (M215) from the blood culture of the patient was sequenced using a NovaSeq platform at Novogene Co., Ltd, Beijing, China at 500 folds coverage. SPAdes v3.14 was used for de novo assembly and further improved using a Sanger pipeline (Bankevich et al., 2012; Page et al., 2016). Annotations of the genome were done using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016). Qualified reads were used to predict the serotype of the strain using SeroBA (Epping et al., 2018). All genomes which are deposited at GenBank and assigned to serogroup 24 referring to the 'Serovar' adopted by Pathogen Detection project (https://www.ncbi.nlm.nih.gov/pathogens/) were included in the present study. Serotypes of these reference genomes were also confirmed using PathogenWatch (https://pathogen.watch/). Multilocus sequence typing (MLST) and Global Pneumococcal Sequencing Cluster (GPSC) were predicted for all genomes with PathogenWatch. In total, 90 genomes of serogroup 24 were combined to call SNPs for phylogenetic analysis and acquired and chromosomal antibiotic resistance genes were predicted using methods described previously (Cao et al., 2019). Additionally, non-β-lactam resistance determinants including those affecting aminoglycosides, cotrimoxazole, fluoroquinolones, macrolides, rifampin, streptothricins, tetracyclines and vancomycin were queried using a previously described pipeline (Metcalf et al., 2016). Penicillin-binding protein (PBP) types and MICs of β-lactam were predicted using one machine learning-based tool with PathogenWatch (Li et al., 2016). The virulence factors were identified using blastp against The Virulence Factor Database (VFDB) (Liu et al., 2019). Additionally, a customized pneumococcal virulence gene database was constructed based on recent reviews on choline binding proteins (CBPs), lipoproteins, LPXTG proteins, non-classical surface proteins (NCSPs) and proteases (Gámez et al., 2018; Marquart, 2021; Pérez-Dorado et al., 2012). These proteins were retrieved from TIGR4 (AE005672.3) and RD6 (AE007317.1). Proteins of all genomes involved in the present study were blast against the customized database with 1e-5 of e-value, 80% identity and 70% coverage as cut-off. Blast results were parsed using in-house script and summarized. The genes defined in regions of diversity (RDs) including RD1-RD13 were extracted from TIGR4 to compare with M215 using blast with 1e-5 of e-value, 80% identity and 70% coverage as cut-off (Obert et al., 2006). The boundaries of RDs in M215 were delineated referring to the blast results. Genes or pathogenicity islands that have been well characterized includes ZmpC (RD1), capsular operon (RD3), pilus islet-1 (PI-1) (RD4), pneumococcal pathogenicity island 1 (PPI-1) (RD6) and PsrP-secY2A2 pathogenicity island (RD10) (Embry et al., 2007). Two key proteins PitA, PitB of pilus islet-2 (PI-2) were involved in the customized pneumococcal surface protein database to confirm that the PI-2 is absent in all strains used in the present study. The RDs in M215 were extracted from genomes and aligned to visualize using Easyfig (Sullivan et al., 2011).

The genome sequence of strain M215 has been deposited in the GenBank under Bioproject PRJNA742224.

3. Results

The isolate was found to be susceptible to penicillin (MIC <0.016 μg/ml) and cefotaxime (MIC <0.016 μg/ml). Disc tests showed resistance to cotrimoxazole and susceptibility to erythromycin, clindamycin and vancomycin.

The genome of strain M215 was assembled. It has a size of 2,083,026 bp and GC content of 39.52%. Blast search revealed that it shared the highest average nucleotide identity (ANI) (99.92%) with the reference *S. pneumoniae* strain ERS518708 (GCA_900193945.1). Phylogenetic and MLST analysis revealed that our strain belongs to GPSC6 and ST162 (allelic profile, *aroE-gdh-gki-recP-spi-xpt-ddl*, 7-11-10-1-6-8-14). It is

most closely related to six strains originating from the Netherlands (n = 3) and United Kingdom (n = 3) in 2009–2013 (Fig. 1). The penicillin binding proteins PBP1a-PBP-2b-PBP2x profile is 2-0-0 with a predicted penicillin MIC ≤ 0.03 μg/ml (susceptible). Mutations in two genes including the *folA* (I100L substitution) and *folP* (two codon insertions), correlating with cotrimoxazole resistance were detected. No other non-β-lactam resistance determinants were detected in M215.

The capsular polysaccharide gene, *cps* locus in strain M215 was assembled. Blast search showed that the *cps* locus shares 100% identity (at 100% query coverage) with that in the serogroup 24 strain MNY584 (GenBank accession MW683297.1) from Germany in 2017 (Supplementary file 1, Fig. S2). Using SeroBA, strain M215 was predicted to belong to serogroup 24. Upon testing with factor antisera of serogroup 24, strain M215 reacted with 24d but not 24c and 24e, resolving the serotype as 24F.

To gain insight into genetic features associated with virulence in the strain, we query the presence of pneumococcal virulence genes and compared it to those in serogroup 24 pneumococcal isolates of other GPSC lineages with at least 5 strains. In total, 139 virulence genes encoding surface proteins and proteases were detected in our strain of which 38 virulence genes were detected by query using the VFDB (Table 1) and an additional 101 virulence genes were detected using our customized database (Supplementary file 1, Table S1 and Supplementary file 2, Table S2). These include genes encoding 12 CBPs, 45 lipoproteins, 14 LPXTG proteins, 48 proteases, 13 NCSPs, 6 PI-1 proteins and 1 other protein (Supplementary file 1, Fig. S3). Except for the PI-1 cluster, similar numbers of genes by groups were detected in isolates of different GPSC lineages (Supplementary file 1, Table S1). In M215, the pattern of virulence genes in our strain is identical to those in the other GPSC6 strains (Table 1). The major virulence genes that were consistently detected in serogroup 24 strains across different GPSCs include choline-binding proteins (*cbpG, lytA, lytB, lytC pce/cbpE*), lipoproteins (*lmb, psaA, slrA, pia,* and *piuA*), LPXTG proteins (*hysA, iga/zmpA, nanA, pfbA*), proteases (*clpP, cppA, groEL, scpB, srtA, tig/ropA*), NCSPs (*eno, htrA/degP, ply, nanB, pavA, plr/gapA*) and *tuf* (a putative complement regulator binding protein). Several virulence genes (including *cbpA, pclA, psrP, pblA*) were absent in the GPSC6 strains (including M215) but were detected in variable frequencies in the other GPSCs (Table 1). The PI-1 gene cluster was detected in strains of GPSC6 but not the other GPSCs. In strain M215, the pilus islet has a size of

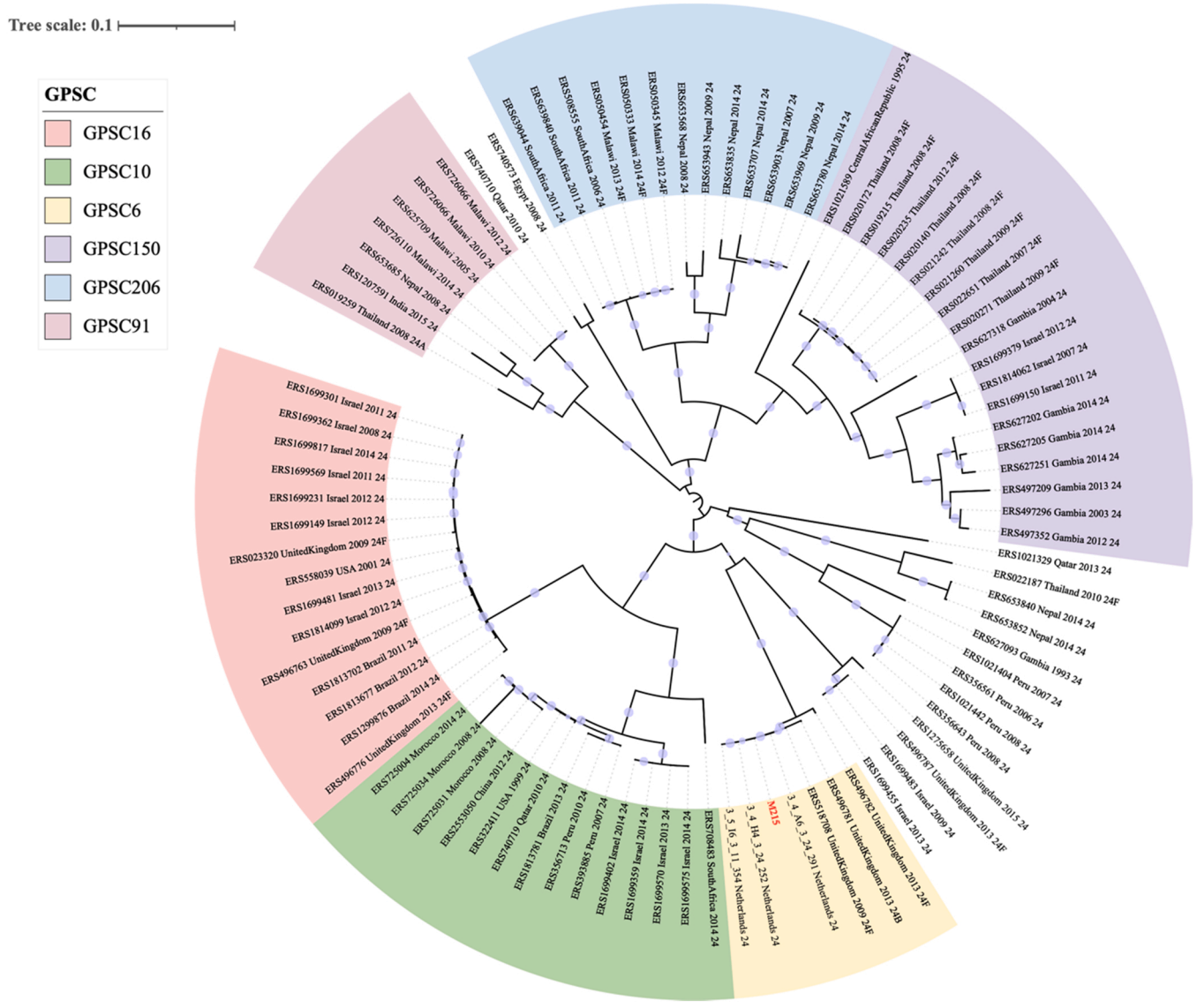

Fig. 1. Maximum likelihood phylogenetic tree of 90 *S. pneumoniae* of serogroup 24 based on total core genome SNP showing short branch lengths. The strain M215 in this study is represented by red font. Each strain is labeled with the name and sequence type (ST). Color shading show strains belonging to GPSC-6, − 10, 91, − 150, and − 206.

Table 1
Selected virulence genes detected in serogroup 24 strains. Heatmap is used to compare presence and absence of gene in strain M215 (orange) in the present study and in serogroup 24 strains of six GPSC lineages (green). Color of the labels on the left panel indicates the groups of the virulence genes: choline binding proteins (yellow), lipoproteins (light green), LPXTG proteins (bisque), proteases (gray), non-classical surface proteins (white), pilus isolaet-I (pink) and other proteins (aqua). Virulence genes detected by query using the VFDB were summarized in this Table. Comparison for the full list of virulence genes is presented in the Supplementary file 2, Table S2.

Protein name or function	Gene	Strain M215	GPSC6 (n=6)	GPSC10 (n=14)	GPSC16 (n=15)	GPSC91 (n=7)	GPSC150 (n=18)	GPSC206 (n=13)
Immunoglobulin A inactivation	*cbpA*	Absent	0%	0%	7%	0%	28%	15%
Choline-binding protein D	*cbpD*	Present	100%	100%	100%	29%	89%	100%
Choline-binding protein G	*cbpG*	Present	100%	100%	100%	100%	100%	100%
Autolysin (N-acetylmuramoyl-l-alanine amidase)	*lytA*	Present	100%	100%	100%	100%	100%	100%
Endo-β-N-acetylglucosaminidase	*lytB*	Present	100%	100%	93%	100%	100%	100%
Lysozyme (1,4-β-N-acetylmuramidase)	*lytC*	Present	100%	100%	100%	100%	100%	100%
Phosphorylcholine esterase	*pce/cbpE*	Present	100%	100%	100%	100%	100%	100%
Pneumococcal surface protein C	*pspC/cbpA*	Present	100%	0%	100%	100%	100%	100%
Pneumococcal surface protein A	*pspA*	Present	100%	0%	0%	71%	33%	100%
Adhesion lipoprotein	*lmb*	Present	100%	100%	100%	100%	100%	100%
Pneumococcal surface adhesin A	*psaA*	Present	100%	100%	100%	100%	100%	100%
Streptococcal lipoprotein rotamase A	*slrA*	Present	100%	100%	100%	100%	100%	100%
Iron-compound ABC transporter	*piaA*	Present	100%	100%	100%	100%	100%	100%
Iron-compound ABC transporter	*piuA*	Present	100%	100%	100%	100%	100%	100%
Hyaluronidase	*hysA*	Present	100%	100%	100%	100%	100%	100%
Inmunoglobulin A1 protease	*iga/zmpA*	Present	100%	100%	100%	100%	100%	100%
Neuraminidase A	*nanA*	Present	100%	100%	100%	100%	100%	100%
Pneumococcal adherence and virulence protein B	*pavB/pfbB*	Present	100%	100%	100%	100%	100%	100%
Pneumococcal collagen-like protein	*pclA*	Absent	0%	36%	93%	86%	78%	100%
Plasmin and fibronectin-binding protein A	*pfbA*	Present	100%	100%	100%	100%	100%	100%
Pneumococcal serine-rich repeats protein	*psrP*	Absent	0%	0%	7%	0%	0%	0%
Zinc metalloprotease	*zmpB*	Present	100%	0%	0%	29%	44%	15%
Clp protease	*clpP*	Present	100%	100%	100%	100%	100%	100%
C3-degrading protease	*cppA*	Present	100%	100%	100%	100%	100%	100%
Major outer membrane protein	*groEL*	Present	100%	100%	100%	100%	100%	100%
C5a peptidase	*scpB*	Present	100%	100%	100%	100%	100%	100%
Housekeeping sortase	*srtA*	Present	100%	100%	100%	100%	100%	100%
Trigger factor	*tig/ropA*	Present	100%	100%	100%	100%	100%	100%
Enolase	*eno*	Present	100%	100%	100%	100%	100%	100%
High temperature requirement A, serine protease	*htrA/degP*	Present	100%	100%	100%	100%	100%	100%
Pneumolysin	*ply*	Present	100%	100%	100%	100%	100%	100%
Neuraminidase B	*nanB*	Present	100%	100%	100%	100%	100%	100%
Pneumococcal adherente and virulence factor A	*pavA*	Present	100%	100%	100%	100%	100%	100%
Glyceraldehyde 3-phosphate dehydrogenase	*plr/gapA*	Present	100%	100%	100%	100%	100%	100%
Pilus-1 tip protein	*rrgA*	Present	100%	0%	0%	0%	0%	0%
Pilus-I backbone protein	*rrgB*	Present	100%	0%	0%	0%	0%	0%
Pilus-1 anchore protein	*rrgC*	Present	100%	0%	0%	0%	0%	0%
Pilus islet-I	*srtC*	Present	100%	0%	0%	0%	0%	0%
Sortase	*srtD*	Present	100%	0%	0%	0%	0%	0%
Pilus islet-I	*srtB*	Present	100%	0%	0%	0%	0%	0%
Complement regulator binding protein	*tuf*	Present	100%	100%	100%	100%	100%	100%
Platelet binding protein	*pblA*	Absent	0%	7%	0%	14%	0%	15%

~11 kb and is flanked by two IS*1167* elements. It contained an array of 7 genes in the same order as in *S. pneumoniae* TIGR4 (GenBank accession AE005672) with an average sequence identity of 98.57% (Supplementary file, Fig. S4). These included the genes encoding sortases (*srtB, srtC, srtD*), one pilus shaft protein (*rrgB*), two minor pilus proteins (*rrgA* and *rrgC*) and one transcriptional regulator (*rlrA*). The PI-2 was absent in M215 and the other serogroup 24 strains. In the customized database analysis, one additional virulence gene (*nanE* encoding an epimerase) was detected in M215/GPSC6 consistently but not the other lineages (non-GPSC6 lineages, 0–14%) (Supplementary file 2, Table S2).

The regions of diversity in M215 were further investigated by alignment with those in TIGR4 as a reference. Five (including RD3, RD4, RD6, RD8 and RD13) of the 13 regions of diversity were detected in strain M215 (Supplementary file 1, Fig. S5). The capsular polysaccharide gene locus, the PI-I and the pneumococcal pathogenicity island-I (PPI-I) was found within RD3, RD4 and RD6, respectively. The genes within RD8 and RD13 in M215 were TIGR-4 like (Supplementary file, Fig. S6). The other eight RDs including RD1 (encoding ZmpC), RD2, RD5, RD7-RD9, RD10 (encoding PsrP-secT2A2 pathogenicity island) and RD11-RD12 were absent in M215.

4. Discussion

The isolate that caused severe pneumococcal infection in a PCV13 vaccinated, healthy 11 months old boy was found to belong to the non-PCV13 serotype 24F and GPSC6/ST162. In our setting, serogroup 24 is rarely (<1%) detected among carriage and invasive isolates before and after introduction of PCV (Ho et al., 2011, 2015, 2019). Among 504

isolates collected in Hong Kong from 1995 to 2001 and 2009–2017 that were sequenced under the Global Pneumococcal Sequencing (GPS) project, only 1 isolate was found to belong to serogroup 24 (24F) but it belonged to a different sequence type (ST8857) (Gladstone et al., 2020). In the phylogenetic analysis, our strain is most closely related to several strains from the Netherlands and United Kingdom. Our patient is the second child of a French family based in Hong Kong. Although there was no history of travel, he may be exposed to pneumococci through close contact with children and adults who had traveled aboard. Person-to-person transmission of pneumococcal carriage among close contacts is well documented (Karppinen et al., 2017).

The serotype of strain M215 was determined using a combination of genome sequencing and typing anti-sera. In serogroup 24, four related subtypes including 24A, 24B, 24C and 24F have been described (Ganaie et al., 2021). Previously, sequence variations in the *abpA*, *abpB* and *wcxG* genes were thought to allow differentiation of the subtypes within serogroup 24 (Bentley et al., 2006). Recent studies, however, have revealed that those sequence variations were not serotype specific (Ganaie et al., 2021). Therefore, subtypes in serogroup 24 that were previously assigned using gene sequences should be interpreted with caution.

In Europe, serotype 24F has been reported as an emerging serotype after PCV use (Hulten, 2018; Kandasamy et al., 2020). In France, a major reduction in IPD incidence was observed following the implementation of PCV13 in 2010 but a rebound in cases caused by non-PCV13 serotypes have been noted since 2015 (Ouldali et al., 2018). In children, the increase was mainly caused by serotype 24F which is recognized to cause serious pneumococcal diseases (Ouldali et al., 2021). In France and Denmark, increase in serotype 24F was reported to be related to the clonal expansion of a ST162 (which belongs to CC156) penicillin-susceptible and cotrimoxazole-resistant lineage (Janoir et al., 2016; Kavalari et al., 2019). Strain M215 is also cotrimoxazole-resistant and our comparison revealed that it shares an identical set of *folA* and *folP* mutations with the other GPSC6/ST162 strains. In Europe, the ST162/serotype 24F clone has been suggested to be related to the PMEN3 clone (Spain^{9V}-CC156) and may arise as a result of capsular switching (Kavalari et al., 2019). In a recent epidemiological analysis, 24 F is one of the serotypes with invasive potential at the upper end of the spectrum (Balsells et al., 2018). Likewise, the clinical course in our patient was severe.

Besides the capsule, the pilus islet-1 in M215 has likely contributed to the severe disease in our patient. The pilus islet-1 that is detected in 20%− 30% of all pneumococcal isolates (Dzaraly et al., 2020). The important contribution of pilus islet-1 in pneumococcal virulence is supported by experimental and epidemiological studies (Iovino et al., 2020; Tabusi et al., 2021). Presence of pilus islets is associated with international pneumococcal clones expressing PCV13 serotypes 19A, 19F, 23F and 7F (Iovino et al., 2020). Most of the piliated pneumococci belong to a few clonal complexes, such as CC156, CC191, CC199, CC271 and CC320 (Dzaraly et al., 2020). Pilus islet-1 has been described to enhance the ability of pneumococci to interact with host epithelial and endothelial cells (Iovino et al., 2020). Using human primary neurons, it has been shown that pneumococci interact with the cytoskeleton protein β-actin through the pilus-1 adhesin RrgA and the cytotoxin pneumolysin (Ply), in promoting neuronal adhesion, invasion and cell death (Tabusi et al., 2021). Presence of the pilus islet-1 may have contributed to the severe meningoencephalitis in our patient. Of note, pilus-I is detected in GSPC6 but not the other GPSC lineages compared in this study, thereby providing a potential explanation for the rapid increase of serotype 24 F-GPSC6/ST162 in Europe. The type I pilus islet is flanked by a pair of IS element, suggesting horizontal gene transfer potentially from a common ancestor or other piliated pneumococci. The strength of this study is the comprehensive analysis of the viriome of pneumococcal virulence factors in M215 with other serogroup 24 strains. On the other hand, the analysis is limited by a lack of virulence gene expression or animal model studies.

In conclusion, this study described the genomic features of a serotype 24F-GPSC6/ST162 *S. pneumoniae* strain causing severe meningoencephalitis. The investigation illustrates the usefulness of using genome sequencing for detecting the introduction of a new clone and for detecting determinants associated with virulence.

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Conflicts of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2021.151543.

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