*Klebsiella pneumoniae* (***Kp***) is Gram-negative bacillus of the *Enterobacteriaceae* family and a component of normal human microbiota and common cause of community- and healthcare-associated infections. The species is characterised by emerging multidrug-resistance (MDR), it belongs to ESKAPE group of pathogens. Its major virulence factors and surface antigens are capsules (antigen K, *e.i.* capsular polysaccharide - CPS and exopolysaccharide - EPS), lipopolysaccharide (LPS, endotoxin, O antigen), and fimbriae. *Kp* O antigens seem to be promising targets for antibody-based therapy (active and passive immunisation) as an alternative to antibiotics. To make such immunotherapy effective, the complete knowledge about O antigens and their distribution among clinical isolates (seroepidemiology) is mandatory. LPS determines O serotype of *Kp.*

**For years random structural analyses of *Kp* O antigens (O-PS) have indicated their surprisingly limited diversity (only 9 O serotypes) what distinguished *Kp* from majority of Gram-negative bacteria.** Contrary to at least 188 *E. coli* O-antigens, only 12 have been identified for *Kp* to date [O1 (variant 1 and 2), O2a, O2afg, O2aeh, O3, O3a, O3b, O4, O5, O7, O8, and O12]. 4 of them have been recently described by Principal Investigator (PI) et al. (O2, O1, O3 variants). It has remains intriguing if there is any room for next new O serotypes among *Kp*. Bioinformatics studies have recently answered this question with "Yes, there is a room for new O serotypes". Depending of the report, from 2.4 to 17% of nontypeable strains have been identified. Unfortunately these data do not include information about exact structures of these potentially new O serotypes. **The project team hypothetises: (i) much higher diversity of *Kp* O serotypes and the presence of novel O antigens, and (ii) correlation between O serotype and phylogenetic lineages, virulence, and epidemic clones, with emphasise on MDR ones.**

The major aims of this project are:

**1. Discovery of novel structures of *Kp* O antigens.** New O serotypes identification will be combined with deciphering of their chemical structures and genes (O loci) responsible for their synthesis.

**2. Determination of *Kp* O serotypes distribution (seroepidemiology) within 2 unique collections** (120 CPKP (carbapenemase-producing *Kp*) strains collected from Polish hospitals (2006-2018) and including severe epidemic clones and 130 European MDR isolates (2008-2011) from hospital in Europe and Israel. provided by prof. M. Gniadkowski from National Institute of Health in Warsaw (Poland).

**3. Identification of O serotype correlations with phylogenetic lineages, geographic spread, virulence load, antimicrobial resistance (AMR) range, and epidemic clones, including the MDR ones.**

**<u>The following arguments support the need for the project implementation:</u>**

1. **The complete knowledge about *Kp* O serotypes diversity is important for further development of therapeutic strategies against *Kp* infections targeting O antigens.**
2. **There is a room for new O serotypes of *Kp*.** The proof of concept for this hypothesis has been provided by initial results.
3. **Contrary to business strategies used by most of R&D start-ups, finding of novel O serotypes cannot be left unresolved by scientific community and identification of possible new O serotypes should be completed.**
4. **Nontypeable strains chosen are characterised by high clinical relevance.**
5. **Even though new O serotypes are characterised by low prevalence, they cannot be neglected due to safety of patients infected by nontypeable strains and dynamics and plasticity of *Kp* genome**.
6. **Simple serotyping, even if combined with genotyping, does not demonstrate molecular structure of the O antigen.**
7. **It is necessary to analyse larger collections of *Kp* isolates of various clinical, epidemiological and geographic origins**.
8. **By implementation of the objectives of the project proposal, the community will be far ahead of possible O-antigen drift consequences described above.**

In 2017 the WHO, CDC, and the UK Department of Health singled out ESBL- i KPC-*Kp* strains as "priority 1. critical" pathogen for health care and in regards to the urgency of need for new antibiotics. **Thus if newly developed O-antigen based strategies against *Kp* infections (therapeutic monoclonal antibodies or vaccines) are going to be effective, the knowledge about novel O serotypes, their diversity and distribution among clinical isolates is mandatory.** Several novel *Kp* O serotypes characteristic for 12 MDR clinical isolates will be provided and characterised by: (ii) O loci genes responsible for novel O antigen synthesis (providing original data for O genotyping). The unique knowledge about O serotype stability and diversity within the complex Polish and European collections of CPKP and MDR *Kp* isolates will be gained. **By implementation of the objectives of the project proposal, the community will be far ahead of possible O-antigen drift consequences described above.**