Final report (2013-09-01 - 2017-08-31) of the **K 108631** OTKA project

The importance of asymptomatic carriage of major Gram-positive pathogens: *Streptococcus pneumoniae, Streptococcus agalactiae* and *Staphylococcus aureus*

This OTKA grant (K108631) was the direct continuation of the previous PD grant (PD75660). In that, only pneumococcal carriage was investigated, which was later extended to two further Gram-positive bacteria. **We have performed the first large-scale epidemiological studies on bacterial carriage in Hungary, providing hundreds of serotyping, genotyping and antibiotic sensitivity data**.

Our work group also expanded during these four years. In the beginning two PhD students were involved as participants. *Adrienn Tóthpál* defended her PhD successfully in April 2015 (in the field of pneumococci) and later obtained a 2-year postdoctoral grant from the Yale University, where she is at the moment. *Krisztina Laub* just submitted her PhD thesis in the beginning of November 2017 (in the field of *S. aureus*). Later on, two new PhD students joined our work group: *Eszter Kovács* and *Judit Sahin-Tóth*, so they were incorporated as participants for the last year of the project.

A graduate student (*Zsófia Párkányi*) has written her diploma work based on the pneumococcal research (AOM) and she also won first prize at the Semmelweis University TDK conference in 2016.

In addition, the results of the carriage studies were presented at several CME courses organised for medical doctors. On 9-10 November 2017, our institute (Inst. of Medical Microbiology, SE) organised the Semmelweis Symposium, where two pneumococcal talks were delivered, one by me (*Orsolya Dobay*, PI) and one by *Dr. Mark van der Linden*, who is our major international collaborative partner and he is also named on the OTKA grant.

Finally, two babies were born to the participants of our work group during this grant!

1. *Streptococcus pneumoniae*

There are two types of pneumococcal vaccines: purified polysaccharide vaccine for adults (Pneumovax) and conjugated vaccines for children (PCV-7, PCV-10 and PCV-13, based on the number of included serotypes). In Hungary, PCV-7 was included in the national immunisation programme (NIP) as a recommended vaccine in April 2009, and in September 2010, PCV-13 replaced it. **PCV-13 became obligatory in July 2014**. These changes make the pneumococcal epidemiological investigations especially interesting, as **due to the vaccine pressure, robust and rapid rearrangement of serotypes could be observed in all countries**. As children receive the vaccines in 3 portions, at the age of 2, 4 and 12 months, by enrolling children aged 1-6 years old in the examinations, **we could very nicely follow these changes also in Hungary.**

1.1. Pneumococcus carriage among healthy children in the post-PCV7 era

S. pneumoniae is a leading human pathogen, still being responsible for the death of half million children aged <5 world-wide [WHO, 2012]. The infection mostly derives from symptomless carriers, usually small children, as this bacterium repeatedly colonizes their nasopharynx [Bogaert et al.,

2004]. Therefore, **we have screened healthy young children, attending day-care centres (DCCs), for pneumococcal colonisation**.

2262 children were involved throughout the study period. Children were divided into two groups according the PCV-7 vaccination rates: the average was 16.4% in GR1 (2009-2010) and 48.0% GR2 (2010-2012). The carriage rates in the two groups were 34.1% and 32.5%, showing only a slight decrease. Partial results about GR1 were reported already in the previous OTKA grant (PD75660), so here we focused on the differences between GR1 and GR2.

Meanwhile the serotype situation in GR1 still reflected the high prevalence of PCV7 serotypes (44.0%), serotype distribution in GR2 showed a drastic rearrangement. The rate of PCV-7 types decreased markedly to 8.1%, and for example serotype 14 vanished completely. On the other hand, the prevalence of non-vaccine types increased considerably. The frequency in ranking order was: 19A, 23A, 15B, 11A, 24F, 35F, 6A, 3, etc.; PCV13-non-PCV-7 types (3, 6A, 19A) were responsible for 24.5% of all strains. One of the most striking differences in the serotype distribution occurred with 19A: while there were only 3 isolates in GR1 (1.4%), its contribution increased to 11.5% in GR2. This corresponds to a more than 8-fold increase in frequency. So we investigated serotype 19A more deeply (see point 1.2).

It was interesting to observe that the overall resistance rate remained stable over time, but meanwhile several serotypes were represented in GR1, the absolute dominance of serotype 19A in GR2 was clear, in case of both penicillin and erythromycin non-susceptibility.

The results of this study were published in several papers and a conference abstract:

- Adrienn Tóthpál, Szilvia Kardos, Krisztina Laub, Károly Nagy, Tamás Tirczka, Mark van der Linden, Orsolya Dobay (2015): Radical serotype rearrangement of carried pneumococci in the first 3 years after intensive vaccination started in Hungary. European Journal of Pediatrics 174:373-381. IF=1.791
- Tóthpál Adrienn, Laub Krisztina, Kardos Szilvia, Nagy Károly, Dobay Orsolya (2014): Invazív és hordozott *Streptococcus pneumoniae* izolátumok vakcina lefedettsége Magyarországon. Lege Artis Medicinae (LAM), 24(4):180–185.
- Dobay Orsolya, Tóthpál Adrienn (2014): Pneumococcus elleni konjugált védőoltások. Gyermekorvos Továbbképzés 13(3):131-133.
- Tirczka Tamás, Tóthpál Adrienn, Berta Brigitta, Dobay Orsolya (2015): *Streptococcus pneumoniae* szerotípus megoszlásának vizsgálata 2013-2015 közötti időszakban Magyarországon. Mikrobiológiai Körlevél 15:14-27.
- A. Tóthpál, E. Kovács, K. Laub, Sz. Kardos, T. Tirczka, M. van der Linden, O. Dobay: Summarized data of carried pneumococcus before and after PCV vaccinations, between 2009-2013, in Hungary (EP0164). 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, Netherlands, April 2016.

1.2. Emergence of serotype 19A among carried pneumococci, due to the effect of PCV-7

Serotype 19A is especially of great concern, as it is known to have high invasive disease potential [ECDC, 2015A] and antibiotic resistance capacity [ECDC, 2015B], and has often been reported world-wide as an emerging serotype after the implementation of PCV7 [Weil-Olivier et al., 2012].

Altogether 61 isolates of serotype 19A (3 in GR1 and 58 in GR2) were detected in our study. Only one isolate was fully resistant to penicillin, but 67.2% of them fell into the intermediate category. More worrisome, almost all were highly resistant to both erythromycin and clindamycin (MIC >256 mg/L).

Based on the information extracted from the questionnaires, two important risk factors could be identified for 19A carriage: male gender (63.9% males) and passive exposure to smoking (49.2%).

The PFGE patterns of the serotype 19A isolates showed several clusters, which shared similar penicillin susceptibility levels. Members of a small PFGE clone proved to be ST320, a famous international resistant clone [Hsieh et al., 2013]. MLST generally showed good correlation with PFGE. Comparison of our new isolates with the previously frequent PMEN clone Hungary19A-6, which typically was resistant to penicillin, showed no similarity.

The results of this study were published in the following paper:

- Adrienn Tóthpál, Krisztina Laub, Szilvia Kardos, Tamás Tirczka, Adrienn Kocsis, Mark van der Linden, Orsolya Dobay (2016): Epidemiological analysis of pneumococcal serotype 19A among healthy children following PCV7 vaccination. Epidemiology and Infection 144: 1563-1573. IF=2.075

1.3. The effect of PCV-13 on pneumococcal carriage

For this study, we have screened either younger children (aged 1-3 years), or children attending DCCs but who were definitely vaccinated already with PCV-13 (and vaccination rate was very high, near 100%). Altogether 749 children were screened: 565 young (1-3 y) children (227 in 2013 and 338 in 2015), and 186 DCC children (3-6 y) in 2015-16. Among the young ones, carriage rate was much higher (45.8%) compared to those observed earlier among DCC children, which is in good correlation with data from the literature, stating that carriage peaks at around 3 years [De Lencastre – Tomasz, 2002].

The serotype distribution reflected the robust impact of PCV-13. This vaccine has six additional serotypes compared to PCV-7, including **19A and 3**. Both serotypes are of high importance. Meanwhile serotype 19A became dominant in the post-PCV-7 period (1.1), now we found only 3 isolates out of the total of 300 carriers (=1.0%) and even **serotype 3 has completely disappeared**! On the other hand, new serotypes are emerging. The major new serotypes in ranking order were: 15B/15C (19.7%), 23A/23B (16.3%), 11A/11F (13.3%), 35F (10.7%), 10A (5.7%) and 24F (5.3%). Most of these types are not present in the Pneumovax vaccine, either. A little worryingly, serotype 19F (an old PCV-7 type) seems to be increasing again; we found 10 isolates (3.3%).

These new types are however more sensitive to antibiotics. No penicillin resistant isolates were detected; the intermediate rate was 20.7%. As usual, erythromycin resistance was relative high (17.3%). The majority of these resistant isolates were of serotype 23A.

The results of this study were summarised in a poster:

- A. Tóthpál, Sz. Kardos, K. Laub, T. Tirczka, M. van der Linden, O. Dobay: Sharp decrease of serotypes 19A and 3 among carried pneumococci from children aged 1-3 years attending nurseries, after PCV13 vaccination in Budapest, Hungary (P0942), 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, May 2015.

In conclusion, monitoring of pneumococcal serotype changes must be continued in the next few years, and there is a continuous need for new vaccines. These could be either higher valency conjugate vaccines (a 15-valent vaccine is under clinical trials, with the two additional types being 22F and 33F, [Caro-Aguilar et al., 2017])**, or other approach, such as whole-cell or protein-based vaccines** [Alderson, 2016].

1.4. Acute otitis media (AOM) isolates

S. pneumoniae is one of the leading causative agents of otitis media, which is the main reason for antibiotic prescription in children in developed countries [Klein, 2000]. According to American data, 80% of <1-year-old children experience at least one episode of AOM, and 40% of them have relapses before the age of seven [Vergison et al., 2010]. The conjugate vaccines (PCVs), although originally were designed to prevent invasive diseases, were shown to have an effect also on the incidence and serotype distribution of AOM. In this study, we wanted to survey the serotype arrangement of AOM isolates in Hungary.

The study included 497 pneumococci from middle ear specimens, referred to the National Center for Epidemiology (NCE), between November 2008 and December 2014. 73.4% of the patients were <5 years old, and 57.7% were men. 44 different serotypes were found in all, but serotypes 3 and 19A together represented half (49.7%) of the isolates. The most prevalent serotypes were the following: 3 (n=130), 19A (n=117), 19F (n=33), 15A (n=17), 11A (n=14), 7F (n=14), 15C (n=13), 15B (n=11). Based on these figures, the PCV-7 types accounted for 13.9% and PCV-13 types for 69.6%.

Our results could be compared to a former study in 2002-2005, involving 73 AOM isolates [Ivády et al., 2009], when PCVs were not used in Hungary yet. Since then, some PCV-7 types have completely disappeared, serotype 3 has remained quite constant, meanwhile 19A peaked in 2011, but started declining in 2013. **These results also reflect very well, how rapidly pneumococci respond to the selective pressure of vaccinations.**

Penicillin resistance of the strains was 5.6% (n=27), erythromycin resistance was 34.9%. **Serotype 19A had high MICs for beta-lactams (providing 25 isolates of the total of 27 penicillin^{R}, 31/34 ampicillin^{R} and 21/21 ceftriaxon^{R} isolates) and macrolides**, while serotype 3 was sensitive to the tested drugs. PFGE examination of the 19A isolates revealed the predominance of a major pattern, members of which belong to the multiresistant ST-320 clone, identified also in our carriage study (see point 1.2).

The results of this study were summarised in a poster (manuscript is in preparation):

- T. Tirczka, Zs. Párkányi, B. Berta, A. Tóthpál, Sz. Kardos, M. van der Linden, O. Dobay: *Streptococcus pneumoniae* AOM isolates from Hungary, over a 6-year period (EP0014). 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, Netherlands, April 2016.

2. *Staphylococcus aureus*

2.1. *S. aureus* carriage

Collection of the isolates started in 2009, when 300 university students were screened for *S. aureus* carriage. Later the screening was continued among healthy young children, attending day-care centres (DCCs), in parallel with the pneumococcal screening. In the first period, 878 children were examined from16 different cities and villages all over Hungary, and subsequently 1390 children were involved in the survey from a single town: Szolnok. In total 2568 individuals were tested and 749 carriers found, resulting in a 29.2% overall carriage rate. Average carriage rate was very similar among students and children (29.3% versus 29.1%), but interestingly, somewhat higher rate was detected among Hungarian students (65/205 = 31.7%), compared to non-Hungarians (23/95 = 24.2%).

We found only six isolates carrying the *mecA* gene, i.e. MRSAs. Oddly, only three of these were also phenotypically oxacillin resistant and one strain was sensitive to both oxacillin and cefoxitin. Five isolates belonged to ST45, these carried the SCC*mec* type IV cassette, and one was ST7, SCC*mec* type V. All six MRSAs were sensitive to ciprofloxacin, gentamycin, tetracycline and mupirocin as well. Otherwise, **the carried MSSA isolates were much more sensitive to antibiotics compared to clinical ones** (data obtained from the National Centre for Epidemiology, NCE); beside the high penicillin resistance (>90%), only macrolide resistance was significant (9-10%).

Male gender was significantly higher among carriers (p=0.003), meanwhile passive smoking was a statistically non-significant risk factor for carriage. The isolates were genetically rather diverse, however several sub-clusters could be identified. Some MSSA isolates shared the same sequence types with the MRSAs. Sometimes the same PFGE patterns could be found in distant cities or different university groups, on the other hand different patterns were present within the same DCC or university groups. **In conclusion, *S. aureus* strains are in a dynamic change over time, which is reflected better by the more sensitive PFGE method within a country of the size of Hungary.**

The results of this study were published in the following 2 papers:

- Krisztina Laub, Adrienn Tóthpál, Szilvia Kardos, Orsolya Dobay (2017): Epidemiology and antibiotic sensitivity of *Staphylococcus aureus* nasal carriage in children in Hungary. *Acta Microbiologica et Immunologica Hungarica* 64:51-62. IF=0.921
- Krisztina Laub, Adrienn Tóthpál, Eszter Kovács, Judit Sahin-Tóth, Andrea Horváth, Szilvia Kardos, Orsolya Dobay (2017): High prevalence of *Staphylococcus aureus* nasal carriage among children in Szolnok, Hungary. *Acta Microbiologica et Immunologica Hungarica*, accepted for publication, doi: 10.1556/030.65.2018.001; IF=0.921

2.2. *S. aureus* and *S. pneumoniae* dual carriage

Among all children's specimen, *S. aureus* and *S. pneumoniae* were screened simultaneously. We detected both bacteria from the same nasal sample in 161 occasions, which equals to 7.1% dual carriage. Considering just *S. aureus carriers*, 24.4% of them were co-colonised with pneumococcus, while among the *S. aureus* non-carriers this ratio was 36.3%. By statistical analysis, this difference proved to be significant ($p=3.9 \times 10^{-8}$), which means that ***S. pneumoniae* colonisation is in negative correlation with *S. aureus* nasal carriage**. Similar negative association between these two bacteria is know from the literature as well (Regev-Yochay et al. 2004; Van Gils et al. 2011; Bosch et al., 2016).

The serotype distribution of the co-carried pneumococci was similar to the general distribution. We also analysed the possible effect of PCV-7 vaccination on *S. aureus* carriage. DCCs were divided into two groups based on the level of vaccination rate: low-level under 50% (average: 24.6%), or high-level above 50% (average: 67.9%). According to statistical analysis, **the positive correlation between high-level PCV-7 vaccination within the children's community and *S. aureus* nasal carriage was obvious** ($p=5.2 \times 10^{-9}$).

These results are not published yet.

2.3. Atypical *S. aureus* isolates

During the large scale screening, we have encountered a few atypical *S. aureus* isolates. Out of the total 751 *S. aureus* strains, we found one methicillin-sensitive, **catalase-negative *S. aureus*** (CNSA). Our CNSA isolate possessed a novel nonsense point mutation in the *katA* gene leading to early truncation of the protein product. Only two nonsense mutations were reported before, one from Hong Kong (To et al., 2011) and one from Chile (Lagos et al., 2016), i.e., from two different continents. Our strain belonged to sequence type 5 (ST5), which is a successful, worldwide spread, usually MRSA clone, associated with haematogenous complications (Fowler et al., 2007).

Catalase has been described as a virulence factor of *S. aureus* strictly required for nasal colonisation (Cosgrove et al., 2007). Accordingly, all previous catalase-negative isolates derived from infections and this is the first report of a CNSA from a symptomless carrier.

In addition, we also found nine **clumping-negative *S. aureus*** strains. Interestingly, they were positive with the tube coagulase test, but repeatedly negative with two different commercial clump-tests. The nine isolates had identical antibiotic sensitivity rates and shared the same PFGE patterns, indicating a clonal origin. In case of both unique phenotypes (i.e., catalase- and clump-negativity), the species identity was confirmed by the presence of the *nucA* gene and by MALDI-TOF analysis.

The catalase-negative *S. aureus* was published in a paper:

- Laub K, Kristóf K, Tirczka T, Tóthpál A, Kardos S, Kovács E, Sahin-Tóth J, Horváth A, Dobay O. (2017) First description of a catalase-negative *Staphylococcus aureus* from a healthy carrier, with a novel nonsense mutation in the *katA* gene. *International Journal of Medical Microbiology* 307:431-434. IF=3.391

2.3. Carriage of coagulase-positive staphylococci (CPS) among healthy owners and their dogs

We made a little investigation whether to what extent could potentially pathogenic bacteria be exchanged between humans and their dogs. We surveyed the carriage of coagulase-positive staphylococci among 102 dogs (collecting nasal, oral and skin specimen) and their 84 owners (only nasal specimen). Meanwhile the most important coagulase-positive species in humans is naturally *S. aureus*, it is *S. pseudintermedius* in dogs. The carriage rate was 23.8% (20/84) in humans and 4.9% (5/102) in dogs regarding *S. aureus*; the *S. pseudintermedius* carriage rate was inversely 2.4% in humans and 34.3% in dogs. In two occasions we detected *S. aureus* from both the owner and the dog, but they shared the same strain in only one occasion. The same *S. pseudintermedius* strain was found in the dog and its owner in one case.

We found no oxacillin-resistant isolates (MRSA or MRSP) in this study. Interestingly, the penicillin- and gentamicin resistance of the *S. pseudintermedius* strains was significantly lower compared to *S. aureus*, but their tetracycline resistance was much higher.

In this study we could prove the transmission of CPS between humans and companion animals, highlighting the risk for potential zoonotic infections caused by *S. pseudintermedius*.

The results of this study were summarised in a poster:

- E. Kovács, K. Laub, Sz. Kardos, O. Dobay, A. Tóthpál: Carriage of coagulase positive staphylococci among healthy owners and their dogs (P0218). 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, Netherlands, April 2016.

3) *Streptococcus agalactiae*

3.1. *S. agalactiae* isolates from the Semmelweis University Obstetrics and Gynaecology Clinics I and II, Budapest

Streptococcus agalactiae (GBS, group B Streptococcus) is one of the most important causes of neonatal sepsis and meningitis (Melin, 2011). The infection of newborns originates mostly from asymptomatic maternal colonisation. In this first GBS project, we have examined 115 GBS isolates collected between 2008-2011, originating from pregnancy screening.

All isolates were fully penicillin sensitive; on the other hand, macrolide resistance proved to be a major problem: 24.3% of the isolates showed the MLSB phenotype, these all carried the *ermB* gene. In addition, 24 strains carried the *ermB* gene, but were phenotypically macrolide sensitive.

We identified the serotypes of the strains with both antisera and PCR. Serotype III was most frequent (40.9%), followed by types V and Ia (24.3% and 17.4%, respectively). No serotypes VI, VII, VIII or IX were found.

We also detected the presence of the major surface proteins (alpha-C, rib, alp2, alp3, and epsilon) by PCR. The serotypes showed strong correlation with the presence of surface proteins: rib was associated mostly with type III, alp2/3 with type V, while epsilon with type Ia.

In the case of *S. agalactiae*, certain group of bacteria are distinguished by their increased disease capacity in neonates, these are called the hypervirulent clones. This is in close correlation with the presence of a surface-exposed protein, Gbs2018, which contributes to the enhanced adhesive properties of this bacterium. It has two major variants: Gbs2018C (or HvgA) is strictly specific for ST17, a major hypervirulent clone of *S. agalactiae* (almost always serotype III), while Gbs2018A (or BibA) is widely distributed among GBS isolates, representing several different clones and serotypes (Lamy et al, 2006; Santi et al, 2007; Tazi et al, 2010). Strikingly, we could identify ST-17 in 37.4%, these were always serotype III. Within the ST-17 clone, PFGE analysis could identify four smaller clusters.

With this study we could hopefully contribute to the better understanding of the epidemiology of *S. agalactiae* in Hungary, and so, enhance the introduction of obligatory pregnancy screening. As 37.4% of the isolates belonged to the ST-17 clone, we suggest that detection of these clones should be included in the screening. Additionally, as in case of penicillin allergy, clindamycin is administered as intrapartum antibiotic prophylaxis, susceptibility testing to macrolides and related drugs should always be routinely performed.

The results of this study were summarised in a poster:

- Sz. Kardos, K. Laub, A. Tóthpál, K. Kristóf, E. Ostorházi, K. Nagy, O. Dobay: Epidemiological survey and characterisation of *Streptococcus agalactiae* isolates from the Semmelweis University Clinics, Budapest, Hungary (P1529). 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, Spain, May 2014.

3.2. *S. agalactiae* isolates from the Dermatology Clinic of Semmelweis University, Budapest

Although GBS still nowadays ranks as the number one infectious cause of neonatal mortality in developed countries, it has recently been more frequently associated also with infections in non-pregnant adults. Some authors suggest that GBS can be transmitted sexually as higher colonization rates were observed among STD patients, even from male urethra. Therefore, we wanted to survey GBS isolates deriving from non-pregnant adults, especially STD patients.

We have examined 96 GBS strains, isolated at the Dermatology, Dermatooncology and Venerology Clinic of Semmelweis University, Budapest, over a 1-year period. The vast majority of the isolates (n=76) derived from the STD ambulance, the others form the general ambulance. Most specimens were either vaginal (n=38), urethral (n=30), or from skin (n=13). The age of patients ranged between 15 and 77 years, with a mean age of 42.4 years. The genders were nearly equalised: 50 females and 46 males.

All isolates were fully penicillin sensitive; on the other hand, macrolide resistance was 41.7%. Thirty-two of these isolates carried the ermB gene (1 isolate had ermB + linB together) and 6 had the mef gene (5 mefE and 1 mefA). Tetracycline resistance was also very high (82.3%); the tetracycline sensitive isolates were also sensitive to macrolides or had M type (i.e., low-level ery R).

In contrast with the vaginal colonisation, where serotype III dominated, here serotype V was most frequent (30.2%), followed by types III and Ia (26.0% and 22.9%, respectively). The other serotypes were less prevalent: IV (10.4%), II (5.2%) and Ib (4.2%). One isolate was not typeable.

The same strong correlation was observed between serotypes and surface proteins as in point 3.1.

Out of the 96 isolates, 21 were hvgA+. Among these, almost all belonged to ST17 (serotype III), but one strain (serotype IV!) proved to be ST291, which is also part of clonal complex 17 (CC17), and differs only in a single nucleotide from ST17 in the pheS allele. The other 75 isolates were bibA+.

In total among the pregnancy screening samples and the dermatology samples, MLST was performed for 13 bibA+ strains. According these results, four isolates belonged to clonal complex 8 (three ST 8, serotype Ib and one ST12, serotype II); four belonged to CC1 (three ST1, serotypes Ib and V and one ST196, serotype IV); two belonged to CC23 (ST23 and ST24, both serotype IV). One strain each was ST110 and ST255, and finally, in case of one strain (serotype V), a completely novel ST was identified, which had a 44-nucleotide deletion in the atr allele.

We have provided the first genotypic data (MLST, surface proteins) about *S. agalactiae* in Hungary, beside, never so many serotyping information was reported before as now.

The results of this study were summarised in a poster (manuscript is in preparation):

- Sz. Kardos, A. Tóthpál, K. Laub, K. Kristóf, E. Ostorházi, F. Rozgonyi, O. Dobay: *Streptococcus agalactiae* isolates from the Dermatology Clinic of Semmelweis University, Budapest, Hungary (P0692). 25th ECCMID, Copenhagen, Denmark, April 2015.

4) Other research topics where our bacterial isolates were used and therefore the OTKA grant was acknowledged in the papers

4.1. Uracil enrichment in bacteria

Uracil is one of the most frequently occurring erroneous bases in DNA and dUTPase is the major enzyme primarily involved in keeping DNA uracil-free. Surprisingly, we have found that in contrast to the generally held opinion, a wide number of bacterial species (such as *Staphylococcus aureus*) lack the dUTPase gene, which might potentially lead to an unusual uracil-enrichment in their genomic DNA.

- Csaba Kerepesi, Judit E. Szabó, Veronika Papp-Kádár, Orsolya Dobay, Dóra Szabó, Vince Grolmusz, Beáta G. Vértessy (2016): Life without dUTPase. Frontiers in Microbiology. 7:1768. IF=4,076

4.2. Bicarbonate inhibits bacterial growth

Bicarbonate directly and indirectly affects lung function, e.g. influences the pH of the airway surface liquid (ASL) via the HCO_3^-/CO_2 buffer system. Especially in cystic fibrosis (CF) bicarbonate plays a key role via the CFTR receptors. It was recently shown that impaired bicarbonate secretion is likely responsible for aggregated mucus and aerosolizing bicarbonate onto the porcine CF airways increased innate bacterial killing in vivo. Thus, we investigated the effects of bicarbonate on CF-related bacteria (such as *S. aureus*). We could show a definite inhibition of bacterial growth not only in planktonic phase, but also on biofilm formation. One possible mechanism could be that bicarbonate increases intracellular cAMP levels, which negatively influences biofilm formation.

- Krisztina Laub, Orsolya Dobay, Balázs Stercz, Adrienn Kéri, Bernadett Balázs, Adrienn Tóthpál, Szilvia Kardos, Paul M. Quinton and Ákos Zsembery (2017): Bicarbonate inhibits bacterial growth and biofilm formation. Journal of Applied Microbiology, accepted for publication. IF=2,099

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