RES-273

Characterisation of Genomic and Phenotypic Antimicrobial Resistance in Paediatric Pneumococcal Carriage Isolates in Malaysia following Pneumococcal Conjugate Vaccine (PCV) Implementation (2021–2023)

Nurul Hanis Ramzi[1,2], Andrew Chun Hoong Tan[2,4], Nur Alia Johari[2,3], Anna Marie Nathan[5], Norhayati Aida Sulaiman[5], Cindy Shuan Ju Teh[6], Mohamad Ikram Ilias[7], Zakuan Zainy Deris[8], Siti Nur Haidar Hazlan[8], Nur Syafiqah Mohamad Nasir[8], Asrar Abu Bakar[9], Muhd Alwi Muhd Helmi[9], Wan Khairunnisa Wan Juhari[9], Norhidayah Kamarudin[10], Chun Wie Chong[11], David W. Cleary[12,13], Rebecca Anderson[14], Alexander J.J. Lister[14], Stephen D. Bentley[15], Lesley McGee[16], Stephanie W. Lo[15,17,18], Harry C.H. Hung[15], Stuart C. Clarke[2,14,19], Lokman Hakim Sulaiman[3,20]

[1]Centre for Cancer and Stem Cell Research, Institute for Research, Development and Innovation, IMU University, Malaysia [2]Institute for Research, Development and Innovation, IMU University, Malaysia [3]Centre for Environmental and Population Health, Institute for Research, Development and Innovation, IMU University, Malaysia [4]Centre of Postgraduate Studies, IMU University, Malaysia [5]Department of Paediatrics, Faculty of Medicine, Universiti Malaya, Malaysia [6]Department of Medical Microbiology, Faculty of Medicine, Universiti Malaya, Malaysia [7]Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Malaysia [8]Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, Malaysia [9]Department of Paediatrics, Kulliyyah of Medicine, International Islamic University Malaysia, Pahang, Malaysia [10]Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Malaysia [11]School of Pharmacy, Monash University Malaysia, Malaysia [12]Department of Microbes, Infection and Microbiomes, School of Infection, Inflammation and Immunology, College of Medicine and Health, University of Birmingham, UK [13]Institute of Microbiology and Infection, University of Birmingham, UK [14]Faculty of Medicine & Institute for Life Sciences, University of Southampton, UK [15]Parasites and Microbes, Wellcome Sanger Institute, UK [16]Division of Bacterial Diseases, Centers for Disease Control and Prevention, USA [17]Milner Centre for Evolution, Department of Life Sciences, UK [18]The Great Ormond Street Institute of Child Health, University College London, UK [19]National Institute for Health and Care Research (NIHR) Southampton Biomedical Research Centre, University Hospital Southampton Foundation NHS Trust, UK [20]Department of Public Health and Community Medicine, School of Medicine, IMU University, Malaysia

BACKGROUND

- The persistence and evolution of multidrug-resistant (MDR) *Streptococcus pneumoniae* (Spn) in nasopharyngeal carriage remains critical for transmission and potential disease development. Following Malaysia's rollout of PCV10 (2020) and PCV13 (2023), limited data are available on antimicrobial resistance (AMR) trends among Spn carriage isolates in children under 5.
- The MY-Pneumo study is a multicentre prospective case-control study designed to evaluate the burden of pneumococcal pneumonia in Malaysian children aged 5 years old and under following the introduction of the 10-valent pneumococcal conjugate vaccine (PCV10) into the National Immunisation Programme (NIP).
- A total of 500 cases of pneumonia and 500 age-matched controls at three tertiary hospitals across three states in Peninsular Malaysia were recruited from October 2021 to August 2023.
- Our study aimed to characterise genomic and phenotypic AMR profiles of Spn carriage isolates post-PCV implementation and identify Global Pneumococcal Sequence Cluster (GPSC)-associated MDR patterns and genotype–phenotype concordance.

METHODS

- **Subject recruitment from 3 teaching hospitals as sentinel sites within Peninsular Malaysia:**
  1. University of Malaya Medical Centre (UMMC), Kuala Lumpur (N=400)
  2. Hospital Universiti Sains Malaysia (HUSM), Kota Bharu, Kelantan (N=400)
  3. International Islamic University Medical Centre (IIUMC), Kuantan, Pahang (N=200)
- Nasopharyngeal swabs (NPS) were collected to test for Spn using culture, multiplex polymerase chain reaction (multiplex-PCR), and whole-genome sequencing (WGS).

PCR Serotyping and WGS

- The gene targets for pneumococcal identification inclusive of the main pneumococcal the capsular biosynthesis gene A (*cpsA*) by multiplex conventional PCR analysis according to CDC (1).
- **Optochin-sensitive** Spn isolates (**n=154**) Isolates were sequenced using a MiSeq (Illumina, UK) to generate 2 × 300 paired-end data.
- Genomes were analysed using the Global Pneumococcal Sequencing (GPS) pipeline to assign GPS clusters (GPSCs), multilocus sequence types (STs), in silico serotypes, AMR determinants across six antibiotic classes, and virulence factor (2).

RESULTS

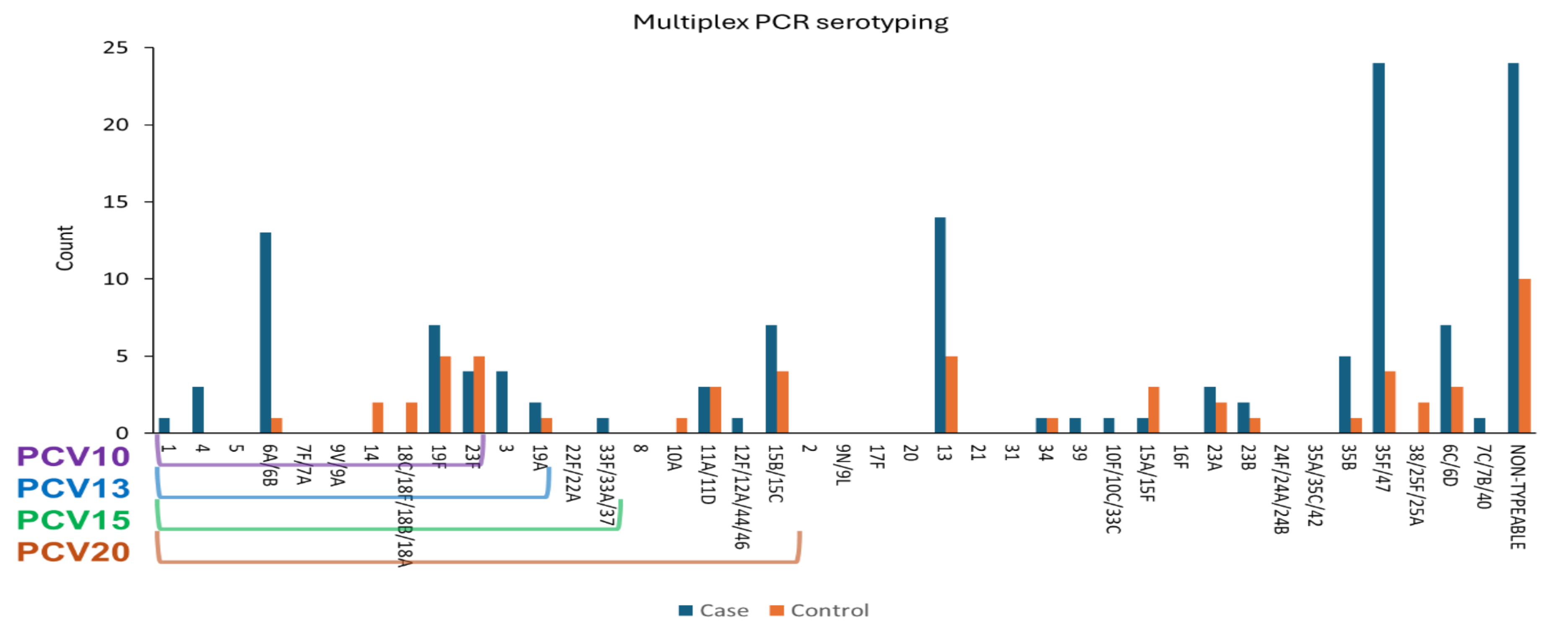

Figure 1 Distribution of Spn serotypes in children <5 years old by Case and Control subject type using multiplex-PCR (n=1000).

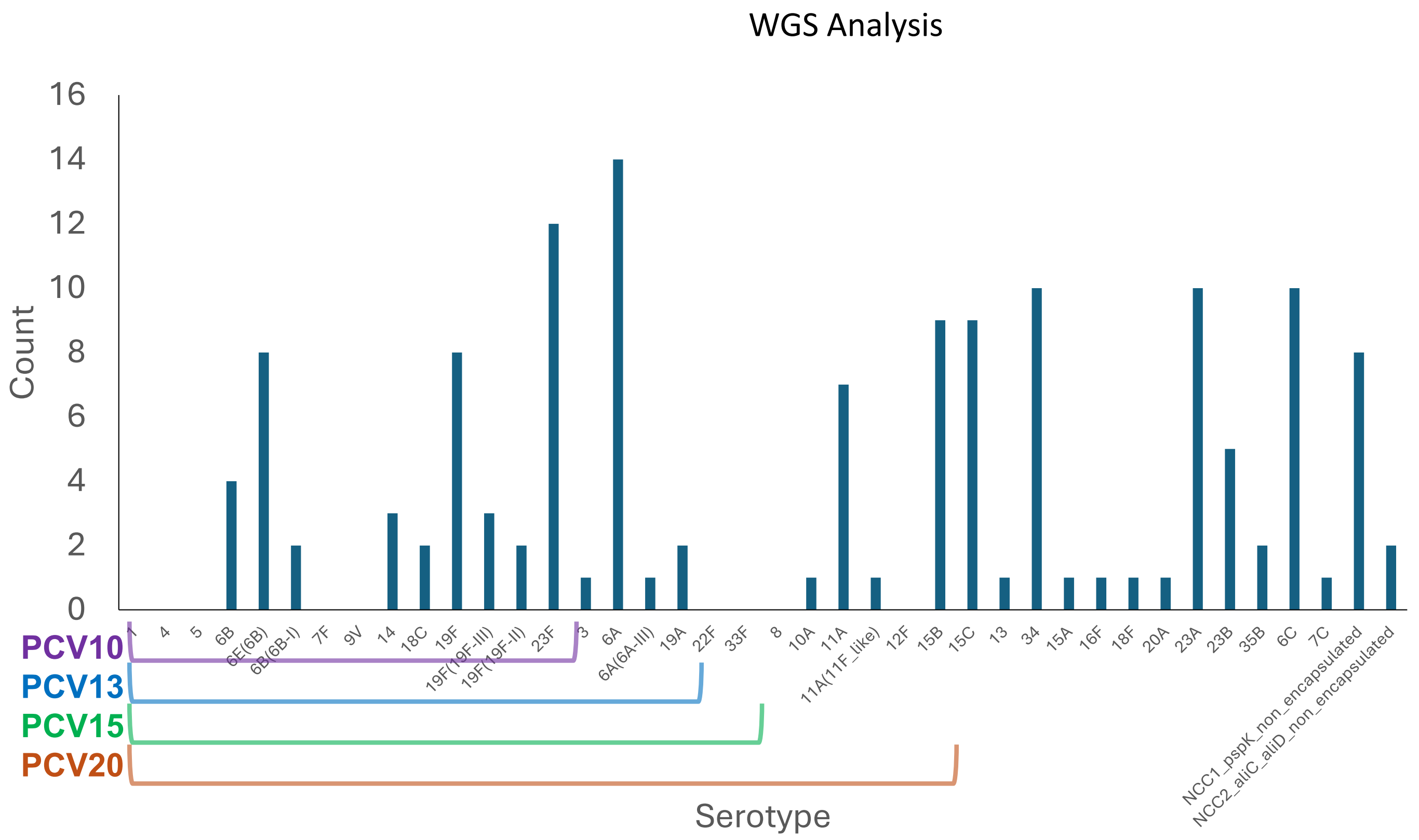

Figure 2 Distribution of optochin-sensitive Spn serotypes in children <5 years old using WGS analysis (n=154).

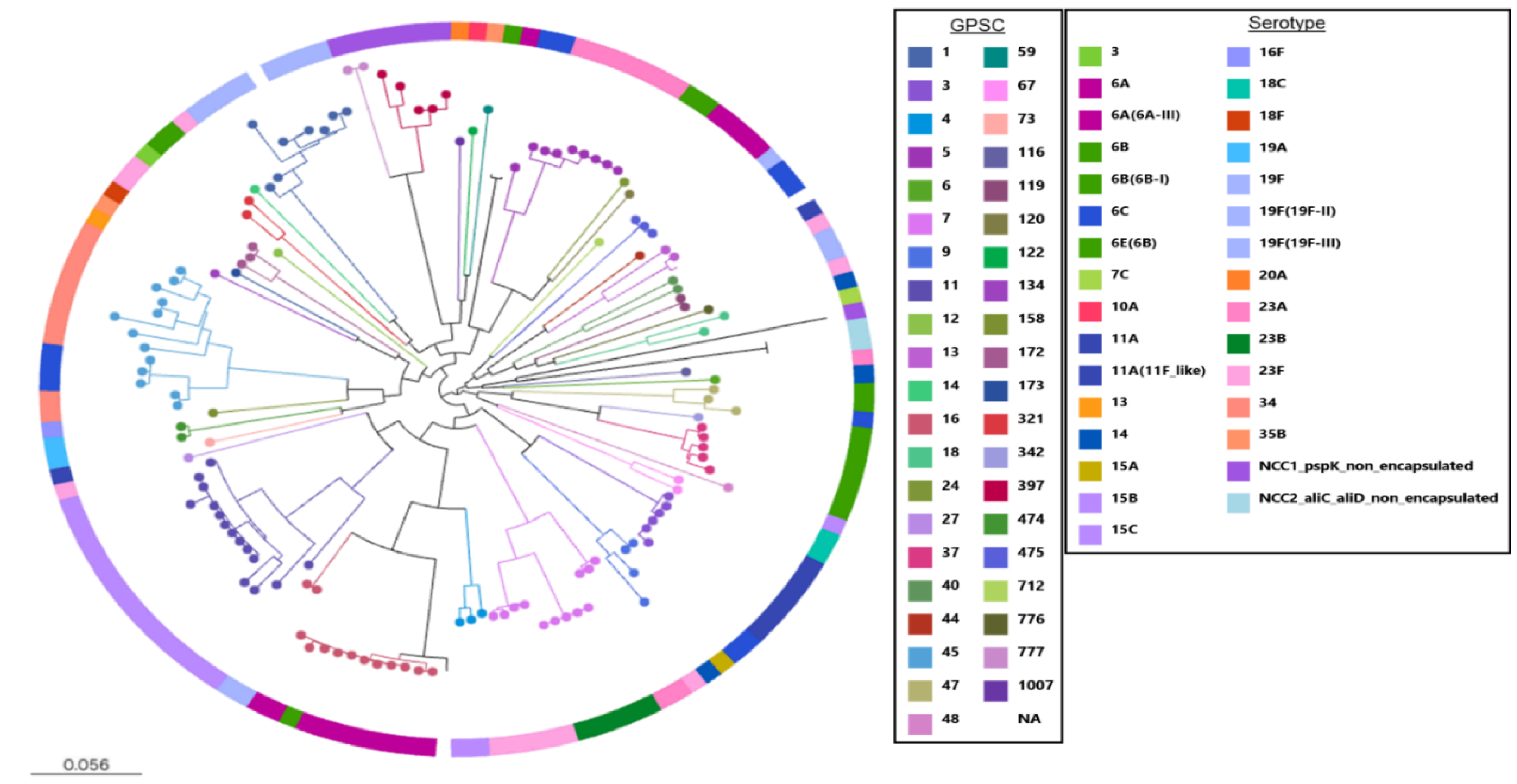

Figure 3 Phylogenetic relationship and serotype distribution of 142 Spn isolates (passed QC for WGS) analysed using the GPSC pipeline (2). The circular phylogeny is annotated with GPSC lineages and serotype calls to illustrate genetic relatedness and capsular diversity within the dataset.

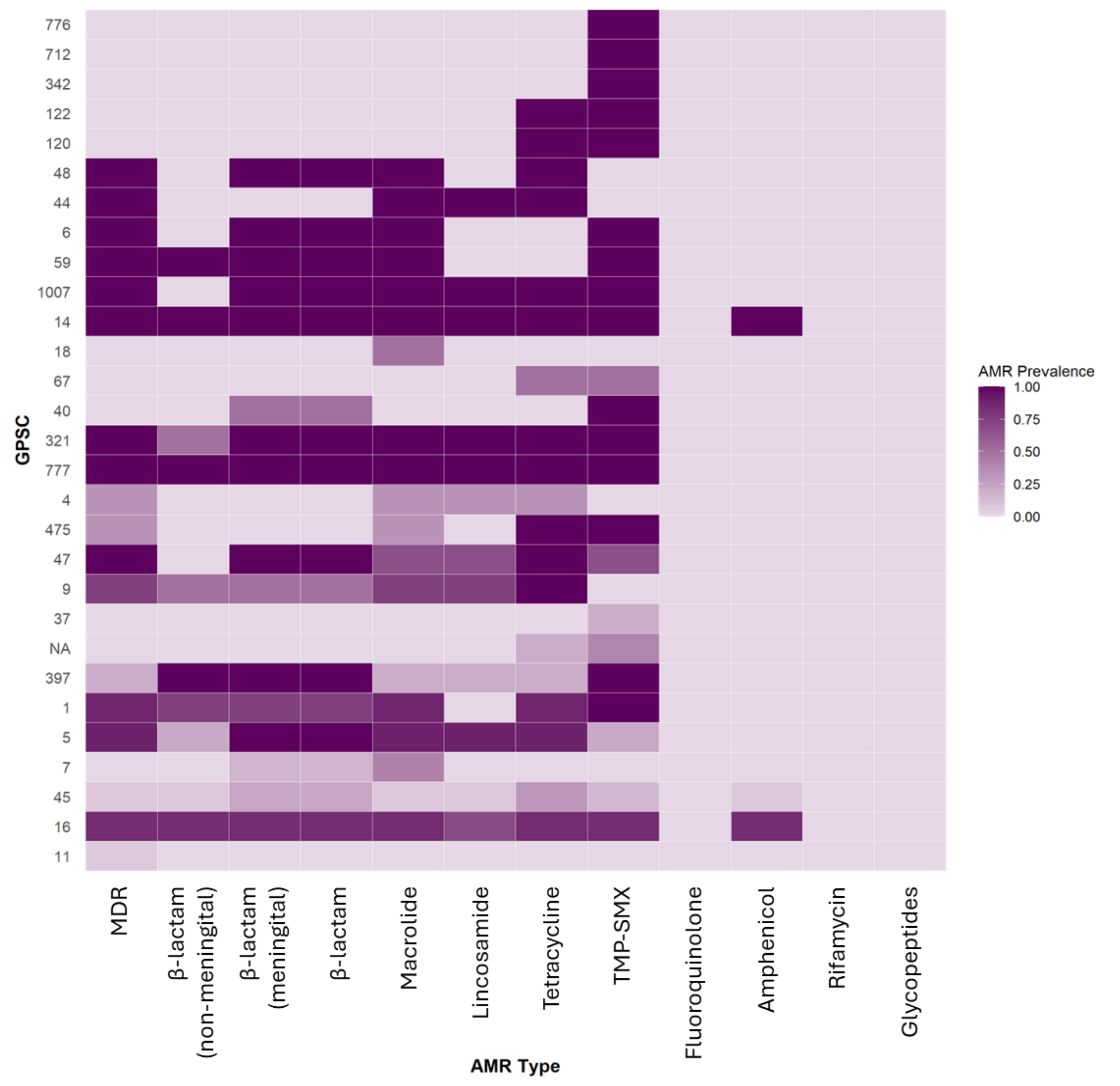

Figure 4 The prevalence index of AMR among the 142 pneumococcal isolates harvested from under 5 in Peninsular Malaysia. Rows represent GPSCs, ordered by their overall prevalence index in the population, while columns represent the AMR type predicted by genetic resistance determinants through WGS. The colour intensity indicates the prevalence of AMR within the GPSCs found, from 0.00 (light purple) to 1.00 (dark purple).

DISCUSSION

- Genotype–phenotype concordance exceeded 90% across key antibiotic classes. Fifty isolates (35.7%) were MDR, with GPSC16 and GPSC5 accounting for 38%. These were predominantly linked to serotypes 6A/6B and 23A/6C. WGS resolved all PCR non-typeable cases.
- The persistence of MDR in non-vaccine serotypes and lineages is especially concerning, as these are less likely to be suppressed by the current PCV10 or PCV13 schedules. Notably, PCV20 coverage was 62.7% compared to 43.7% for PCV13, indicating that the adoption of higher-valency conjugate vaccines may help to limit the expansion of MDR lineages such as GPSC16, GPSC5, and GPSC1.

CONCLUSION

- WGS offers high-resolution AMR and serotype profiling, supporting its integration into national pneumococcal surveillance strategies in the post-PCV era.
- Its integration into national surveillance frameworks is crucial for guiding evidence-based interventions in the post-PCV landscape.

References

1) CDC Streptococcus Laboratory. *Streptococcus pneumoniae* carriage study protocol – nasopharyngeal (NP) swab processing. NCIRD/DBD/RDB-Centers for Disease Control and Prevention; 2010. Available from: https://www.cdc.gov/streplab/pneumococcus/resources.html#carriage-protocols
2) Hung HCH, Kumar N, Dyster V, Yeats C, Metcalf B, Li Y, Hawkins PA, McGee L, Bentley SD, Lo SW. GPS Pipeline: portable, scalable genomic pipeline for *Streptococcus pneumoniae* surveillance from Global Pneumococcal Sequencing Project. Nat Commun. 2025;16:8345. https://doi.org/10.1038/s41467-025-64018-5

Acknowledgement

This work is supported in part by a research grant from Investigator-Initiated Studies Program of Merck Sharp & Dohme Corp. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme Corp. We thank all the parents/guardians and children who participated in the study.