

2618. Determination of the Chemical Structure of a Novel Pneumococcal Serotype, 39X

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Background: *Streptococcus pneumoniae* produces a diverse group of capsular polysaccharides (serotypes) that are important for the virulence of the organism and for the serotype-specific prevention of pneumococcal disease. As a consequence of widespread PCV usage and pneumococcal genome plasticity, the distribution of pneumococcal serotypes is changing with an increase in non-vaccine serotypes post-vaccine introduction, a phenomenon known as serotype replacement. Recently, a potentially novel serotype was described and was provisionally named as serotype 39X. Genetic studies suggest that this novel serotype may be a hybrid of serotypes 6C and 39/10A.

Methods: Three 39X strains with the distinct serological and genetic description of the *cps* biosynthetic loci were obtained from the Global Pneumococcal Sequencing project (www.pneumogen.net). Capsular polysaccharide from one (Camb.853/MNZ2334) of the 39X strains was purified by sequential ethanol precipitation followed by ion-exchange chromatography. To detect polysaccharide fractions during purification, an inhibition ELISA assay was developed using factor serum 10d. The chemical structure of the 39X repeating unit was determined using one-dimensional (1D) and 2D nuclear magnetic resonance (NMR).

Results: All three isolates were confirmed to have the 39X genotype by PCR amplification and sequencing of the 39X specific region (*wciN_{6C}-wcrO-wcrC₃₉*) of the *cps* locus. (Figure 1). The 39X capsule PS fractions were detected during purification and pooled for structural studies (Figure 2). 1D-NMR for 39X showed it to be chemically distinct (Figure 3). 2D-NMR studies revealed that five of the sugar residues in 39X PS are identical to those in 39 PS, except the acetylation (Figure 4). The remaining part of the structure is being investigated.

Conclusion: The 39X capsular PS has a distinct chemical structure in addition to its distinct serologic and genetic properties. Given that serotype 39X is a new serotype, it becomes the 100th pneumococcal serotype. The chemical structure supports the genetic depiction of serotype evolution as a result of recombination between well-characterized and unrelated serotypes. Structural elucidation of the 39X capsule PS will help facilitate our understanding of serotype replacement and vaccine development.

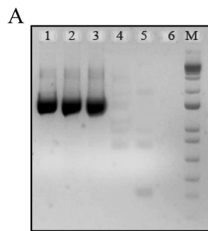


Figure 1. Confirmation of 39X genotype. (A). PCR amplification of *wciN_{6C}-wcrO-wcrC₃₉* fragment of *cps* loci. Lane 1: PATH 4346/MNZ332; Lane 2: Camb.657/MNZ2333; Lane 3: Camb.853/MNZ2334 (Lane 1-3: Serotype 39X); Lane 4: MJC705 (Serotype 6C); Lane 5: SSI Serotype 39; Lane 6: No template control; M: 100bp marker. (B). Sequencing of the amplified PCR product. Gray shaded and bold region indicates *wciN_{6C}* gene fragment, Black shaded region indicates *wcrO* gene. The sequence in bold and italics represents *wcrC₃₉* gene fragment.

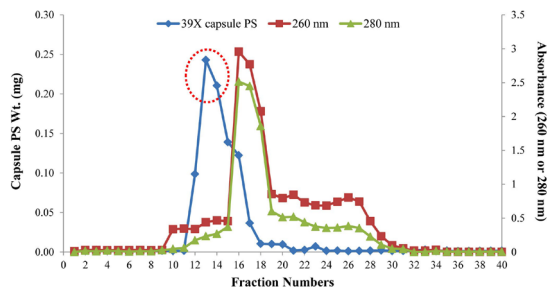


Figure 2. Purification of capsule PS from Camb.853/MNZ2334. Capsule PS fractions were detected by inhibition ELISA assay after ion exchange chromatography. Absorbance at 260 nm (Red line), and absorbance at 280 nm (Green line) indicates the nucleic acid and protein impurities, respectively. The fractions 13 & 14 encircled were pooled and lyophilized for NMR studies

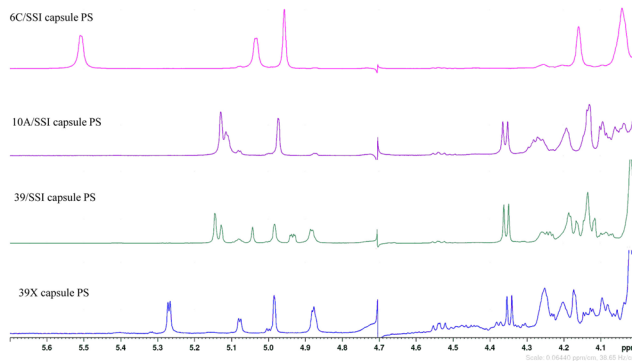


Figure 3. 1H NMR spectra of the anomeric region (4.8 to 5.6 ppm) of serotypes 6C, 10A, 39 and 39X capsular PS. 6C, 39 and 10A capsular PSs were obtained from (Staten's Serum Institute (SSI)). The NMR spectra indicates that 39X capsule PS is chemically distinct from 6C, 10A and 39 capsule PS.

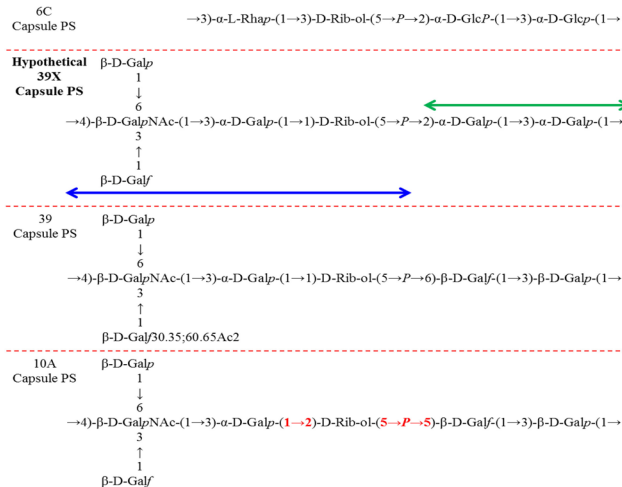


Figure 4. Hypothetical structure of 39X compared to 6C, 39 and 10A structures. The blue arrow indicates the portion of 39X repeat unit which has been confirmed to be identical to 39 PS, except the acetylation. The green arrow indicates the part of 39X structure being studied. Red color in 10A PS indicates differences from 39 PS.

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2619. Clinical Characteristics and Etiology of Community-Acquired Pneumonia in Children: A Contemporary, Prospective, Multicenter Study in Ohio, 2015–2018

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Background: Worldwide, pneumonia is the leading cause of death in children <5 years of age and the second most common reason for hospitalization in children in the United States and Europe. This study was designed to describe the clinical characteristics and etiology of community-acquired pneumonia (CAP) in children.

Methods: We conducted a prospective, multicenter, observational study of CAP among previously healthy children aged 2 months through 18 years in six children's hospitals in Ohio. Blood, pleural fluid, and nasopharyngeal swabs were collected for pathogen detection by culture and/or molecular diagnostics. Patient clinical management including antibiotic therapy and respiratory support, followed the standard of care at each study site. Follow-up information regarding clinical outcomes was collected via a survey 6–8 weeks after enrollment.

Results: We enrolled 441 children (n = 380, 86% hospitalized) with CAP from 2015 to 2018. Median age was 5 years (IQR: 2.1–8.9y). Intensive care and respiratory support