O antigen structural studies and O *loci* identification of nontypeable clinical isolates of *Klebsiella pneumoniae*

Klebsiella pneumoniae is a nosocomial pathogen and one of the priority species, pointed out by the World Health Organisation (WHO) as critical regarding highly limited options of treatment of infections caused by the species. Lipopolysaccharide (LPS) and capsular polysaccharide (CPS, K antigen) are its major virulence factors and surface antigens, determining O and K serotypes and encoded by O or K *loci*, respectively. Contrary to CPS, the species has been perceived as of limited variety of O antigens (11 O serotypes identified to date) and that trait makes LPS an attractive target for antibody-based therapies (vaccines and passive immunization) as an alternative to antibiotics. To make such immunotherapy effective, knowledge about O antigen structures, drift, and distribution among clinical isolates is important. At present, the structural analysis of O antigens is efficiently supported by bioinformatics. O and K *loci*-based genotyping by polymerase chain reaction (PCR) or whole-genome sequencing WGS has been proposed as a diagnostic tool, including the Kaptive (https://kaptive-web.erc.monash.edu/), and obtained results have indicated indicating higher diversity of the O antigen *loci*.

The research included the group of eleven LPSs isolated from clinical isolates of *K. pneumoniae* (BIDMC 7B, ABC152, ABC122, BC738, BC13-986, 3936/19, Kp164, Kp165, Kp166, Kp174, Kp177). Strains were selected as nontypeable strains considering their reactivity with antibodies against known O serotypes or differences in O *loci* sequences. This PhD project was part of the OPUS16 grant founded by National Science Center (2019-2025) and entitled "Nontypeable O antigens of *Klebsiella pneumoniae* – structures and seroepidemiology".

Lipopolysaccharides were isolated, purified and analysed as a native molecules by ^{1}H, ^{13}C HR-MAS NMR (*high-resolution-magic angle spinning nuclear magnetic resonance*). For selected strains, O-specific polysaccharides were isolated and analysed by chemical methods and NMR spectroscopy. Additionally, structural analyses were supported by molecular biology and O *locus* prediction by Kaptive. DNA was isolated from all 11 nontypeable *K. pneumoniae* isolates, sequenced and obtained contigs analysed by Kaptive. Moreover, multi-*locus* sequence typing (MLST) analyses were performed for each isolate.

The spectra of selected LPSs were compared with the spectra of *K. pneumoniae* LPSs of known O serotypes, which allowed the LPSs to be assigned to four groups:

i) group 1 (strains BIDMC 7B, ABC122) representing O2 variant 1 (O2v1) phenotype with insertion sequence in *rfb* regions predicted by Kaptive as O2v2 genotype; ii) group 2 (strains ABC152, BC738, BC13-986, 3936/19) characterised by identical and novel O13 serotype; iii) group 3 (strains Kp164, Kp165, Kp166) represented known O4 serotype, and iv) group 4 (strains Kp174, Kp177) expressing rough LPS devoid of O-specific polysaccharide.

Two original achievements have been made and published. For BIDMC 7B and ABC122 strains the reasons for discrepancies in O2 serotyping between Kaptive-based predictions (indicating the following O antigen repeating unit →3)-β-D-Gal*f* -(1→3)-[α-D-Gal*p*-(1→4)]-α-D-Gal*p*-(1→ (O2v2 serotype)) and the phenotype (→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→ (O2v1)) have been identified. As a reason, insertion sequences (ISs) were identified within *rfb* regions in the gene *gmlB* responsible for biosynthesis of the terminal α-D-Gal*p* residue. Discovered phenomenon were reported by *in silico* analysis of 8130 genomes available in public databases (December 3, 2019) for ~10% and ~28% of O1v2 and O2v2 genomes, respectively indicating a broader distribution of ISs (e.g. ISR1, IS903B, ISKpn14, or ISKpn26) in *rfb* regions that may influence the O antigen chemical structure (Artyszuk *et al*. 2020).

As a second accomplishment, one of the novel *K. pneumoniae* O *loci*, for which the antigen structure has not been elucidated so far, OL101 *locus*, have been characterised by identification of encoded O antigen structure. In this study, four clinical isolates predicted by Kaptive as OL101 (ABC152, BC738, BC13-986, 3936/19) were characterized and found to have the O antigen structure composed of β-Kdo*p*-[→3)-α-L-Rha*p*-(1→4)-α-D-Glc*p*]$_n$, representing a novel serotype O13 and occurring in ~6,55% of isolates in the dataset of *K. pneumoniae* 71377 genomes (July 27, 2023) screened by *in silico* analysis (Artyszuk *et al*. 2024).