# Structural elements of carbohydrate antigens shared by bacteria of the genus *Bordetella* as universal vaccine components


## SUMMARY

Whooping cough is a highly contagious disease caused by *Bordetella pertussis*. A pathognomonic symptom of pertussis is a severe spasmodic and unproductive cough that worsens at night and is accompanied by vomiting, apnea and cyanosis. Among vaccinated children, adolescents and adults, the symptoms are milder and less specific, and therefore more difficult to diagnose (WHO 2014). In the pre-vaccination era, tens of thousands of pertussis cases were diagnosed each year in Poland. Whole-cell pertussis vaccine (DTwP) has been highly effective in reducing morbidity and mortality. However, DTwP vaccines are reactogenic as they contain lipopolysaccharide (LPS) and they have been partly or completely replaced, by acellular pertussis vaccines (DTaP) that instead contain purified bacterial protein antigens. In spite of the sustained high coverage of vaccinations, there is an increase of whooping cough in all age groups. New diagnostic methods allow to identify other *Bordetella* species that cause pertussis-like symptoms, i.e. *B. parapertussis* and *B. holmesii* (Guthrie *et al*. 2010; Rodgers *et al*. 2013). *B. holmesii* have become the second pertussis etiological factor, but the currently used vaccines do not provide a cross-protection. The imperfection of existing vaccines is the reason for development of improved and new components of vaccines against whooping cough. One of the novel approaches is to focus on surface carbohydrates antigens of *Bordetellae*.

Bacterial polysaccharides can be divided into 3 groups. The first group comprises of LPS which are composed of the lipid A, an oligosaccharide core and an O-antigen. LPS is an integral component of the outer membrane of Gram-negative bacteria and induces the production of specific and bactericidal antibodies (Rappuoli 2004). LPS seems to be an ideal vaccine antigen, but its reactogenicity excludes using it in its native form. LPS-derived polysaccharide fragments are devoid of endotoxic activity and still maintain antigenic properties. Polysaccharide vaccines have been developed for 50 years, but their main disadvantage is that the polysaccharide itself as a T-independent antigen induces a weak and short-lived immunity. Polysaccharides become T-dependent antigen by conjugation with an immunogenic protein carrier. Glycoconjugate vaccines combine the antigenic properties of carbohydrate and protein components. Glycoconjugates

containing *B. pertussis* LOS-derived oligosaccharide fragments induce the production of bactericidal antibodies (Koj *et al*. 2015). Bacteria of the "*B. bronchiseptica* cluster" are closely related, but they produce different lipopolysaccharides, containing some highly conserved segments related to the core OS.

Previously *Escherichia coli* OS studies have shown that antibodies directed against the OS core of rough-strains cross-react with smooth-strains cores (Lukasiewicz *et al*. 2003). Therefore, it is essential to identify common antigenic motifs that in the isolated forms could become components of universal vaccines protecting against *Bordetella* infections. The second group of bacterial glycans, which are also used as vaccine components, are capsular polysaccharides (CPS). CPS protect bacterial cells from the host defense mechanisms and from enviromental conditions (Roberts 1996). CPS are bound to the cell via a lipid anchor. CPS molecules released into environment constitute the third group of so-called exopolysaccharides (EPS). According to published data, some *Bordetellae* (*B. pertussis*, *B. parapertussis* and *B. bronchiseptica*) produce Bps exopolysaccharide (*Bordetella polysaccharide*), made of the GlcNAc homopolymer, which is supposed to play an important role in the biofilm development (Conover *et al*. 2010). Other publications suggest that *B. pertussis* produces a microcapsular polysaccharide, a form of the GalNAcA homopolymer that might resemble Vi antigen (Neo *et al*. 2010; Hoo *et al*. 2014). However, none of these publications presented direct analyses of the isolated EPS and CPS. The genes responsible for the CPS synthesis have been identified in the *Bordetellae* genomes, but to the date no one has been able to determine whether the capsular polysaccharides are actually produced by *Bordetella* species. These uncertainties were the reason for our interest in EPS and CPS structural features of *Bordetellae.*

In this study, structural and serological analyses of heteropolysaccharide parts of LPS among various *Bordetellae* species (*B. pertussis* 186, *B. pertussis* 606, *B. parapertussis* 529, *B. bronchiseptica* 530, and *B. bronchiseptica* 1943) were performed. This work identifies the structure and describes the chemical shifts for *B. pertussis* 606 oligosaccharide for the first time. The lack of cross-reactivity with anti-glycoconjugate sera (anti-penta-PT, anti-penta-TTd and anti-disach-HSA) recognizing the distal trisaccharide, confirms that the OS of *B. pertussis* 606 is a nonasaccharide devoid of distal trisaccharide. *B. bronchiseptica* 530 and *B. bronchiseptica* 1943 strains were identified as rough strains not synthesizing the O-antigen. Minimal OS of *B. bronchiseptica* 530 and 1943 is a heptasaccharide and is the shortest OS among the analysed species, which has also been confirmed by very weak cross-reactions with sera

recognizing OS of *B. pertussis* 186 (anti-OS-TTd and anti-OS-PT). *B. parapertussis* 529 was defined as a smooth strain. Anti-glycoconjugate sera (anti-penta-TTd and anti-disach-HSA) cross-recognize the *B. pertussis* 186 immunodominant epitope in the LPS of *B. parapertussis* 529, suggesting that a distal trisaccharide is present in the *B. parapertussis* LPS structure. Two forms have been distinguished among structures of the OS core of *B. parapertussis* 529. Minimal OS is an octasaccharide, while the complete structure of OS is a dodecaccharide. The minimum OS core for *Bordetellae* was defined as a hexasaccharide. It has been established that the basic core OS structure is conserved among *Bordetella* species. The modifications included the loss of some residues or an additional substitution by non-sugar substituents. An interesting observation is the fact that during the performed analyses no pentasaccharide linker was identified. According to Preston et al. this linker attaches the O-antigen to OS (Preston *et al*. 2006). This can be explained by the lack of O-antigens in *B. bronchiseptica* strains, but this type of linker should be present in *B. parapertussis* LPS, which synthesizes the O-antigen.

*B. holmesii* ATCC 51541 is a novel and important etiological factor of pertussis. Almost nothing is known regarding the structure of carbohydrate antigens of this bacterium and their possible applications as new glycoconjugate pertussis vaccines. Here the structure of the OS core and O-antigen of *B. holmesii* ATCC 51541 is presented. Based on the NMR and MALDI-TOF MS spectra it was established that the OS core of *B. holmesii* is identical to this of *B. pertussis* 606. *B. holmesii* OS is a nonasaccharide devoid of distal trisaccharide in its structure. This observation was also confirmed by the lack of serological cross-reactions with antibodies directed against the immunodominant epitope of *B. pertussis* 186. The NMR spectroscopy, mass spectrometry and chemical methods allowed to determine the structure of the O-antigen of B. *holmesii* ATCC 51541. It has been identified as a polysaccharide composed of pentasaccharide subunit (mass of 974.32 Da) with the following structure: [→2)-α-L-Rha-(1→3)-α-D-Glc*p*-(1→4)-[β-D-Glc*p*NAc-(1→3]α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→]$_n$

Due to the divergent literature data on the EPS produced by *Bordetellae* and the lack of complete structural data, analyses of EPS isolated from the post-culture medium of the tested *Bordetellae* species were performed. Long-chain polysaccharides were detected in the post-culture medium separated using size exclusion chromatography. Polysaccharides were identified as two glucose homopolymers substituted at the 4 and 6 positions [→4)-α-D-Glc*p*-(1→] and [→6)-α-D-Glc*p*-(1→]. In the chromatograms

exooligosaccharide-like fractions was also detected. The main oligosaccharide fraction contains a new hexasaccharide with a mass of 1235.5 Da, identified using mass spectrometry techniques. The structure was characterized by NMR spectroscopy and chemical analysis. The deduced structure differed substantially from hypothetical Bps and microcapsule structures, thus it has been named "*Bordetella* oligosaccharide" (BOS). BOS is universal among all the *Bordetella* species studied. BOS structure was defined as α-Glc*p*NAc-(1→4)-α-Gal*p*NAcAN-(1→4)-[β-D-Glc*p*-(1→3)]-α-Gal*p*NAcAN-(1→4)-α-Glc*p*NAcA-(1→3)-α/β-Glc*p*NAc. In the NMR spectra of BOS-containing fraction -CH, $-CH_2$ and $-CH_3$ signals were also identified, resembling fatty acids spin systems. They could be explained by the presence of an anchoring element for the BOS in the outer membrane. At the BOS reducing end, GlcNAc has been identified in both α and β configurations. This could be a place where the lipid anchor might be attached. However, no direct connection between BOS and possible anchor could be identified, because no BOS-containing fraction connected to the lipid anchor was identified.

Anti-glycoconjugate sera (anti-penta-PT, anti-OS-PT and anti-disach-HSA) and sera containing polyclonal antibodies directed against whole bacterial cells of *B. pertussis* (anti-SM Bp 186 and anti-SM Bp mix) that recognize LOS on the cell surface, showed a weak cross-reaction with BOS. In both structures, the terminal GlcNAc was identified, which may be the crucial epitope for the cross-recognition. Reactivity of sera to whole-cells support the hypothesis that BOS may be a capsule. Similarly anti-Vi serum, which according to published data, specifically recognizes GalNAcA that form a microcapsule, wakly reacts with BOS.

Understanding of the antigenic properties of BOS, its role in colonization and transmission of bacteria, as well as the possibie application of BOS neoglyconjugate as a new potential component of broad-spectrum pertussis vaccines requires further research.