The asymptomatic carriage of four potentially pathogen respiratory tract bacteria in children attending communities

PhD thesis

Eszter Tamara Kovács

Doctoral School of Pathological Sciences

Semmelweis University

Supervisor: Orsolya Dobay, PhD

Official reviewers: Levente Emődy, MD, DSc

Tibor Zelles, MD, PhD

Head of the Final Examination Committee: Edit Buzás, MD, DSc

Members of the Final Examination Committee: Csire Márta, PhD

Lohinai Zsolt, MD, PhD

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Introduction

Streptococcus pneumoniae (*S. pneumoniae*), *Staphylococcus aureus* (*S. aureus*), *Haemophilus influenzae* (*H. influenzae*) and *Moraxella catarrhalis* (*M. catarrhalis*) are common pathogens found in the respiratory tract. They can cause a wide range of infections from mild to severe, life-threatening diseases like bacteremia, meningitis and pneumonia. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* are often associated with acute otitis media, which is the number one reason for antibiotic prescription worldwide. Due to the armada of virulence factors possessed by *S. aureus*, it can cause a variety of diseases which can be classified into two groups: exotoxin-mediated ones and direct bacterial organ invasion associated diseases. Despite their strong pathogenic potential, they are often carried asymptomatically, especially by preschool children attending communities. In contrast to children, adults are less often colonized, consequently, may be infected upon exposure, for example after contact with grandchildren carrying pathogens. Thus, carrier children are significant sources of infections in the surrounding population.

Currently, vaccination is available against *S. pneumoniae* and *H. influenzae* serotype b (Hib). There is a 23-valent pneumococcal polysaccharide vaccine which contains purified capsule polysaccharides from 23 of the currently known more than 95 serotypes. Unfortunately, polysaccharide vaccines are poorly immunogenic under two years of age because B cell responses are T cell dependent mainly, but responses to polysaccharides are T- independent, therefore memory B cells are not developed.

To avoid this problem, pneumococcal conjugated vaccines (PCVs) were developed. First, PCV7 was implemented in the USA in 2000, later its spectrum was extended, PCV10 and PCV13 were marketed. PCV7 successfully decreased PCV7 serotypes among invasive diseases, but on the other hand, an increased incidence of non-PCV7 types, especially 19A was observed.

In Hungary, vaccination with PCV7 started in 2005 and it was replaced by PCV13 in 2010, which was then made mandatory in July 2014.

Conjugated Hib vaccine is composed of the type b capsule of *H. influenzae* and diphtheria toxoid. Similarly to the above mentioned pneumococcal conjugate vaccine, addition of protein toxin was necessary to activate T lymphocytes. In Hungary, Hib vaccination is mandatory in a 3+1 scheme since 1999.

Penicillin resistance or non-susceptibility is rapidly disseminating among *S. pneumoniae* isolates. Penicillin resistance is related to structurally modified penicillin-binding proteins.

Penicillin resistance in *S. aureus* can also occur. Here, the biggest problem is the presence of methicillin resistant *S. aureus* (MRSA) strains as they are resistant to penicillinase-resistant penicillins and all β-lactam antibiotics. The first MRSA strain was reported in 1961, two years after methicillin was introduced into clinical practice. For the eradication of nasal *S. aureus* as well as MRSA carriage, mupirocin ointment is used most commonly.

H. influenzae isolates are also frequently resistant to β-lactam antibiotics showing different resistance mechanisms. Regarding *M. catarrhalis*, almost 100% of the strains can produce β-lactamase.

Also resistance to other antibiotics appeared, therefore the treatment of infections caused by the investigated four species has become more difficult and expensive.

Objectives

The objective of this study was to survey the nasal carriage rates of four important respiratory pathogens (*S. pneumoniae, S. aureus, M. catarrhalis* and *H. influenzae*) in three different age groups of children attending communities, in 2015-2016. This was the first time in Hungary to conduct a survey about the asymptomatic carriage of *M. catarrhalis* and *H. influenzae*. Besides their individual carriage rates, we could also examine the co-carriage of the four species.

We tried to specify certain risk factors for their nasal carriage as well as determine their antibiotic resistance.

Serotype distribution was also investigated where applicable. The aim of this study was to follow the effects of the pneumococcal conjugate vaccine on pneumococcal serotype replacement, as well as on the carriage prevalence of the other three species. Hungary is a good model for vaccine efficacy monitoring as it is a country with a very strict vaccination policy, unlike some European countries which have a significant level of vaccine sceptics.

Genetic relatedness was determined for all *H. influenzae* and *S. aureus* isolates and some selected serotypes of *S. pneumoniae* to gain an insight into their clonal spread. In case of *S. pneumoniae*, four specific serotype 19F isolates were more closely analysed by multilocus sequence typing (MLST), as this type was represented in a surprisingly high proportion despite that it was already included in PCV7 it is due to its elevated capability to cause invasive diseases.

Clinical pneumococcal isolates were obtained from the same time period (2015-2016) to compare serotype distribution and antibiotic resistance pattern of clinical and carried isolates.

Epidemiological surveys are valuable tools in predicting changes regarding dominant serotypes, genotypes in the near future, estimating their prevalence among invasive disease causing types. They can also provide crucial information facilitating prevention and control of diseases, such as vaccine development.

Materials and methods

Study population

In total, 580 asymptomatic children belonging to three different age groups (1-3y, 3-6y, 6-13y) were tested between March 2015 and May 2016 deriving from nurseries, day-care centers (DCCs) and a primary school. In total, the genders were absolutely equally represented with 288 males and 288 females.

As a second part of the study, we obtained 146 clinical pneumococcal isolates from the Institute of Laboratory Medicine, Semmelweis University, Budapest in the same time frame. We received pure cultures on blood agar plates after routine laboratory identification.

Nasal samples were taken from both nostrils with soft cotton swabs and inserted into active charcoal containing Amies transport media (Transwab, Medical Wire & Equipment, Corsham, UK). The swabs were transported to the microbiology laboratory within 24 hours. A questionnaire was filled in by the parents of the participants anonymously with questions related to risk factors.

Identification of *S. pneumoniae*, *S. aureus*, *H. influenzae* and *M. catarrhalis*

The collected samples were inoculated onto Columbia blood agar plates (for *S. pneumoniae* and *S. aureus*) and onto vancomycin containing chocolate agar plates for the selective cultivation of *H. influenzae* and *M. catarrhalis*. Colonies showing typical phenotypes were chosen to produce pure cultures. These species-specific phenotypes were the following: In case of pneumococcus, the mucoid or flat colonies which were collapsed in the middle and showed α-haemolysis on blood agar were further tested for optochin sensitivity (5µg discs, Mast Group Ltd., Bootle, UK). *S. aureus* was suspected if the colony had a β-haemolytic zone and had a positive catalase and clump test (Pastorex Staph-Plus Kit, Bio-Rad, Marnes-la-Coquette, France). On the chocolate agar plates supplemented with X- and V-factors, the smooth, round, colorless or greyish colonies were identified as *H. influenzae,* subsequently confirmed with a positive catalase and oxidase test. *M. catarrhalis* often grew as a pure culture on the chocolate agar plates. The typical snow white colonies with irregular edge were able to show the "hockey puck sign" (i.e., sliding along the surface without hindrance if taken by an inoculation loop) and were oxidase positive. The phenotypically confirmed isolates were frozen and stored at -80˚C on cryobeads (Cryobank, Mast Group Ltd., Bootle, UK) until further testing.

On genetic level, species specific gene targets were amplified in a polymerase chain reaction (PCR) reaction to identify each species: *lytA* gene for *S. pneumoniae*, *nucA* for *S. aureus, ompP2* for *H. influenzae* and a specific *16S rRNA* sequence for *M. catarrhalis*.

Serotyping

To determine the serotypes of the pneumococcal isolates, the Pneumotest Latex Kit (Statens Serum Institut, Copenhagen, Denmark) was used. Factor determination was done either by PCR by us, or by the Quellung method at the National Public Health Center and the German National Reference Centre for Streptococci (GNRCS).

For the capsular typing of *H. influenzae and M. catarrhalis,* PCR was applied.

Antibiotic susceptibility testing

Basically, agar dilution method was used to determine the minimum inhibitory concentration (MIC) of all isolates. The following antibiotics were tested, where appropriate: penicillin, ampicillin, amoxicillin-clavulanic acid, oxacillin, cefotaxime, imipenem, tetracycline, erythromycin, clindamycin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, vancomycin, mupirocin and trimethoprim/sulfamethoxazole (TMP/SMX). The *S. aureus* isolates with an oxacillin MIC ≥0.25 mg/L were also screened with disc diffusion method using 30 μg cefoxitin discs (Bio-Rad). Mupirocin susceptibility of *S. aureus* isolates was tested by gradient strips (E-test) (Liofilchem, Roseto degli Abruzzi, Italy). In all cases the EUCAST (The European Committee on Antimicrobial Susceptibility Testing) guidelines and breakpoints were applied.

Mupirocin resistant *S. aureus* isolates were further investigated by PCR to distinguish low-level and high-level resistance.

In an in-house duplex PCR, *mecA* primer pair was concomitantly added to the *nucA* PCR mix in order to screen for methicillin resistant *S. aureus* isolates.

Macrolide resistant *S. pneumoniae* isolates were checked for four resistance genes (*ermA, ermB, ermTR, mefE/A*) by PCR.

Ampicillin resistant *H. influenzae* isolates were tested using nitrocefin discs (Sigma-Aldrich, St. Louis, USA) to detect the beta-lactamase production.

Genotyping by pulsed-field gel electrophoresis (PFGE)

PFGE was used to get information about the clonal relatedness of *S. pneumoniae*, *S. aureus* and *H. influenzae* isolates. Complete bacterial genome was digested with *SmaI* restriction enzyme. The analysis of the PFGE profiles (normalisation, gel comparison and dendrogram creation) was performed with the BioNumerics software version 2.5 (Applied Maths, Sint-Martens-Latem, Belgium).

Multi-locus sequence typing of *S. pneumoniae* isolates

MLST was also performed for four selected 19F *S. pneumoniae* isolates, based on their PFGE dendrogram. Well defined sections of seven housekeeping genes (aroE, gdh, gki, recP, spi, xpt, ddl) were amplified by PCR, using the primers provided on the MLST website. The products were purified and sent for sequencing to the BIOMI Ltd., Gödöllő, Hungary. The allele sequences were compared to the MLST database and the sequence types identified.

Statistical analysis

The Fisher's exact test of independence was employed to determine statistical significance of risk factors due to the small numbers. Applying a 95% confidence interval, p value < 0.05 was considered significant.

Results

Carriage rate

Out of the 580 screened children, 442 (76.2%) carried at least one of the four bacterial species. There was a clear age related bacterial prevalence: *M. catarrhalis*, *S. pneumoniae* and *H. influenzae* carriage decreased with age, meanwhile *S. aureus* prevalence showed an inverse tendency (Figure 1).

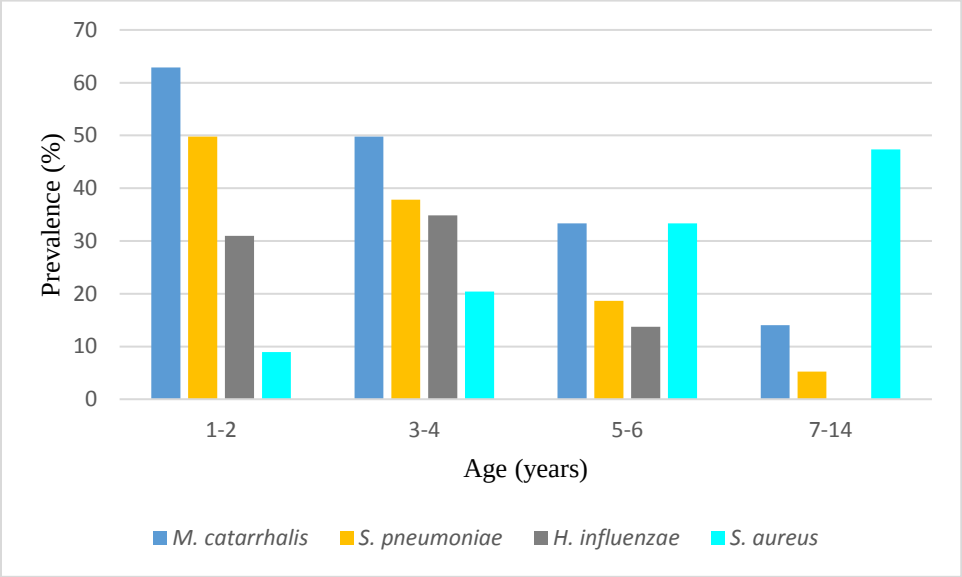


Figure 1. The prevalence of the four bacterial species

Multiple carriage occurred more frequently without *S. aureus* which was most obvious in case of triple and double carriage (Figure 2).

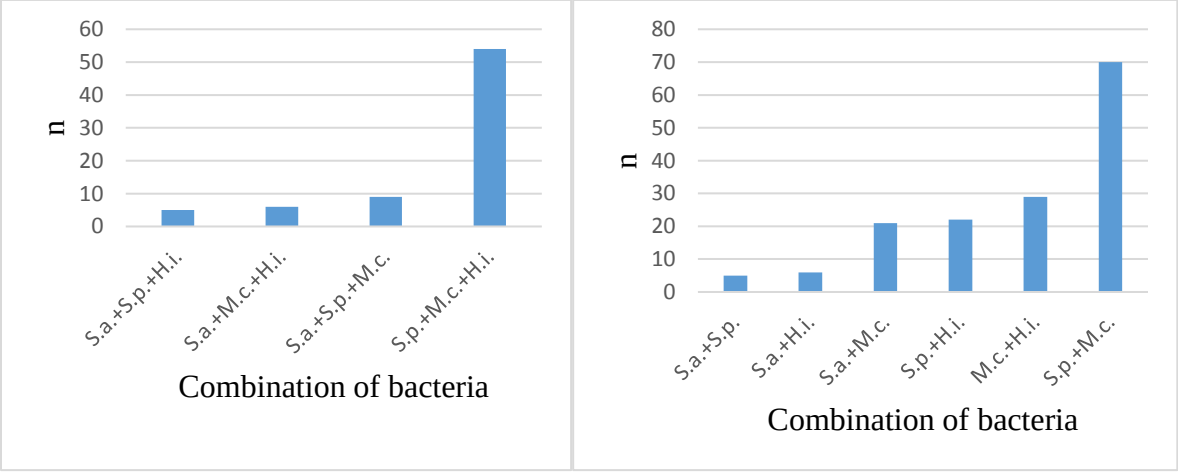


Figure 2. Triple and double carriage prevalence

During statistical analysis, we found a significant negative association between *S. aureus* and *S. pneumoniae* or *M. catarrhalis*, and it was nearly significant with *H. influenzae.* On the other hand, positive association existed between *S. pneumoniae-H. influenzae*, *S. pneumoniae-M. catarrhalis* and *H. influenzae-M. catarrhalis.*

Risk factors

Regarding gender, boys seemed to be colonized with *S. aureus* more frequently in the primary school group. Furthermore, having siblings was associated positively with *S. aureus* carriage in the summarized group (data from nurseries, DCCs and primary school were summarized). However, antibiotic exposure in the past two weeks affected negatively its carriage (cumulative data). Taking antibiotics also reduced *H. influenzae* carriage (but only in DCC group). Finally, passive smoking was negatively associated with *S. pneumoniae* carriage. *M. catarrhalis* was not influenced by any of the investigated factors.

Serotype distribution

S. pneumoniae

Among 208 pneumococcus carriers, 210 strains were isolated because double carriage was detected in case of two girls. PCV13 serotypes were hardly present in the three groups: 4.8% (19F, 19A) in nurseries, 9.8% (19F, 9V) in DCCs and 25.0% (7F) in primary school, while PPV23 serotypes were more prevalent: 34.5% (15B, 11A, 10A, 9N, 33F, 22F), 39.0% (11A, 15B, 10A, 17F) and 0.0%, respectively. The leading serotypes were 15B (17.0%) in nurseries and 11A (26.8%) in DCCs. An increasing prevalence with age was observed in case of serotype 23B (2.4% in nurseries, 9.8% in DCCs and 25.0% among school children), serotype 11A (6.7% in nurseries vs. 26.8% in DCCs) and 6C (1.8% vs. 9.8%), while 15B and 24F showed decreasing tendencies (17.0% vs. 4.9% and 11.5% vs. 0.0%, respectively). Comparing nurseries and DCCs, both PCV13 (4.8% vs. 9.8%) and PPV23 (34.5% vs. 39.0%) coverage was higher in DCCs.

We divided the clinical specimens into two groups: deriving from patients <7 years (P1) and ≥7 years (P2) to make an easier comparison between carriers of the same age group and to monitor the patients (P2) who were probably not vaccinated against pneumococcus. In P1, PCV13 and PPV23 serotype coverage was 17.5% and 22.2% while in P2, it was 32.5% and 28.9%. Carriers <7 years showed a spectacular tendency in vaccine coverage rate: only 5.8% for PCV13 and 35.4% for PPV23.

19F was represented in all three groups (Carriers <7 years, P1 and P2) as a prominent PCV13 serotype, whereas serotype 3 was completely absent among carriers. On the other hand, type 3 was the leading serotype in older patients (15.7%) explaining the high PCV13 coverage. Among PPV23 serotypes, 15B was the most frequent among carriers (14.6%) and in P1 (9.5%) whereas in P2 it had only 2.4%. Interestingly, 11A was the leading PPV23 serotype in P2 (12.0%) and it was the second leading in P1 (7.9%) and in carriers (10.7%).

H. influenzae

H. influenzae was not isolated in primary school children. In DCCs (n=37), 33 isolates (89.2%) were non-typeable *Haemophilus influenzae* (NTHi) and 4 (10.8%) were serotype f. In nurseries (n=115), 110 (95.7%) NTHi were found, 3 (2.6%) f and 2 (1.7%) e. Type b was not found throughout the study.

M. catarrhalis

All isolates (n=9) deriving from primary school children belonged to serotype A. In DCCs (n=70), serotype A was represented in 68.6% (n=48), serotype B in 18.6% (n=13), serotype C in 2.9% (n=2) and 10.0% (n=7) were non-typeable (NT). Out of 202 isolates, also serotype A was the most prevalent in nurseries with 89.1% (n=180), followed by B and C with 9.4% (n=19) and 1.0% (n=2). The remaining 1 isolate (0.5%) was NT.

Antibiotic susceptibility

S. pneumoniae

Among *S. pneumoniae* isolates, penicillin resistance was not detected. Only low level penicillin intermediate resistance was observed: 13.1%, 17.5% and 25.3% among carriers, patients <7 y and patients >7 y, respectively. Erythromycin resistance was 17.5%, 19.0% and 28.9% in the three groups, respectively. The macrolide resistant isolates (n=36 among carriers, n=12 in P1 and n=24 in P2) were further checked for *erm(B)* and *mef* genes. *Erm(B)* was detected in 38 cases which showed MLSB phenotype: high MIC values for both erythromycin and clindamycin. *Mef(E)* was found in 29 cases which had lower MIC values (8–16 mg/L) for erythromycin and were susceptible to clindamycin, corresponding the classic M phenotype. In addition, both genes were present in three cases (one strain each of serotype 19A, 19F and NT). Besides 11A (always M phenotype), 19F, 15A, 15C and 19A (MLSB type) were the major macrolide resistant serotypes. Serotype 3, 10A, 23A, 23B, 24F, 35B and 35F isolates were sensitive mostly to all tested antibiotics.

S. aureus

No MRSA isolates were found in our study. On the other hand, high-level mupirocin resistance was observed in three cases (2.4% of all strains), all of them originated from DCCs in Pápa. Regarding vancomycin, 100% sensitivity was measured. Penicillin resistance was very high in all three groups: 73.7% in nurseries, 76.8% in DCCs and 82.8% in primary school, respectively.

H. influenzae

Nine isolates in the nursery group (7.8%) and one from DCC (2.7%) were ampicillin resistant. All 10 strains had a MIC value >8mg/L, but all were susceptible to amoxicillin-clavulanic acid. This refers to beta-lactamase production which was confirmed by a positive nitrocefin disc test in every case. The highest resistance was detected for TMP/SMX (11.3% in the nurseries and 24.3% in the DCCs). To highlight the nine serotypeable isolates, all of them were fully susceptible to all tested antibiotics.

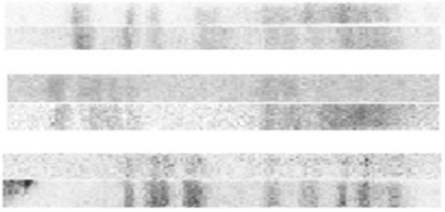
M. catarrhalis

In general, 100% sensitivity was documented in the case of amoxicillin-clavulanic acid, cefotaxime and moxifloxacin. Non-susceptibility to macrolides and TMP/SMX was also below 5% and always with low MIC values.

Genotyping results

S. pneumoniae

Fully identical PFGE profiles of clinical and carried isolates were found in case of serotypes 11A, 19A and 19F (Figure 3).



Strain	Origin	Serotype	Peni	Ery	Clinda	Date of isolation
16775	clinical	11A	<0.015	<0.06	<0.5	Apr-2015
BT10	carried	11A	<0.015	0.125	<0.5	Apr-2016
14749	clinical	19A	<0.015	0.125	<0.5	Apr-2015
BT75	carried	19A	0.03	0.125	<0.5	Apr-2016
45354	clinical	19F	0.03	256	128	Oct-2015
BT7	carried	19F	0.06	>256	>128	Apr-2016

Figure 3. Identical restriction pattern of clinical and carried isolates among serotypes 11A, 19A and 19F

Serotype 3

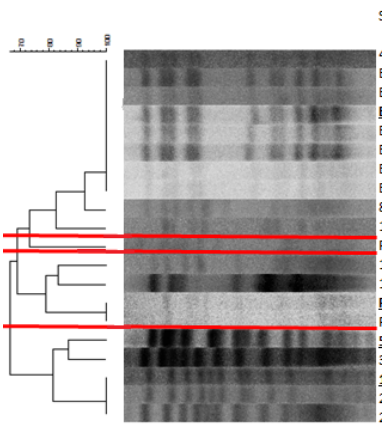
Regarding serotype 3, half of the isolates were indistinguishable (sharing the same pattern) and the rest of the clinical isolates were also closely or possibly related. Besides their general susceptibility to antibiotics, the two penicillin intermediate isolates (16744, 52128) did not belong to the same clone.

Serotype 19A

The restriction pattern of serotype 19A isolates was very variable. One isolate (8754) had the same pattern as a previously isolated strain which belonged to the worldwide circulating resistant ST320 clone. Not only their restriction pattern but their MIC values were identical as well. In addition, isolate 8754 expressed *erm(B)* and *mef(E)* genes together.

Serotype 19F

Their PFGE patterns showed three major clusters which corresponded well with their antibiotic sensitivity. The penicillin susceptible and high level macrolide and lincosamide resistant isolates formed the first and largest cluster, all of them deriving from Budapest. The second, smaller cluster comprised of fully susceptible isolates. Finally, cluster 3 contained only clinical isolates which had penicillin intermediate level resistance and low level erythromycin resistance, with one exception. Isolate 57103 (the only strain with elevated penicillin MIC and MLSB phenotype) stood alone in a separate subcluster. One representative of each three clusters and this latter strain (indicated with bold face and underlining on Figure 4) were chosen for MLST analysis. Based on this, cluster 1 strains belonged to ST179, cluster 2 to ST180. Isolate 57103 proved to be a member of the ST320 sequence type. Similarly to the previously mentioned ST320 isolate among serotype 19A isolates, 57103 also possessed *erm(B)* and *mef(E)* genes. The MLST type of the other cluster 3 isolate was ST651. ST651 belongs to the same clonal complex as ST320 (CC-271) which means they differ only in one out of the seven housekeeping loci.



Strain	Serotype	Peni	Ery	Clinda	Date of isolation	
45354	19F	0.03	256	128	Oct-2015	
BT7	19F	0.06	>256	>128	Apr-2016	
BT8	19F	0.06	256	>128	Apr-2016	
BT104	19F	0.06	>256	>128	May-2016	
BT112	19F	0.06	>256	>128	May-2016	Cluster 1
BT115	19F	0.06	>256	>128	May-2016	
BT122	19F	0.06	>256	>128	May-2016	
BT123	19F	0.06	>256	>128	May-2016	
8431	19F	<0.015	>256	>128	Feb-2015	
11204	19F	0.03	256	>128	Mar-2015	
PP273	19F	0.03	0.125	<0.5	Feb-2016	
18124	19F	0.06	0.125	<0.5	Apr-2015	
11406	19F	<0.015	<0.06	<0.5	Mar-2015	
PP266	19F	0.03	0.25	<0.5	Feb-2016	Cluster 2
PP272	19F	0.03	0.25	<0.5	Feb-2016	
57103	19F	1.5	>256	>128	Dec-2015	
38198	19F	1	8	<0.5	Aug-2015	
18017	19F	1	8	<0.5	Apr-2015	Cluster 3
24536	19F	2	8	<0.5	Jun-2015	
23535	19F	1	8	<0.5	2015	

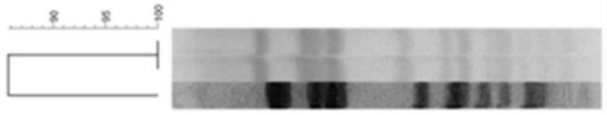
Figure 4. PFGE dendrogram of serotype 19F strains

Serotype 11A

Two major clusters could be detected: The first one contained isolates representing M phenotype, the second one comprised of sensitive isolates.

S. aureus

High-grade diversity was observed with many smaller clusters comprising of 2-5 members. Out of the three mupirocin resistant strains, two of them had the very same pattern and their antibiotic resistance pattern completely correlates with this partition (Figure 5). The third isolate (PP264) had a slightly modified PFGE and antibiotic profile (it was sensitive to erythromycin) but this was also in close relationship with the other two. It is supported by the information that all three isolates derived from Pápa, but the third one from a different DCC.



Strain	Peni	Ery	Clinda	Date of isolation
PP159	4	>256	0.5	Jan-2016
PP160	8	>256	0.5	Jan-2016
PP264	4	2	0.5	Feb-2016

Figure 5. PFGE pattern of the three mupirocin resistant *S. aureus* isolates

H. influenzae

NTHi isolates differed a lot genetically, only a few major clusters could be determined. Four of the seven *H. influenzae* serotype f isolates – originating from the same DCC – were indistinguishable, furthermore, their MIC values were the same. The serotype e isolates differed only in one band so they were closely related.

Conclusions

- This is the first study in Hungary investigating the asymptomatic carriage of *H. influenzae* and *M. catarrhalis*
- This is the first study in Hungary investigating the asymptomatic carriage of four respiratory pathogens together
- Carriage prevalence of bacteria changes along with the age of children
- Carriage prevalence of *S. aureus* shows an inverse pattern compared to the carriage of the other three investigated species
- The pneumococcal vaccination had caused a drastic serotype arrangement within the pneumococcal population, the most important changes being the following:
    - Serotypes 3 and 6A have completely disappeared from carriage in Hungary
    - Serotype 3 is still the leading serotype among elderly patients
    - Serotype 19A shows decreasing tendency while serotype 19F seems to re-appear
    - The replacing non-vaccine types (NVTs) have low invasive potential and lower proportion of antibiotic resistance
- The pneumococcal vaccination also had an effect on the prevalence of the other carried bacterial species
- The clinical pneumococcal isolates respond with delay to the selection pressure of conjugate vaccines
- No MRSAs were found among the carried *S. aureus* isolates
- All four species were more susceptible to the tested antibiotics compared to clinical isolates from Hungary in the same time period
- The two Gram-negative species were basically more sensitive than the two Gram-positive ones
- There were examples for the presence of isolates belonging to the same PFGE clone in different cities, indicating that certain clones have spread in a larger geographical area
- Some clones (e.g. pneumococcal ST320) could be detected already several years ago in previous carriage studies, indicating the long-term circulation of certain successful clones in Hungary

List of publications

Papers related to the thesis

Kovács E., Sahin-Tóth J., Tóthpál A., Linden M., Tirczka T., Dobay O. (2020) Co-carriage of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* among three different age categories of children in Hungary. PLOS ONE, 15: (2) Paper e0229021

Kovács E., Sahin-Tóth J., Tóthpál A., Kristóf K., Linden M., Tirczka T., Dobay O. (2019) Vaccine-driven serotype-rearrangement is seen with latency in clinical isolates: Comparison of carried and clinical pneumococcal isolates from the same time period in Hungary. Vaccine, 37: (1) pp 99-108

International congress abstracts related to the thesis

Kovács E., Sahin-Tóth J., Tirczka T., Tóthpál A., Dobay O. (2017) Nasal bacterial carriage among Hungarian children with a focus on *Staphylococcus aureus* (P0128) 27th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Vienna, Austria, April 2017

Papers not related to the thesis

Sahin-Tóth J., Kovács E., Tóthpál A., Juhász J., Forró B., Bányai K., Havril K., Horváth A., Ghidán Á., Dobay O. (2021) Whole genome sequencing of coagulase positive staphylococci from a dog-and-owner screening survey. PLOS ONE, 16: (1) Paper e0245351

Kovács E., Horváth A., Sahin-Tóth J., Kaptás Á., Huber A., Dobay O., Juhász E., Kristóf K. (2020) Tünetmentes meningococcus-hordozás felmérése Magyarországon egyetemisták és középiskolások körében. Gyermekgyógyászati Továbbképző Szemle, 25: (4) pp 14-16

Laub K., Tóthpál A., Kovács E., Sahin-Tóth J., Horváth A., Kardos S., Dobay O. (2018) High prevalence of *Staphylococcus aureus* nasal carriage among children in Szolnok, Hungary. Acta Microbiologica et Immunologica Hungarica, 65: (1) pp 59-72

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: (8) pp 431-434

International congress abstracts not related to the thesis

Tóthpál A., Kovács E., Laub K., Kardos S., Tirczka T., Linden M., Dobay O. (2016) 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.

Kovács E., Laub K., Kardos S., Kristóf K., Dobay O., Tóthpál A. (2016) 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.