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Structural diversity of *Klebsiella pneumoniae* O-antigen glycoforms

Summary

Klebsiella pneumoniae is a Gram-negative, highly virulent rod-shaped bacterium, primarily attacking immunocompromised individuals. This bacterium can cause pneumonia, pyogenic liver abscess and urinary tract infections. *K. pneumoniae* together with *Escherichia coli* and *Pseudomonas aeruginosa* is one of the leading Gram-negative bacteria in the development of sepsis. A characteristic feature *of K. pneumoniae* is antibiotic resistance associated with the ability to produce beta-lactamases hydrolysing the β-lactam ring of antibiotics. Recently, the emergence of isolates producing carbapenemases has raised alarm because carbapenems are often treated as last resort drugs in dealing with *K. pneumoniae* infections.

Lipopolysaccharide (LPS, endotoxin) and capsular polysaccharide (K antigen, CPS) are major surface carbohydrate antigens of *K. pneumoniae*. The LPS molecule consists of three distinct regions: lipid A (anchoring LPS in the outer *membrane* bilayer), core oligosaccharide and the O-specific polysaccharide (O-antigen, O-PS). LPS toxicity is related to the lipid portion, while the polysaccharide part exhibits immunogenicity and determines the O serotype.

Due to the limited options of preventing and treating infections caused by *K. pneumoniae*, there is a need for new therapeutic strategies. Capsular antigens and O-specific polysaccharides were identified as potential antigens in immunotherapy. Compared with other members of *Enterobacteriaceae*, defined O-serotypes of *K. pneumoniae* show limited diversity. The number of O serotypes (7 and their subtypes) is significantly lower than the number of K antigens of this species (approximately 78) and thus O-antigen-based immunotherapy seems to be a more promising approach.

This PhD thesis was part of the European Klebsicure project (EUROSTARS, E! 7563, 2012 – 2015), implemented by a consortium of two biotechnology companies - Arsanis Biosciences GmbH (Vienna, Austria), GATC Biotech AG (Constance, Germany) and Max Planck Institute for Infection Biology (Berlin, Germany) and the Institute of Immunology and Experimental Therapy Polish Academy of Sciences in Wroclaw. The aim of the project and its title was “Development of monoclonal antibody-based passive immune therapy and companion diagnostics for severe *Klebsiella* infections”. The antigens that were selected for the therapeutic target of the antibodies were O-specific polysaccharides of *K. pneumoniae*. Therefore, an integral part of the project, and thus the overriding aim of my PhD thesis, was isolation, purification, structural analysis, degradation of selected LPS *K. pneumoniae* and then purification and structural analysis of selected O-antigens. This was the stage preceding the preparation of biotinylated O-PSs used for selection of monoclonal antibodies and further characterization of

these antibodies. Initially it was assumed that there was a limited number of O serotypes, but preliminary serological tests indicated the variety of O1, O2, O3 serotypes which were the main subject of this PhD thesis (Guachalla i in. 2017; Stojkovic i in. 2017; Szijártó i in. 2016).

The complete structural analysis of selected O-specific polysaccharides belonging to the O2 and O1 serotypes (strains: Kp26 – O2 *gml*–, PCM-27 – O2 *gml*+, Kp4 – O1 *gml*–, Kp24 – O1 *gml*+) were performed by interpretation of one- and two-dimensional NMR spectra. The preliminary tests performed by the consortium leader suggested that the *gmlABC* operon encode for a modification of O-specific polysaccharide subunit [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→] described as D-galactan-I (gal-I) present in O1 and O2 serotypes. Structural analyses of O-specific polysaccharides from *gml*+ strains (PCM-27 – O2 and Kp24 – O1) revealed branched galactose trisaccharide structure, termed as galactan-III (gal-III), in which the gal-I backbone is decorated by the terminal α-D-Gal*p*. O-PS isolated from *gml*– strains (Kp26 – O2, Kp4 – O1) was devoid of a branching terminal α-D-Gal*p*. The structures of isolated O-PSs were also confirmed by chemical methods (sugar and methylation analyses). An important part of this research were structural analyses of O-PS from transcomplemented mutants. This study confirmed that the presence of *gmlABC* operon is responsiible for modifying gal-I structure to gal-III.

O-specific polysaccharides of *K. pneumoniae* 5505Δ*cps*, PCM-11 and Kp81 (belonging to O3 strains) were analysed by NMR spectroscopy and MALDI-TOF mass spectrometry to determine structures and the average molecular weights of the O-PS repeating units. Analysis indicated the presence of penta-mannose form within the O-antigen repeating units ("traditionally" called O3) for strain 5505Δ*cps*, tetra-mannose form for strain PCM-11 (novel O3a) and three-mannose form for strain Kp81 (novel O3b).

In order to analyze O-PS structures directly on bacterial surface and using non-degraded LPS molecules, HR-MAS NMR was used. HR-MAS NMR spectra of bacteria and non-degraded LPS were obtained and compared to previously established NMR spectra of isolated O-antigens. It was shown that relatively fast identification of *K. pneumoniae* serotype was possible based on ^{1}H and ^{1}H,^{13}C HSQC-DEPT of LPS using HR-MAS NMR method. The identification of serotype based on spectra obtained exclusively for bacteria was significantly impeded by the presence of additional signals from contaminants.