VILNIUS UNIVERSITY

INDRĖ STACEVIČIENĖ

NASOPHARYNGEAL COLONISATION OF *STREPTOCOCCUS PNEUMONIAE* IN PRESCHOOL CHILDREN AND ITS INFLUENCE ON THE COURSE OF ACUTE RESPIRATORY TRACT INFECTIONS

Summary of Doctoral Dissertation

Biomedical Sciences, Medicine (06B)

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VILNIAUS UNIVERSITETAS

INDRĖ STACEVIČIENĖ

STREPTOCOCCUS PNEUMONIAE KOLONIZACIJA IKIMOKYKLINIO AMŽIAUS VAIKŲ NOSIARYKLĖJE IR JOS ĮTAKA ŪMINIŲ KVĖPAVIMO TAKŲ INFEKCIJŲ EIGAI

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Abbreviations

DCC – day-care centre;

ED – Emergency Department of Children's Hospital, an Affiliate of Vilnius University Hospital Santariškių Klinikos;

EUCAST – European Committee on Antimicrobial Susceptibility Testing;

IPD – invasive pneumococcal disease;

MDR – multidrug resistance;

MIC – minimal inhibitory concentration;

PCC – primary care centre;

PCV – pneumococcal conjugate vaccine;

PISP – penicillin intermediate *S.pneumoniae* (MIC >0.06–2.0 mg/l);

PNSP – penicillin non-susceptible *S.pneumoniae*;

PRSP – penicillin resistant *S.pneumoniae* (MIC >2 mg/l);

PSSP – penicillin susceptible *S.pneumoniae* (MIC ≤0.06 mg/l);

RTI – respiratory tract infection;

S.pneumoniae – *Streptococcus pneumoniae*;

URTI – upper respiratory tract infection;

WHO – the World Health Organization.

1. INTRODUCTION

1.1. *S.pneumoniae* infection – a serious public health concern

Streptococcus pneumoniae (*S.pneumoniae*, pneumococcus) is one of the major bacterial pathogens colonising the nasopharynx, which can cause a wide spectrum of illnesses from upper respiratory tract infection (URTI) to invasive pneumococcal disease (IPD), or the colonisation can be asymptomatic [1, 2].

S.pneumoniae infection is a serious public health concern in the world, especially among infants and young children. This infection is associated with high mortality – an estimated 1.6 million people, including 0.7–1 million children under the age of five years, die of pneumococcal diseases each year worldwide [3]. *S.pneumoniae* is one of the major causes of acute otitis media, pneumonia, sepsis and meningitis [4-8]. Most of these diseases often require antimicrobial treatment and *S.pneumoniae* has shown an increasing resistance to commonly used antimicrobials (macrolides, cephalosporins), which is another public health concern [9].

Pneumococcal disease is preceded by asymptomatic colonisation: the colonisation rate is especially high in children under the age of six years [1, 10]. In addition, nasopharyngeal carriage is a major factor in the horizontal transmission of *S.pneumoniae* strains and nasopharyngeal isolates reflect currently circulating strains in the community [11]. Young children are thought to be the most important source in horizontal dissemination of pneumococcal strains due to the high frequency of pneumococcal colonisation and high crowding in day-care centres (DCC) and families [1].

There is a wide variation in *S.pneumoniae* capsule polysaccharides: currently, 97 serotypes have been identified [12]. Different *S.pneumoniae* serotypes have different propensities to cause disease [13]. The distribution of *S.pneumoniae* colonisation rate, serotypes and antimicrobial resistance vary by country, age, ethnic group, study design and other factors [11, 14-20].

1.2. Relevance and scientific novelty

Data on *S.pneumoniae* carriage rate, incidence of pneumococcal disease, *S.pneumoniae* serotype distribution and resistance to antimicrobials in Lithuania and in neighbouring countries are limited.

The National Public Health Surveillance Laboratory in Vilnius collects all invasive pneumococcus strains nationwide since 2006. The amount of serotyped *S.pneumoniae* strains is very low and varies from 18 to 68 per year among children and adults and from 1 to 15 among children under the age of 5 years [21, 22]. Similarly, a small amount of *S.pneumoniae* strains (altogether 37-87 per year) is tested for antimicrobial resistance [21, 22]. As the amount of data is low, however, the findings do not necessarily reflect the actual *S.pneumoniae* serotype distribution and antimicrobial resistance among IPD paediatric patients in Lithuania.

Three studies of *S.pneumoniae* nasopharyngeal carriage in healthy children have been performed in Vilnius (in 1999, 2001 and 2006), when a total of 1,625 children from the same 13 day-care centres were enrolled [23-26]. These studies were rather limited because all of them were performed during a short period of time (in February and March) and the data presented were from only one city of the country. Another study was conducted in Kaunas in 2004-2005, in which a total of 601 healthy children from child care institutions were enrolled [27]. According to available data, there were no more studies performed on this subject in Lithuania.

Our study included other geographically important cities in Lithuania for the first time. It was ongoing for a full year so we were able to evaluate the influence of seasons for the carriage of *S.pneumoniae* in our country. Pre-school children were the subjects in our study as they are the most important source of *S.pneumoniae* and carry the biggest risk of pneumococcal infection [28-33]. Previous studies included healthy children, whereas we included children diagnosed with acute respiratory tract infections for the first time in Lithuania. This allowed to evaluate the influence of *S.pneumoniae* nasopharyngeal colonisation on the course of acute respiratory tract infections. These data are scarce not only in Lithuania, but also worldwide.

1.3. Aim and objectives

The aim of the research was to evaluate the nasopharyngeal colonisation of *Streptococcus pneumoniae* in preschool children and its influence on the course of acute respiratory tract infections.

Objectives:

1. Determination of the nasopharyngeal colonisation rate of *S.pneumoniae* in preschool children with acute respiratory tract infections.
2. Determination of the serotypes of identified *S.pneumoniae* strains.
3. Evaluation of the influence of *S.pneumoniae* nasopharyngeal colonisation on the course of acute respiratory tract infections.
4. Determination of the antimicrobial susceptibility of identified *S.pneumoniae* strains.
5. Determination of the theoretical coverage of pneumococcal serotypes by conjugate pneumococcal vaccines which are used for children in Lithuania.

1.4. Practical significance

Knowledge of the *S.pneumoniae* nasopharyngeal colonisation rate, ęserotype distribution and antimicrobial resistance will help to diagnose and treat the disease more rationally, and to avoid severe cases by expanding use of pneumococcal conjugate vaccines (PCVs).

The study was performed before the implementation of the universal programme of PCV vaccination in Lithuania in October 2014. It provides a basis for future comparisons of *S.pneumoniae* carriage, serotype distribution and antimicrobial resistance between the pre- and post-vaccination era in the country. Besides, our results add to the European data on *S.pneumoniae* carriage, serotype distribution and antimicrobial resistance.

The direct benefit for our study subjects was that by nasopharyngeal culture and testing for antibiotic susceptibility of *S.pneumoniae*, a possible causing agent of acute respiratory tract infection was determined and the most suitable treatment (antimicrobials in that case) was prescribed.

2. MATERIALS AND METHODS

The prospective study was carried out from February 2012 to March 2013. Eight primary care centres (PCC) in Lithuania's five cities (Vilnius (n=2), Kaunas (n=2), Klaipėda (n=2), Panevėžys (n=1) and Alytus (n=1)) from all main regions of the country

and the emergency department (ED) of Children's Hospital, Affiliate of Vilnius University Hospital Santariškių Klinikos in Vilnius were involved in examining children for *S.pneumoniae* nasopharyngeal carriage, serotype distribution and antimicrobial susceptibility.

The approval of Vilnius Regional Biomedical Research Ethics Committee was obtained (2011-11-08 No. 158200-11-418-118) and the parents or legal representatives were asked to sign an informed consent form before the child was enrolled in the study.

This study was supported by an Investigator initiated Research grant from Pfizer.

All subjects satisfied all of the following criteria at study entry:

1. Aged 0–71 months old at the day of sampling;
2. Visited the primary health care provider regarding an acute respiratory tract infection (acute onset, fever of 37.2^0C or higher and/or symptoms of respiratory tract infection – runny nose, sneezing, cough and sore throat);
3. Signed by parents or legal representatives of the Patient Informed Consent and Written Agreement to participate in the study form.

Subjects that met any of the exclusion criteria listed below were not included in the study:

1. Another known cause of fever is identified (e.g., urinary tract infection, gastrointestinal tract infection, etc.);
2. History of vaccination with any pneumococcal vaccine;
3. Treatment with antimicrobials within one-month prior to the enrolment.

General information, demographic data (date of birth, gender, siblings aged less than 6 years, DCC attendance, and use of antimicrobials 1-6 months prior the enrolment) were collected and recorded to the Case report forms. The signs and symptoms assessed by the physician were fever, rhinitis, cough, lung auscultation findings, and inflammation of the pharynx and the palatine tonsils.

Diagnoses were coded according to the International Statistical Classification of Diseases and Related Health Problems, 10^{th} Revision, Australian Modification (ICD-10-AM), which is used in all healthcare institutions in Lithuania since the 1^{st} of April, 2011 as per requirement of the Ministry of Health (No. V-164).

A total of 900 children were included in our study: 636 patients at the PCCs and 264 at the hospital ED. The participants were enrolled throughout the study period. Two thirds of them were enrolled during spring and autumn (35.0% (n=315) and 32.9% (n=296), respectively), the others – during winter (22.0% (n=198) and summer (10.1% (n=91)). The distribution of enrolled children was similar in all the cities, with the exception of Alytus (Table 1), which may be due to a very small number of study participants in this city (n=18).

Table 1. Demographic specifics of the study population (n=900).

Characteristic	Number (%)*							
	Site							
	Vilnius			Kaunas	Klaipėda	Panevėžys	Alytus	
	PCC	ED	PCC and ED	PCC	PCC	PCC	PCC	
Children enrolled	173 (19.2)	264 (29.3)	437 (48.6)	159 (17.7)	63 (7.0)	223 (24.8)	18 (2.0)	900 (100)
Female	84 (48.6)	110 (41.7)	194 (44.4)	78 (49.1)	28 (44.4)	104 (46.6)	4 (22.2)	408 (45.3)
Male	89 (51.4)	154 (58.3)	243 (55.6)	81 (50.9)	35 (55.6)	119 (53.4)	14 (77.8)	492 (54.7)
Mean age, years (SD)	2.23 (1.398)	2.31 (1.386)	2.28 (1.390)	2.30 (1.594)	2.78 (1.497)	2.69 (1.467)	3.72 (1.274)	2.45 (1.474)
DCC attendance	110 (64.7)	149 (56.7)	259 (59.8)	113 (71.1)	45 (73.8)	177 (79.4)	14 (87.5)	608 (68.2)
Siblings aged less than 6 years	61 (35.3)	122 (46.4)	183 (42.0)	73 (45.9)	23 (37.7)	48 (21.5)	7 (41.2)	334 (37.3)
Antimicrobial use 1-6 months' prior the enrolment	58 (33.9)	41 (16.3)	99 (23.4)	14 (10.4)	17 (38.6)	70 (31.7)	12 (66.7)	212 (25.2)

ED: emergency department of Children's Hospital, Affiliate of Vilnius University Hospital Santariškių Klinikos; PCC: primary care centre; SD: standard deviation.

* Unless otherwise indicated.

The subjects were arranged according to age into 3 groups: 0-23 months (28.7%, n=258), 24-47 months (47.0%, n=423) and 48-71 months (24.3%, n=219).

Upper respiratory tract infections (ICD-10-AM codes J00-J06) were diagnosed in 628 (69.8%) cases. Bronchitis, pneumonia, and acute otitis media (ICD-10-AM codes

J20, J18 and H65-H66, respectively) were diagnosed in 213 (23.7%) cases. There were 4 cases (0.4%) with other specified diagnoses and 55 cases (6.1%) had no diagnosis specified.

Nasopharyngeal swabs were taken at the time of enrolment in the study using culturette with Amies transport medium (*Deltalab*, Spain) and transported to a certified laboratory of Children's Hospital, Affiliate of Vilnius University Hospital Santariškių Klinikos in Vilnius within 48 h from collection.

Classic cultural methods (cultivation in 5% CO_2, colony morphology, Gram staining, catalase test, optochin sensitivity) were used to isolate *S.pneumoniae* from the swabs [34, 35]. All the isolates were sensitive to optochin. The bacterial antigen rapid latex agglutination test (*Wellcogen*, Remel Europe Limited, United Kingdom) was used for the confirmation.

Serotypes were determined by means of latex agglutination reaction using the Pneumotest-Latex kit and selected Latex Factor sera: 6b, 6c, 7b, 9g, 18c, 18f, 19b, 19c and 23b (Statens Serum Institut, Copenhagen, Denmark). The expected theoretical protection of PCVs was calculated by comparing the isolated *S.pneumoniae* serotypes with the serotypes included in the currently available PCVs.

Susceptibility to penicillin, erythromycin, clindamycin, trimethoprim–sulphamethoxazole, norfloxacin and vancomycin was determined by the disk-diffusion method. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing recommendations (EUCAST, 2012) [36].

Susceptibility of *S.pneumoniae* to penicillin was determined using a 1 μg oxacillin disk (*Bio-Rad*, France). Isolates showing inhibition zones ≤19 mm were confirmed by the penicillin Etest (*bioMerieux*, France). Breakpoints of minimal inhibitory concentrations (MICs) were interpreted according to EUCAST, 2012 [36]. *S.pneumoniae* strains were defined as penicillin susceptible *S.pneumoniae* (PSSP, MIC ≤0.06 mg/l), penicillin intermediate *S.pneumoniae* (PISP, MIC >0.06–2.0 mg/l) and penicillin resistant *S.pneumoniae* (PRSP, MIC >2 mg/l). PISP and PRSP were grouped as penicillin non-susceptible *S.pneumoniae* (PNSP). Multidrug resistance (MDR) was defined as non-susceptibility to penicillin and ≥2 other non–β-lactam antimicrobial classes [37].

Children were followed-up either via phone call or an additional visit during a 4-week period. The data obtained during the follow-up were the final diagnosis, hospitalisation, treatment with antimicrobials, duration of illness (recovery duration) and number of days absent from DCC. Physicians were notified of the results of nasopharyngeal culture.

The data were analysed using the SPSS (*Statistical Package for the Social Sciences*) software version 24 (license no. 43775936a4321226cddb). Descriptive statistics were used to systemise the study results, namely the mean, standard deviation, minimal and maximal values. A Chi-squared test (x^2) was used to test the statistical significance of differences between the two groups. A Fisher's exact test was used when cell values in the SPSS table had an expected frequency of five or less. Statistical significance was defined by $p < 0.05$. A univariable and multivariable Poisson regression with robust variance estimation was used to analyse the associations among various factors affecting the colonisation of *S.pneumoniae*, as well as the serotype distribution, antimicrobial resistance and the influence on the course of acute respiratory tract infection.

3. RESULTS

3.1 *S.pneumoniae* nasopharyngeal colonisation rate

A total of 367 *S.pneumoniae* strains (one per patient) were isolated from the 900 samples collected, giving a colonisation rate of 40.8%.

The colonisation rate was higher in Vilnius (47.4%) than in Kaunas (32.7%, p=0.001), Panevėžys (36.8%, p=0.009) and Alytus (11.1%, p=0.002). There was also a higher colonisation rate in Vilnius than in Klaipėda (38.1%), but the difference was not statistically significant (p=0.168).

The youngest child with a positive pneumococcal sample was two months old. The colonisation rate of *S.pneumoniae* in infants was 28.0%. A peak level was reached at two to three years of age (48.8% and 45.4%, respectively). A slight decrease in the colonisation rate was found among children aged four to five years (38.1% at the age of four years and 30.7% at the age of five years).

The sex of the patients had no influence on the pneumococcal colonisation rates: 40.4% of the girls and 41.1% of the boys carried *S.pneumoniae* in their nasopharynx.

Seasonality differences were detected in *S.pneumoniae* colonisation rates: they were higher in spring (43.2%) and autumn (44.6%) than in summer (35.2%, p>0.05 for both comparisons) and winter (33.8%, p=0.035 and p=0.017, respectively).

The colonisation of *S.pneumoniae* was significantly higher in children who were attending DCC than in those who were not (44.4% vs 33.1%, p=0.001). Siblings aged less than 6 years or any antimicrobial in 1 to 6 months prior to the nasopharyngeal sample had no influence on the pneumococcal colonisation rates. Similar results were found, using univariable and multivariable Poisson regression analyses (Table 2).

3.2 *S.pneumoniae* serotype distribution

Of the 367 *S.pneumoniae* strains isolated, 22 different serotypes were detected. The most common serotypes were 6B (15.8%, n=58), 19F (13.9%, n=51), 23F (13.9%, n=51), 15 (10.1%, n=37), 14 (9.5%, n=35), 6A (9.3%, n=34), 11 (4.6%, n=17), 3 (3.0%, n=11) and 18C (3.0%, n=11). Other *S.pneumoniae* serotypes constituted 16.9% (n=62) of all isolates and were as follows: serotypes 23 (non-23F) (2.7%, n=10), 19A (2.2%, n=8), 9V (1.6%, n=6), 9 (1.1%, n=4), 10 (1.1%, n=4), 22 (0.8%, n=3), 6C (0.5%, n=2) and single isolates of 4, 7, 7F, 12, 17 and 19 serotypes; 5.2% (n=19) were non-typable.

A slightly different distribution of *S.pneumoniae* serotypes was found in the study sites (Figure 1). Serotype 6B was more prevalent in Panevėžys, compared with Vilnius (p=0.004), Kaunas (p=0.011) and Klaipėda (p=0.045). Serotype 19F was more common in Klaipėda, compared with Vilnius (p=0.018) and Panevėžys (p=0.009). Serotype 23F was more prevalent in Kaunas, compared with Panevėžys (p=0.035). Serotype 6A was not found in Klaipėda but was observed in Vilnius, Kaunas, Panevėžys and Alytus; serogroup 11 was not found in Panevėžys but was identified in the other cities studied. Alytus was excluded from this comparison because of the small number of *S.pneumoniae* isolates (n=2).

The study showed an age-related *S.pneumoniae* serotype distribution (Figure 2), with serotypes 6A and 11 being more common in the youngest age group (0–23 months), while serogroup 3 and serotype 18C was more prevalent in the older age group (48–71 months). There were no sex-related differences, except for 6B, which was more common in girls than boys (20.0% vs 12.4%, p=0.049).

Table 2. *Streptococcus pneumoniae* colonisation rate in relation to various factors.

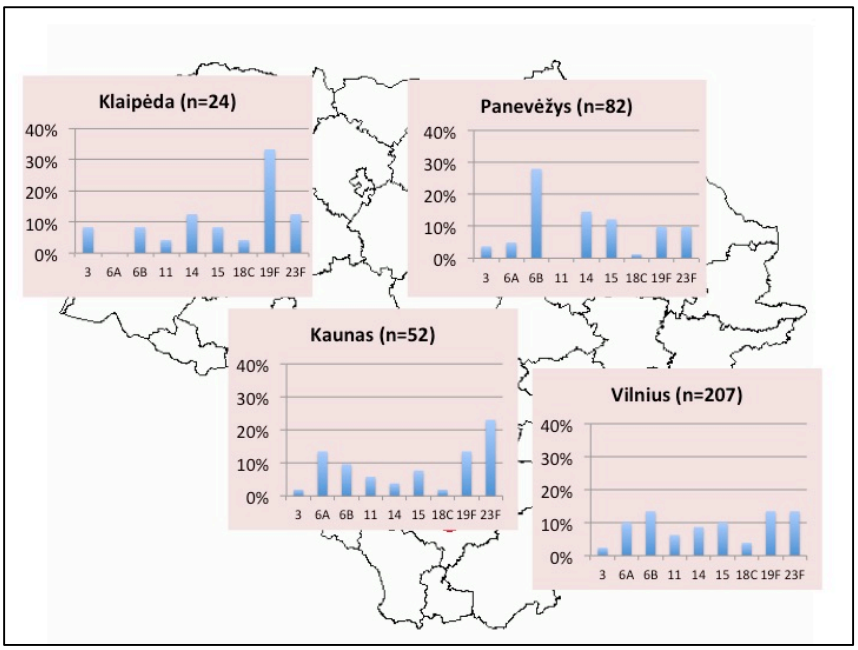
Characteristic	*S.pneumoniae* colonisation rate, n/N (%)	Univariable Poisson regression analysis		Multivariable Poisson regression analysis	
		PR (95% CI)	p value	PR (95% CI)	p value
Cities of Lithuania					
Vilnius[a]	207/437 (47.4)	1.0		1.0	
Kaunas	52/159 (32.7)	**0.690 (0.541-0.881)**	**0.003**	**0.697 (0.541-0.899)**	**0.05**
Klaipėda	24/63 (38.1)	0.804 (0.578-1.119)	0.196	0.795 (0.547-1.156)	0.229
Panevėžys	82/223 (36.8)	**0.776 (0.637-0.947)**	**0.012**	**0.732 (0.584-0.917)**	**0.007**
Alytus	2/18 (11.1)	**0.235 (0.063-0.870)**	**0.030**	**0.249 (0.070-0.878)**	**0.031**
Age groups in months					
0-23	92/258 (35.7)	1.028 (0.805-1.312)	0.828	**1.476 (1.084-2.010)**	**0.014**
24-47	199/423 (47.0)	**1.356 (1.101-1.669)**	**0.004**	**1.411 (1.137-1.752)**	**0.002**
48-71[a]	76/219 (34.7)	1.0		1.0	
Sex					
Female[a]	165/408 (40.4)	1.0		1.0	
Male	202/492 (41.1)	1.015 (0.867-1.189)	0.852	1.034 (0.884-1.211)	0.637
Season					
Spring	136/315 (43.2)	**1.276 (1.011-1.610)**	**0.040**	1.237 (0.966-1.584)	0.092
Summer	32/91 (35.2)	1.039 (0.739-1.460)	0.825	0.911 (0.632-1.314)	0.619
Autumn	132/296 (44.6)	**1.318 (1.044-1.663)**	**0.020**	**1.285 (1.017-1.624)**	**0.036**
Winter[a]	67/198 (33.8)	1.0		1.0	
Day care centre attendance					
Attending	270/608 (44.4)	**1.342 (1.112-1.619)**	**0.002**	**1.529 (1.178-1.983)**	**0.001**
Non-attending[a]	94/284 (33.1)	1.0		1.0	
Siblings under 6 years of age					
Living with siblings	145/334 (43.4)	1.114 (0.949-1.308)	0.187	1.109 (0.942-1.304)	0.214
Living without siblings[a]	219/562 (39.0)	1.0		1.0	
Antimicrobial use in 1 to 6 months prior to the nasopharyngeal sample					
Treated with antimicrobials	98/212 (46.2)	1,166 (0,979-1,388)	0,084	1,149 (0,962-1,373)	0,125
Not treated with antimicrobials[a]	249/628 (39.6)	1.0		1.0	

PR (95% CI) - prevalence ratio and 95% confidence interval of *Streptococcus pneumoniae* colonisation rate

[a] Reference group for each comparison

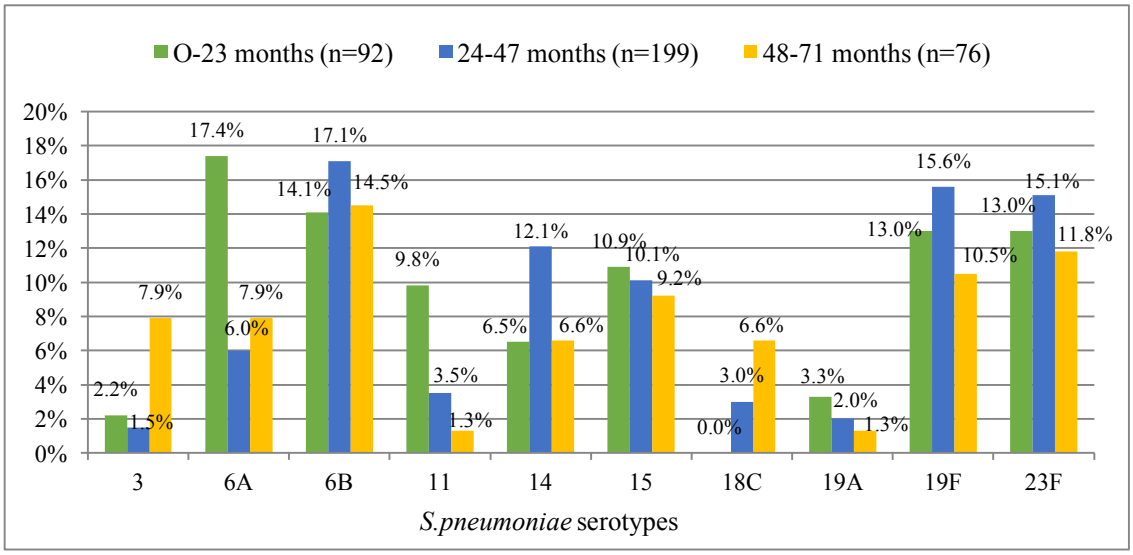
Highlighted (blue) results are statistically significant

Figure 1. Distribution of the most common *Streptococcus pneumoniae* serotypes in the study sites[a] of Lithuania (n=365).



[a] *Note that Alytus was excluded from this comparison because of the small number of S.pneumoniae isolates (n=2).*

Figure 2. Distribution of the most common *Streptococcus pneumoniae* serotypes by age group of enrolled children.



Using a Chi-squared test and univariable Poisson regression analysis, significant differences were found in these comparisons: serotype 3 was more prevalent in children 4-5 years compared to children 2-3 years (PR (95% PI) – 5.237 (1.343-20.413), p=0.017); serotype 6A was more prevalent in children under the age of 2 years compared to children aged 2-3 years (p=0,002); serogroup 11 was more prevalent in children under the age of 2 years compared to children aged 2-3 years (PR (95% PI) – 2.781 (1.069-7.237), p=0.036) and 4-5 years (p=0.021); serotype 18C was more prevalent in children 4-5 years compared to children under the age of 2 years (p=0.018).

The prevalence of serotypes 6B, 19F and 23F varied during the seasons. Serotype 6B was more prevalent during autumn (22.0% (29/132) and winter (19.4% (13/67) as compared with spring (11.0% (15/136), p=0.018 and p=0.108, respectively) and summer (3.1% (1/32), p=0.014 and p=0.03, respectively). Conversely, serotype 19F reached its peak (25.0% (8/32)) during summer; serotype 23F peaked during spring (21.3%, 29/136). The fluctuation rates of other *S.pneumoniae* serotypes according to the season were not statistically significant. The numbers are small, however, thus limiting the ability to meaningfully compare between the seasons.

Serotype 6B was more prevalent among DCC attendees (18.9% (51/270) vs 7.4%, (7/94) p=0.009), while serotype 6A and serogroup 11 were more prevalent among children not attending DCC (14.9% (14/94) vs 7.4% (20/270), p=0.032 and 10.6% (10/94) vs 2.6% (7/270), p=0,003, respectively). Siblings aged less than 6 years or antimicrobial use in 1 to 6 months prior to the nasopharyngeal sample had no influence on the *S.pneumoniae* serotype distribution.

3.3 Influence of *S.pneumoniae* nasopharyngeal colonisation on the course of acute respiratory tract infections

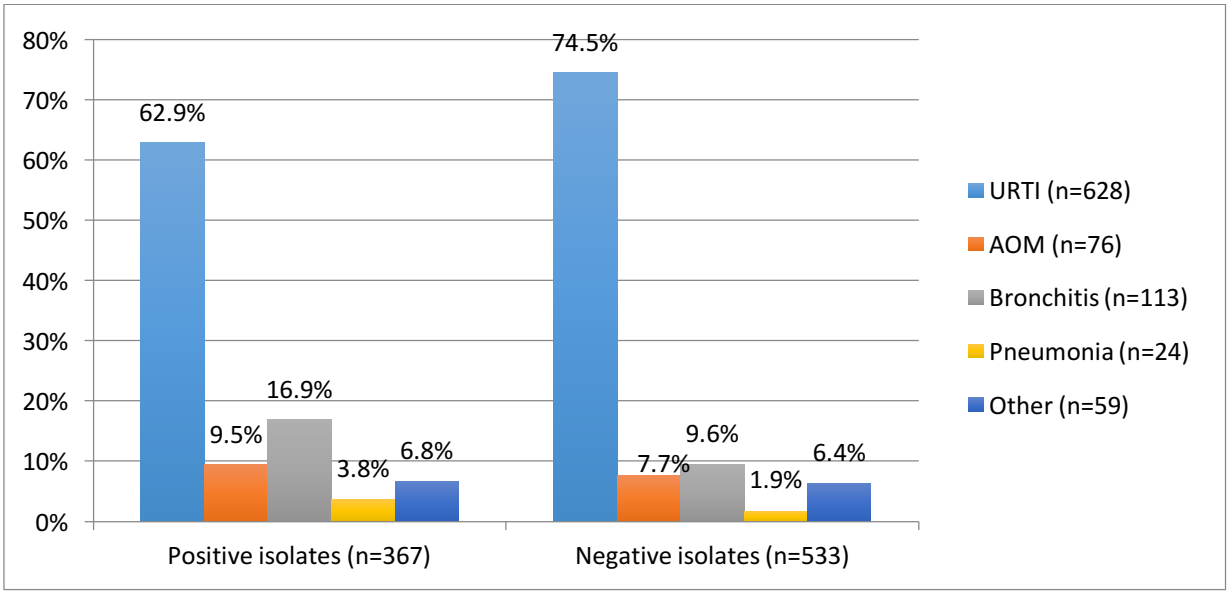
S.pneumoniae nasopharyngeal colonisation had a negative impact on the course of respiratory tract infection. Significantly more children colonised with *S.pneumoniae* had a longer recovery duration (of 3 to 4 weeks) compared to the children with negative cultures (34.0%, 114/335 vs 19.9%, 98/492, respectively, PR (95% CI): 1,496 (1,194-1,876), p=0,000). Serotypes 6A and 19F, as well as serogroups 14, 15 and 23 (non-23F serotypes) were associated with longer recovery duration as compared to the non-colonised cases.

Children with positive *S.pneumoniae* cultures were longer absent from DCC due to the respiratory tract infection. Females with positive *S.pneumoniae* cultures were absent for 1.9 days longer (95% CI: 0.042–3.764, p=0.045); males – 1.0 days longer (95% CI: 0.937–3.052, p=0.298); both sexes combined – 1.5 days longer (95% CI: 0.138–2.867, p=0.031).

Acute otitis media, bronchitis and pneumonia were more common in the *S.pneumoniae*-positive than in the *S.pneumoniae*-negative group (30.2%, 111/367 vs.

19.1%, 102/533, p=0.000). Conversely, more URTIs were diagnosed in the *S.pneumoniae*-negative than in the *S.pneumoniae*-positive group (74.5%, 397/533 vs. 62.9%, 231/367, p=0.000). A more detailed comparison is presented in Figure 3.

Figure 3. Distribution of the diagnoses based on the *Streptococcus pneumoniae* colonisation (n=900).



URTI – upper respiratory tract infection, AOM – acute otitis media.

Using a univariable Poisson regression analysis, significant differences were found in these comparisons: bronchitis was more common in the S.pneumoniae-positive group (PR (95% CI) – 1.416 (1.077-1.860), p=0.013); more URTI were diagnosed in the S.pneumoniae-negative group (PR (95% CI) – 1.359 (1.100-1.680), p=0.005).

The most frequently carried *S.pneumoniae* serogroups/serotypes were 23F, 6B and 19F among children with URTI, serotypes 19F, 6A and 6B – among children with acute bronchitis and serogroups/serotypes 14, 6B and 23F – among children with pneumonia (Table 3).

Significantly more subjects with *S.pneumoniae* colonisation were treated with antimicrobials during the current episode of respiratory tract infection compared to children with negative *S.pneumoniae* cultures (57.5%, 204/355 vs 30.5%, 157/514, PR (95% CI) – 1.901 (1.540-2.346), p=0.000).

Table 3. Distribution of *Streptococcus pneumoniae* serotypes based on the diagnosis.

Serotypes	Diagnosis, n (%)				
	URTI (n=231)	**AOM (n=35)**	**Bronchitis (n=62)**	**Pneumonia (n=14)**	**Other (n=25)**
PCV-10 serotypes/serogroups (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F)					
4	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
6B	34 (14.7)	4 (11.4)	13 (21.0)	2 (14.3)	5 (20.0)
7F	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
9V	3 (1.3)	1 (2.9)	2 (3.2)	0 (0.0)	0 (0.0)
14	17 (7.4)	3 (8.6)	8 (12.9)	5 (35.7)	2 (8.0)
18C	6 (2.6)	2 (5.7)	1 (1.6)	0 (0.0)	2 (8.0)
19F	28 (12.1)	9 (25.7)	9 (14.5)	1 (7.1)	4 (16.0)
23F	40 (17.3)	1 (2.9)	7 (11.3)	2 (14.3)	1 (4.0)
Total	**130 (56.3)**	**20 (57.1)**	**40 (64.5)**	**10 (71.4)**	**14 (56.0)**
Additional PCV-13 serotypes/serogroups (3, 6A and 19A)					
3	5 (2.2)	3 (8.6)	2 (3.2)	0 (0.0)	1 (4.0)
6A	20 (8.7)	6 (17.1)	5 (8.1)	1 (7.1)	2 (8.0)
19A	5 (2.2)	0 (0.0)	1 (1.6)	0 (0.0)	2 (8.0)
Total	**160 (69.3)**	**29 (82.9)**	**48 (77.4)**	**11 (78.6)**	**19 (76.0)**
Other serotypes/serogroups, that are not included in PCVs (6C, 7[a], 9[a], 10, 11, 12, 15, 17, 19[a], 22 and 23[a]) **or were non-typable**					
Total	**71 (30.7)**	**6 (17.1)**	**14 (22.6)**	**3 (21.4)**	**6 (24.0)**

URTI – upper respiratory tract infection, AOM – acute otitis media,

PCV - pneumococcal conjugate vaccine

[a] – except those serotypes that are in PCVs (7F, 9V, 19A, 19F, 23F).

3.4. Antimicrobial susceptibility of *S.pneumoniae*

All isolated strains of *S.pneumoniae* (n=367) were tested for antimicrobial susceptibility. About a half (56.7%, n=208) of *S.pneumoniae* strains were susceptible to all the antibiotics tested.

The highest penicillin MIC was 2 mg/l. Hence, according to the 2012 EUCAST breakpoints [36], none of the strains fell into the resistant category, but 15.8% (n=58) were PISP. Only 12.1% (n=7) of PISP were susceptible to other antibiotics tested while other PISP were concomitantly non-susceptible to erythromycin (82.8%, n=48), clindamycin (77.6%, n=45) or trimethoprim-sulphamethoxazole (34.5%, n=20). In addition, 8.6% (n=5) of PISP showed dual resistance, while 51.7% (n=30) and 27.6% (n=16) were resistant to 3 or 4 antibiotics tested, respectively.

S.pneumoniae resistance to clindamycin was 16.9 % (n=62). Higher rates of

resistance were present to erythromycin: 21.0% (n=77) were resistant and 0.3% (n=1) were intermediately susceptible. The highest non-susceptibility rates of pneumococci were found to trimethoprim-sulphamethoxazole, whereas 21.0% (n=77) were resistant and 6.3% (n=23) intermediately susceptible. None of the tested isolates was resistant to norfloxacin and vancomycin.

A single drug resistance was observed in 24.0% (n=88) of *S.pneumoniae* isolates with a predominance of non-susceptibility to trimethoprim-sulphamethoxazole (19.3%, n=71). Dual resistance was present in 5.2% (n=19) and MDR – in 12.5% (n=46) of *S.pneumoniae* isolates.

Antimicrobial non-susceptibility of *S.pneumoniae* varied in different studied sites of Lithuania. Higher rates of non-susceptibility to penicillin, erythromycin and clindamycin and MDR were found in Panevėžys, while a non-susceptibility to trimethoprim-sulphamethoxazole was more common in Vilnius.

Sex, age, season, DCC attendance, antimicrobial use in 1 to 6 months prior to the enrolment were not significantly associated with the carriage of non-susceptible *S.pneumoniae* strains.

The highest non-susceptible *S.pneumoniae* carriage rates were in children diagnosed with pneumonia as compared to children with other diagnosis (Table 4).

Table 4. The colonisation rate of non-susceptible *S.pneumoniae* based on the diagnosis.

Diagnosis	**Antimicrobials**			
	Penicillin	**Erythromycin**	**Clindamycin**	**TMP-SMX**
URTI (n=231)	13.9%	16.9%	13.9%	28.6%
Acute otitis media (n=35)	14.3%	22.9%	17.1%	31.4%
Bronchitis (n=62)	12.9%	24.2%	16.1%	25.8%
Pneumonia (n=14)	50.0%	57.1%	57.1%	21.4%

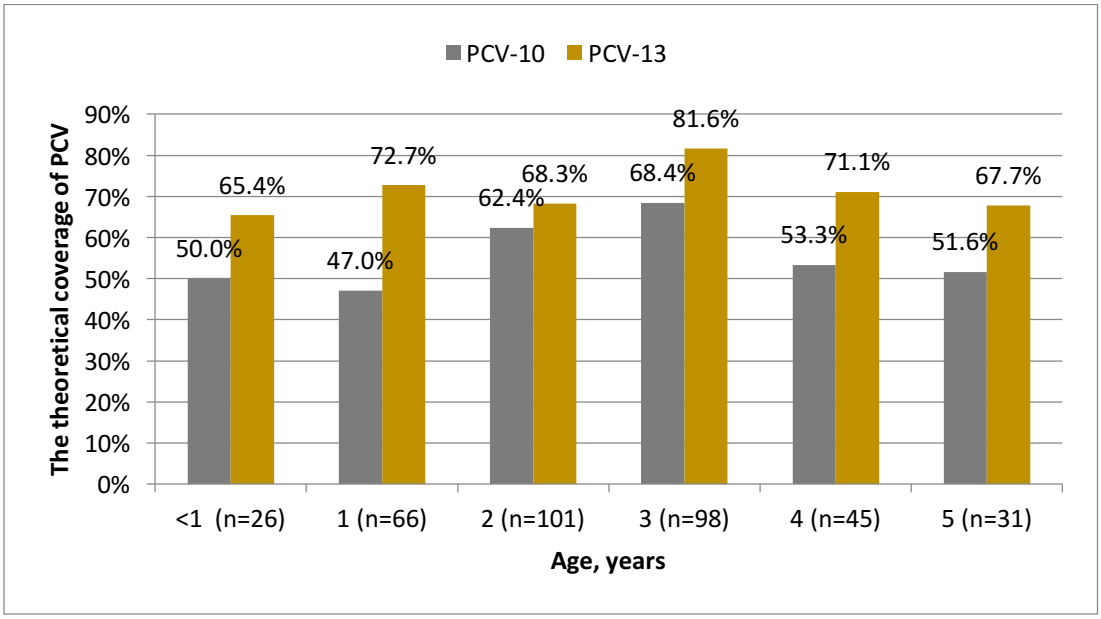
URTI – upper respiratory tract infection, TMP-SMX - trimethoprim-sulphamethoxazole.

Serotype 14 was the most prevalent among the *S.pneumoniae* strains that were non-susceptible to penicillin, erythromycin and clindamycin and MDR, whereas serotype 19F was the most prevalent among the *S.pneumoniae* strains that were non-susceptible to trimethoprim-sulphamethoxazole.

3.5. Theoretical coverage of pneumococcal conjugate vaccines

Among all serotypes isolated from our 367 patients, 58.3% (n=214) were present in PCV-10 and 72.8% (n=267) in PCV-13. The theoretical coverage of PCVs was high in each age group with a peak at the age of three years (Figure 4).

Figure 4. The theoretical coverage of pneumococcal conjugate vaccines based on age.



PCV - pneumococcal conjugate vaccine

Among the non-susceptible serotypes, 67.9% (108/159) were present in PCV-10 and 82.4% (131/159) in PCV-13. Among the MDR serotypes, 73.9% (34/46) were present in PCV-10 and 84.8% (39/46) in PCV-13. More detailed results are presented in Table 5.

Table 5. The theoretical coverage of pneumococcal conjugate vaccines based on *S.pneumoniae* strains that were non-susceptible to different antimicrobials

Antimicrobials	**PCV-10 n/N (%)**	**PCV-13 n/N (%)**
Penicillin	41/58 (70.7)	51/58 (87.9)
Erythromycin	54/78 (69.2)	66/78 (84.6)
Clindamycin	46/62 (74.2)	52/62 (83.9)
Trimethoprim-sulphamethoxazole	69/100 (69.0)	80/100 (80.0)
Multidrug resistant	34/46 (73.9)	39/46 (84.8)

PCV - pneumococcal conjugate vaccine

4. DISCUSSION

Quality of treatment

Our study was conducted in five cities from all main regions of the country for the first time, so it allowed us to evaluate the carriage and serotype distribution of *S.pneumoniae* in different regions of the country. We were able to reach a sufficient sample size and derive representative results during a one-year period.

The nasopharyngeal sampling method that we used in our study meets the WHO recommendations [35]. However, skim milk-tryptone-glucose-glycerol (STGG) remains the medium of choice for transportation and storage of nasopharyngeal swabs in lieu of *Amies* transport medium that we chose [34, 35]. We consider it as one of the shortcomings of the study. However, Hare K.M. et al. found *Amies* transport medium to be a suitable alternative [38].

Usual standardised and WHO recommended laboratory techniques were used for the identification of *S.pneumoniae*. A limitation of our study is that we did not test for other pathogens in the nasopharynx of our subjects, thus we could not determine the actual cause of the RTI. Serotypes were determined by means of latex agglutination reaction, which is an alternative method for *S.pneumoniae* serotyping, not the standard one [34, 35]. This could potentially reduce the value of the study. However, eleven European laboratories have found a high level of congruence between the Quellung reaction and other serotyping methods (including latex agglutination reaction and gel diffusion) [39]. Susceptibility to antimicrobials was determined by standardised methods and the results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing recommendations (EUCAST, 2012) [36].

Our data were compared to the studies from other countries before the implementation of the universal programme of pneumococcal vaccination. The shortcoming of our approach is the difference in time, because most of the studies in other countries were performed much earlier than in Lithuania. After the beginning of vaccination with PCVs, many countries have reported significant changes. As it is impossible to match both time and beginning of vaccination criteria, we chose to compare our data with studies that resemble ours, i.e., to examine pre-school children for

the *S.pneumoniae* nasopharyngeal carriage before the introduction of the universal pneumococcal vaccination.

S.pneumoniae colonisation rate

In our findings, the *S.pneumoniae* colonisation rate among young children with acute RTI in Lithuania was high – 40.8%. The pneumococcal carriage rate varied in different sites of the study. There were more pneumococcal carriers (47.4%) in Vilnius compared to other cities (e.g., 32.7% in Kaunas and 36.8% in Panevėžys). These results are comparable with earlier data from our country. The colonisation of *S.pneumoniae* varied from 55% (in 2001) to 43% (in 2006) in healthy DCC attending pre-school children in Vilnius [23, 25, 26]. A lower colonisation rate of 31% was among children with frequent RTI in the same city (in 2007-2008) [23, 26]. Similar results were found in Kaunas (in 2004-2005), where children from state-care institutions were enrolled. Pneumococcal carriage rates were 37.4% and 29.2% among those healthy children under the age of 3 years and 3 to 7 years, respectively [27].

S.pneumoniae carriage rates among preschool children varied, with a wide range from 6.5% [40] to 97% [41] among different studies worldwide before the implementation of the universal programme of pneumococcal vaccination. A number of studies were performed in Europe, with pneumococcal carriage rates ranging from 25.3% in Romania [42] to 78.4% in Norway [43]. Our data fall somewhere in the middle as compared to other countries and was most comparable to the Czech Republic (38.1%) [44], Hungary (39.2%) [16], Estonia (44%) [14] and Sweden (45%) [45]. It is important to note that most studies have focused on the *S.pneumoniae* carriage in healthy children, while in our study children with RTI were enrolled.

S.pneumoniae colonisation rates vary within the country as well [14, 41, 46-50]. We found the highest rate of pneumococcal carriage in Vilnius. Estonians and Greeks also suggest differences among cities, with Tartu [14] and Heraklion (Crete) [48] displaying higher *S.pneumoniae* colonisation rates.

The youngest child with a positive pneumococcal sample was two months-old. A few studies tested *S.pneumoniae* carriage from the 1st day of life and detected the first colonisation at approximately 2 months [51, 52]. Early *S.pneumoniae* colonisation is more prevalent in developing countries. For example, pneumococcal carriage among

infants is up to 70-98% in India [53], Gambia [41] and Kenya [54]. Our results (carriage rate of 28.0% in infants) are close to the *S.pneumoniae* nasopharyngeal carriage rate of Finnish infants (9-22%) [55].

Younger children (especially up to 3-4 years of age) carry a higher risk of *S.pneumoniae* colonisation [14, 43, 44, 54, 56-58]. We also found the highest *S.pneumoniae* nasopharyngeal carriage rate in children at two to three years of age. However, a few studies from Greece and Romania proposed different results, pneumococcal carriage being higher among children older than 3 years of age [42, 48]. An accurate comparison with other studies is difficult because subjects have been arranged into different groups according to their age.

The sex of the patients had no influence on the pneumococcal colonisation rates. These findings are in the accordance with earlier Lithuanian data [26, 27] and with the results from other countries [41, 43, 44, 48, 58-68].

Seasonality differences were detected in *S.pneumoniae* colonisation rates: they were higher in spring and autumn. It could be due to more common viral RTIs in spring and autumn. Similarly, spring appeared to be a favorable season for the colonisation of *S.pneumoniae* in comparison to winter in neighbouring Poland [69]. On the contrary, pneumococcal carriage was more common in June-July (rainy season) compared to March (dry season) in Kenya [54]. Other studies have found the seasonal effect to be negligible or absent [29, 64, 70-72].

DCCs are the main factor of pneumococcal transmission in a population as extensively shown by Hoti et al. [73]. DCC attendance predisposes children to *S.pneumoniae* colonisation which may spread among their families and then further within the adult population. Our results are in accordance to previous findings [45, 60, 63, 64, 66, 71, 74] as we found DCC attendees having a higher prevalence of *S.pneumoniae* colonisation.

Some studies suggest that growing up with siblings increases the risk of a *S.pneumoniae* colonisation [46, 51, 59, 64, 66, 75, 76]. We also found pneumococcal carriage to be higher if the subject had been growing up with siblings (under the age of 6 years); however, the results were not statistically significant. Similarly, the number of siblings had no impact on the prevalence of *S.pneumoniae* colonisation in Norway, Greece, Turkey and Gambia [41, 43, 48, 67]. However, we did not take into

consideration the DCC attendance status of older siblings, a factor found to be significant for *S.pneumoniae* spread to younger siblings by Otsuka et al. [76].

Children who had taken antibiotics during the previous month were not enrolled into the study, as usage of antibiotics during the sampling or one month before decreases the incidence of pneumococcal carriage [54, 64, 74]. The colonisation of *S.pneumoniae* was higher among subjects who used antibiotics in 1-6 months prior to the study; however, the difference was not statistically significant. Similarly, the highest pneumococcal carriage rates were detected among children who received antimicrobials 3 months before the studies in Greece [48] and Japan [66]. A common use of antimicrobials was also associated with higher pneumococcal carriage rates in Poland [60].

We evaluated the most important risk factors for the colonisation of *S.pneumoniae*. The results resemble those from the other countries and confirm the influence of geographical areas, age and DCC attendance on pneumococcal carriage.

S.pneumoniae **serotype distribution**

In our study, the most predominant colonising serotypes/serogroups were 6B, 19F, 23F, 15, 14 and 6A, which accounted 72.5% of the isolates. Previously reported data on *S.pneumoniae* colonisation show that the same serotypes/serogroups 6A, 6B, 14, 19F and 23F were the most prevalent among pre-school *S.pneumoniae* carriers before the widespread use of PCV routine vaccination, with some differences in their rates [11, 15, 16, 19, 43, 45, 65]. Serotypes 6A, 6B, 19F and 23F were also predominant among nasopharyngeal isolates in healthy Lithuanian children both in Vilnius [23, 25, 26] and Kaunas [27].

S.pneumoniae serotype fluctuation during different study years has been observed in Lithuania. The prevalence of serotype 3 was 13% in 1999 [26] and only 2.4% in our study, while serogroup 14 became a few times more common. This serogroup constituted 3.2% of all isolated pneumococci in Vilnius in 2006 [26] and was not detected in Kaunas in 2004-2005 [27], whereas it constituted 8.7% in Vilnius and 3.8% in Kaunas according to our data. The prevalence of serotype 15 was 9% in 1999; it was 2.5% in 2006 [26] and 10.1% in our study. The distribution of other *S.pneumoniae* serotypes remained stable, with small differences in their rates. It is important to note,

however, that the study sites and the type of children studied differed, which limits the comparison.

Influence of *S.pneumoniae* colonisation on the course of acute respiratory tract infections

Previously reported data show that *S.pneumoniae* colonisation is more common among children with RTIs (especially with lower respiratory tract infections) [68, 77-79]. It is difficult to interpret whether RTIs predispose higher *S.pneumoniae* colonisation or, conversely, *S.pneumoniae* colonisation leads to more cases of RTIs.

While we could not determine the aetiology of the RTI from the data we obtained in our study, we found that the nasopharyngeal colonisation with *S.pneumoniae* was associated with longer recovery duration in children under the age of 6 years. The study conducted by Kristo A et al. evaluated the effects of nasal colonisation with pathogenic bacteria (including *S.pneumoniae*) on the course of acute RTIs in children. Although they used nasal middle metal specimens to identify bacteria and studied children aged at least 6 years, their findings show that pneumococcal colonisation during acute RTI was predictive of a longer course of illness [80].

A longer duration of disease results in a longer absence from DCC. Children colonised with *S.pneumoniae* during acute RTI were 1.5 days longer absent from DCC than children with negative cultures. The longer disease duration and longer absence from DCC are likely to increase caregiver's absence from work which is a significant burden to the families and society.

We found *S.pneumoniae* colonisation to be associated with higher frequencies of acute otitis media, bronchitis and pneumonia. These findings may imply that nasopharyngeal colonisation with *S.pneumoniae* predisposes children to diseases such as acute otitis media, bronchitis and pneumonia, possibly by means of co-interaction of various potential pathogens (both viral and bacterial) in the nasopharynx of the host. Our finding of more frequent lower RTI in children with nasopharyngeal colonisation with *S.pneumoniae* are in accordance with previous findings [78, 79]. Furthermore, more subjects with *S.pneumoniae* colonisation were treated with antimicrobials during the current episode of RTI compared to children with negative *S.pneumoniae* cultures.

To sum up, *S.pneumoniae* nasopharyngeal colonisation had a negative impact on the course of acute RTI, likely because of *S.pneumoniae* being the cause of the disease or a complicating factor. It was associated with and may be responsible for higher frequencies of acute otitis media, bronchitis, pneumonia, and the need of antimicrobial treatment, longer duration of illness and absence from DCC.

Susceptibility of *S.pneumoniae* to antimicrobials

In our findings, the rate of PSSP (penicillin susceptible *S.pneumoniae*) was quite high (84.2%) and there were no PRSP (penicillin resistant *S.pneumoniae*) strains found in the nasopharynx. Similar results were observed among invasive *S.pneumoniae* isolates from children and adults in our country during the study years: 83.8% of PSSP in 2012 and 76.3% in 2013 [21, 81]. However, our findings indicate lower PSSP rates than those in healthy children aged 2–7 years in the previous nasopharyngeal carriage studies in Vilnius: 93.8% in 1999, 90.0% in 2001 and 90.4% in 2006 [23].

Results of the pre-vaccination studies on *S.pneumoniae* nasopharyngeal carriage have found no PRSP in many countries [14, 43, 44, 65, 82-84], while there was a wide geographical variation in the prevalence of PNSP (penicillin non-susceptible *S.pneumoniae*). The rates of PNSP seen in our study (15.8%) was higher to those observed in healthy children in Norway (1.8%) [43], the Netherlands (2.7%) [83], the Czech Republic (3%) [44] and Estonia (6%) [14]. Contrarily, results of pre-vaccination studies conducted in Greece [85], Poland [60] and Romania [19] have shown higher rates of PNSP in healthy children: 34.7%, 39.2% and 83%, respectively. Later, a decrease of resistance to penicillin was reported due to PCV vaccination [86, 87].

According to our results, the susceptibility of *S.pneumoniae* to erythromycin and clindamycin was also high (78.7% and 83.1%, respectively). However, previously reported rates of the nasopharyngeal *S.pneumoniae* susceptibility to erythromycin and clindamycin were even higher among healthy preschool children in Vilnius (90.4% and 98% in 2006, respectively) [23]. A significant decrease (from 100% to 75%) of the susceptibility to macrolides of invasive *S.pneumoniae* isolates was also observed during the period 2006–2013 in Lithuania [21, 81]. It may be due to an increased use of macrolides in the clinical practice.

The non-susceptibility of non-invasive *S.pneumoniae* strains to erythromycin and clindamycin varies from 1.2% [44] to 72.5% [42] and from 0.6% [44] to 49% [88] in different European countries, respectively. Our results are similar to those observed among non-invasive *S.pneumoniae* strains in paediatric population in Poland (29.5 and 29.2%, respectively) [60] and Russia (16.7 and 19.3 %, respectively) [57] before routine PCV vaccination was begun in these countries.

High levels of pneumococcal resistance to trimethoprim-sulphamethoxazole were observed previously among non-invasive *S.pneumoniae* strains in healthy preschool children in Lithuania. Trimethoprim-sulphamethoxazole is not widely used to treat RTIs in children, therefore the non-susceptibility to trimethoprim-sulphamethoxazole decreased from 60.0% in 1999 to 46.0% in 2006 [23] and it was 27.3% in the current study. Pneumococcal resistance to trimethoprim-sulphamethoxazole was found to be high up to 66–67% in Estonia [14] and Romania [19], while the rates were lower in Norway (4.6%) [89], Sweden (9.8%) [45], the Netherlands (12.9%) [83] and the Czech Republic (15.7%) [44].

Our data suggest that multidrug resistance of non-invasive *S.pneumoniae* among children in Lithuania (12.5%) is intermediate as compared with other European countries in the pre-PCV-vaccination era. Higher rates of MDR strains were found in Greece (25.0%) [85], Poland (34.1%) [60] and Romania (67%) [19], while lower rates were observed in the Netherlands (1.9%) [83], Estonia (4%) [14] and Norway (4.5%) [89]. A decrease of resistance to macrolides, trimethoprim-sulphamethoxazole and MDR was reported due to the PCV vaccination [86, 89].

Resistance of *S.pneumoniae* varies not only among the countries. We found a geographical variation of *S.pneumoniae* resistance within the cities of the country; therefore, the attention should be paid to the use of penicillin and macrolides in Panevėžys and to the use of trimethoprim-sulphamethoxazole in Vilnius.

To conclude, the rates of nasopharyngeal *S.pneumoniae* susceptibility to penicillin and macrolides are high among preschool children in Lithuania, however they are lower as compared with previous studies. A strict policy with respect to antibiotic prescription together with a widespread use of vaccination could potentially reduce the carriage rate of antibiotic-resistant pneumococci in our country.

Theoretical effectiveness of pneumococcal conjugate vaccines

The serotypes found in a population might be an important predictor of the likely effectiveness of a pneumococcal vaccine in that population. Our findings suggest a rather high theoretical coverage (58.3–72.8%) of nasopharyngeal pneumococcal isolates by the currently available PCVs (PCV-10 and PCV-13). The lowest theoretical coverage by PCV-10 and PCV-13 has been reported in Turkey (17.3%) [62] and Denmark (57%) [90], respectively, the highest - in Poland (73.7% and 80.1%, respectively) [11].

Our data suggest that vaccination could potentially reduce the carriage rate of antibiotic-resistant pneumococci in Lithuania as a majority (67.9%–82.4%) of non-susceptible *S.pneumoniae* serotypes belonged to serotypes included in PCVs. The previously highly prevalent resistant serotypes seem to be successfully suppressed in the PCV-vaccination era, and an emergence of the new, often less resistant, but sometimes more virulent and invasive serotypes is observed [16].

Final remarks

S.pneumoniae colonisation rate, serotype distribution and susceptibility to antimicrobials varies in different geographical regions. Differences are even noted in the same region depending on time of the study, methodology, subject characteristics, vaccination approach and policy of antimicrobial use.

Our results supplement the data about the *S.pneumoniae* colonisation rate, serotype distribution and susceptibility to antimicrobials in the Eastern European region. The study was performed before the implementation of the universal programme of PCV vaccination in Lithuania; therefore, it provides a basis for future comparisons of *S.pneumoniae* carriage, serotype distribution, antimicrobial resistance between the pre- and vaccination era in the country. It would be also helpful in improving the antimicrobial policy in Lithuania.

5. CONCLUSIONS

1. *S.pneumoniae* nasopharyngeal colonisation rate was high (40.8%) in preschool children with acute respiratory tract infections;
2. The most prevalent *S.pneumoniae* serotypes (6A, 6B, 14, 15, 19F and 23F) in Lithuania were the same ones that had been predominant in other European

countries before the introduction of pneumococcal vaccines into the national immunization programme;

3. *S.pneumoniae* nasopharyngeal colonisation had a negative impact on the course of acute respiratory tract infections:
    - longer recovery duration;
    - longer absence from day-care centres;
    - higher rates of acute otitis media, bronchitis and pneumonia;
    - more frequent use of antimicrobials;
4. Most *S.pneumoniae* strains were sensitive to commonly used antimicrobials (e.g., penicillin, macrolides);
5. Currently commercially available pneumococcal conjugate vaccines could be effective for children in Lithuania.

6. PRACTICAL RECOMMENDATIONS

The nasopharyngeal colonisation of *S.pneumoniae* was common in children with acute RTIs. Therefore, doctors should consider pneumococcus as a possible cause when upper or lower bacterial RTI is suspected.

Penicillin should be the first choice for the empiric treatment of pneumococcal infection. Macrolides should be given only in the case of penicillin allergy. An unnecessary use of antimicrobials should be avoided as pneumococcal resistance is increasing.

It is important for both doctors and parents to get acquainted with our data (high *S.pneumoniae* nasopharyngeal colonisation rates, predominant serotypes and high theoretical coverage of PCVs), in order to promote PCV vaccination among children as currently the most effective preventive measure.

Invasive pneumococcal diseases represent only the tip of the iceberg. Therefore, we propose creating and updating a Lithuanian or European database that would include not only invasive pneumococcal diseases, but would also gather data on other pneumococcal diseases, *S.pneumoniae* colonisation, serotype distribution and antimicrobial resistance. This could help to observe the dynamics and improve vaccination and antimicrobial treatment policies.

7. SANTRAUKA

DAV – dauginis atsparumas vaistams;

EUCAST – Europos jautrumo antimikrobinėms medžiagoms tyrimų komitetas (angl. *European Committee on Antimicrobial Susceptibility Testing);*

KPV – konjuguotoji pneumokokinė vakcina;

MSK – minimali kolonijų dauginimąsi slopinanti koncentracija;

NIP – nacionalinė imunoprofilaktikos programa;

PI – pasikliautinieji intervalai;

PS – priėmimo skyrius;

PSPC – pirminės sveikatos priežiūros centras;

SD – standartinis nuokrypis;

ŠS – šansų santykis;

ŪKTI – ūminė kvėpavimo takų infekcija;

VULSK – VšĮ Vilniaus universiteto ligoninės Santariškių klinikos.

Įvadas

Streptococcus pneumoniae (*S.pneumoniae*, pneumokokas) yra viena iš dažniausių nosiaryklę kolonizuojančių patogeninių bakterijų. Ši kolonizacija yra besimptomė, tačiau pneumokokų suaktyvėjimas gali sukelti įvairių patologinių būklių – nuo viršutinių kvėpavimo takų infekcijų iki sunkių invazinių ligų [1, 2].

S.pneumoniae infekcija yra aktuali visuomenės sveikatos problema visame pasaulyje, ypač tarp kūdikių ir mažų vaikų. Pasaulio sveikatos organizacijos duomenimis, kasmet apie 1,6 mln. žmonių, iš jų apie 0,7–1 mln. vaikų iki 5 metų amžiaus miršta nuo pneumokokinės infekcijos [3]. Ji yra viena iš pagrindinių ūminio vidurinės ausies uždegimo, plaučių uždegimo, sepsio ir meningito priežasčių [4–8]. Didėjantis pneumokokų atsparumas dažnai vartojamiems antibakteriniams preparatams (pvz., makrolidams, cefalosporinams) ir dauginis atsparumas – dar viena aktuali visuomenės sveikatos problema [9].

Lietuvoje trūksta duomenų apie *S.pneumoniae* kolonizacijos ir infekcijos dažnumą, cirkuliuojančius serotipus ir atsparumą antibakteriniams preparatams. Tiriamų invazinių *S.pneumoniae* kultūrų skaičius mūsų šalyje labai nedidelis, svyruoja nuo 18 iki

68 per metus tarp vaikų ir suaugusiųjų ir nuo 1 iki 15 tarp vaikų iki 5 metų amžiaus [21]. Toks nedidelis invazinių padermių kiekis galimai nerodo tikrosios padėties.

S.pneumoniae nešiojimas Lietuvos vaikų nosiaryklėje tirtas tik keliuose miestuose. Vilniuje 1999, 2001 ir 2006 metų vasario kovo mėnesiais tirti sveiki vaikai (n = 1625), lankantys ikimokyklines ugdymo įstaigas [23–26]. Kaune 2004–2005 metais tirtas sveikų globos įstaigose augančių vaikų (n = 601) *S.pneumoniae* nešiojimas [27].

Mūsų tyrimas pirmą kartą apėmė ir kitus geografiškai svarbius Lietuvos miestus. Jis buvo atliekamas visus metus, o tai leido įvertinti metų laikų įtaką *S.pneumoniae* nešiojimui mūsų šalyje. Tirti ikimokyklinio amžiaus vaikai, kurie yra pagrindiniai pneumokokų nešiotojai ir turi didžiausią *S.pneumoniae* infekcijos riziką [28–33]. Ankstesniuose tyrimuose tirti sveiki vaikai, o į šį tyrimą pirmą kartą mūsų šalyje įtraukti ūminėmis kvėpavimo takų infekcijomis (ŪKTI) sergantys vaikai. Be to, įvertinta *S.pneumoniae* kolonizacijos įtaka ŪKTI eigai. Tokių duomenų trūksta ne tik Lietuvoje, bet ir kitose pasaulio šalyse.

Svarbu paminėti, kad tyrimas atliktas prieš konjuguotosios pneumokokinės vakcinos (KPV) įtraukimą į nacionalinę imunoprofilaktikos programą (NIP). Tai galimybė įvertinti skiepijimo KPV būtinumą ir teorinį veiksmingumą. Be to, didelės imties duomenys iš įvairių Lietuvos regionų sudaro tvirtą pagrindą tolesniems tyrimams. Ateityje bus galima įvertinti KPV įtaką *S.pneumoniae* nešiojimui, serotipų pasiskirstymui ir jautrumui antibakteriniams preparatams.

Darbo tikslas

Įvertinti ikimokyklinio amžiaus vaikų, sergančių ūminėmis kvėpavimo takų infekcijomis, *S.pneumoniae* kolonizacijos nosiaryklėje ypatumus ir įtaką ūminių kvėpavimo takų infekcijų eigai.

Darbo uždaviniai

1. Nustatyti *S.pneumoniae* kolonizacijos dažnumą ikimokyklinio amžiaus vaikų, sergančių ūminėmis kvėpavimo takų infekcijomis, nosiaryklėje.
2. Nustatyti tyrimo metu identifikuotų *S.pneumoniae* serotipus.
3. Įvertinti *S.pneumoniae* kolonizacijos įtaką ūminių kvėpavimo takų infekcijų eigai.

4. Nustatyti tyrimo metu identifikuotų *S.pneumoniae* jautrumą antibakteriniams preparatams.
5. Įvertinti potencialų konjuguotosios pneumokokinės vakcinos veiksmingumą Lietuvos vaikams.

Tiriamieji ir tyrimo metodai

Biomedicininis tyrimas atliktas, gavus Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimą 2011 m. lapkričio 8 d., Nr. 158200-11-418-118.

Vaikai dėl *S.pneumoniae* nešiojimo nosiaryklėje buvo tiriami nuo 2012 m. vasario 1 d. iki 2013 m. vasario 28 d. imtinai. Tyrime dalyvavo aštuoni pirminės sveikatos priežiūros centrai (PSPC) penkių Lietuvos miestų (Vilniaus (n = 2), Kauno (n = 2), Klaipėdos (n = 2), Panevėžio (n = 1), Alytaus (n = 1)), iš visų šalies regionų, taip pat Vaikų ligoninės VšĮ Vilniaus universiteto ligoninės Santariškių klinikų (VULSK) filialo Priėmimo skyrius (PS).

Tyrimui atlikti buvo gauta tarptautinės *Pfizer* kompanijos tyrėjų inicijuoto tyrimų fondo parama (angl. *Investigator initiated Research grant from Pfizer).*

Įtraukimo į tyrimą kriterijai:

1. Vaiko amžius įtraukimo į tyrimą ir mėginio ėmimo metu nuo 0 iki 72 mėnesių (t. y. iki 5 metų 11 mėnesių 29 dienų) amžiaus.
2. Vizitas pas gydytoją dėl ŪKTI:
    - staigi pradžia,
    - kūno temperatūra, viršijanti 37,2 °C,
    - ir / arba ŪKTI simptomai – sloga, čiaudulys, kosulys, ryklės skausmas.
3. Pasirašyta vaiko tėvų arba globėjų informuoto asmens sutikimo forma.

Neįtraukimo į tyrimą kriterijai:

1. Kita galima karščiavimo priežastis (pvz., šlapimo takų ar virškinamojo trakto infekcija).
2. Vaiko skiepijimas bet kuria pneumokokine vakcina.
3. Gydymas antibakteriniais vaistais vieną mėnesį iki tyrimo.

Į tyrimą įtraukta 900 vaikų. Pasiskirstymas pagal lytį tolygus (45,3 proc. mergaičių ir 54,7 proc. berniukų). Tirtų vaikų amžiaus vidurkis metais – 2,45 ± SD 1,474. Daugiausia vaikų į tyrimą įtraukta Vilniaus mieste (48,6 proc.), Panevėžyje (24,8 proc.) ir Kaune (17,7 proc.), mažiausia – Klaipėdoje (7,0 proc.) ir Alytuje (2,0 proc.).

Daugumai tiriamųjų (69,8 proc.) diagnozuota ūminė viršutinių kvėpavimo takų infekcija. Ūminis vidurinės ausies uždegimas (8,4 proc.), ūminis bronchitas (12,6 proc.) ir plaučių uždegimas (2,7 proc.) sudarė apie ketvirtadalį visų diagnozių.

Tiriamiesiems vaikams mėginys iš nosiaryklės imtas steriliu lanksčiu vienkartiniu tamponu. Tyrime naudota *Amies* sterili transportinė terpė. Paimti mėginiai iš visų tirtų Lietuvos miestų per 48 val. nuvežti į Vaikų ligoninės VšĮ VULSK filialo mikrobiologijos laboratoriją, kurioje *S.pneumoniae* identifikuota, naudojant įprastus standartinius laboratorinius metodus [34, 35]. *S.pneumoniae* serotipavimas atliktas, taikant latekso agliutinacijos reakciją, naudojant *Pneumotest-Latex* rinkinį. Detalesnis tipavimas atliktas, naudojant papildomus pneumokokų identifikavimo serumus: 6b, 6c, 7b, 9g, 18c, 18f, 19b, 19c ir 23b.

S.pneumoniae jautrumas penicilinui, eritromicinui, klindamicinui, trimetoprimui-sulfametoksazoliui, norfloksacinui ir vankomicinui tirtas diskų difuzijos metodu. Rezultatai interpretuoti pagal Europos jautrumo antimikrobinėms medžiagoms tyrimų komiteto (angl. *European Committee on Antimicrobial Susceptibility Testing, EUCAST*) 2012 metų rekomendacijas [36]. *S.pneumoniae* padermės vertintos kaip jautrios penicilinui, kai minimali kolonijų dauginimąsi slopinanti koncentracija (MSK) buvo ≤0,06 mg/l, vidutiniškai jautrios penicilinui, kai MSK buvo > 0,06–2,0 mg/l, ir atsparios penicilinui, kai MSK viršijo 2 mg/l. Antibiotikams vidutiniškai jautrūs ir atsparūs pneumokokai vertinti kaip nejautrūs. Dauginis atsparumas vaistams (DAV) apibrėžtas kaip nejautrumas penicilinui ir nejautrumas 2 ir daugiau ne β-laktaminių antimikrobinių vaistų klasėms [37].

Tyrimų rezultatams sisteminti naudota aprašomoji statistika. Chi-kvadrato (x^2) testas taikytas nustatant statistiškai reikšmingus skirtumus tarp grupių. Univariacinė ar multivariacinė Puasono regresinė analizė naudota vertinant įvairių veiksnių, turinčių įtakos *S.pneumoniae* kolonizacijai, serotipų pasiskirstymui, atsparumui antibakteriniams preparatams ar ŪKTI eigai, ryšį.

Rezultatai

Iš 900 tirtų vaikų nosiaryklės tepinėlių išaugo 367 *S.pneumoniae* padermės. (nešiojimo dažnis – 40,8 proc.).

S.pneumoniae nosiaryklėje nešiojančių vaikų daugiau buvo Vilniuje (47,4 proc.) nei Kaune (32,7 proc., p = 0,001), Panevėžyje (36,8 proc., p = 0,009), Klaipėdoje (38,1 proc., p = 0,168) ar Alytuje (11,1 proc., p = 0,002). Taip pat *S.pneumoniae* nešiojimas nosiaryklėje dažnesnis buvo pavasarį (43,2 proc.) ir rudenį (44,6 proc.), palyginti su vasara (35,2 proc.), atitinkamai p = 0,172 ir p = 0,111) ir žiema (33,8 proc., atitinkamai p = 0,035 ir p = 0,017).

Pneumokokų nešiojimas nosiaryklėje buvo dažnesnis 2–3 metų amžiaus vaikų (47 proc.), palyginti su jaunesniais vaikais iki 2 metų amžiaus (35,7 proc., p = 0,004) ir vyresnių vaikų amžiaus grupe (34,7 proc., p = 0,003). *S.pneumoniae* nosiaryklėje nešiojančių vaikų daugiau buvo tarp lankančių kolektyvą (44,4 proc.) nei tarp nelankančiųjų (33,1 proc., p = 0,001). Lytis, tai, kad tiriamieji turi brolių ar seserų (iki 6 metų), ar iki tyrimo 1–6 mėnesius vartoti antibakteriniai preparatai *S.pneumoniae* nešiojimui neturėjo įtakos.

Šeši *S.pneumoniae* serotipai (6B, 19F, 23F, 15, 14 ir 6A) sudarė 72,5 proc. rastų serotipų. Serotipų pasiskirstymas tirtuose Lietuvos miestuose nevienodas. 6B serotipas buvo dažnesnis Panevėžyje (28 proc.), palyginti su Vilniumi (13,5 proc. p = 0,004), Kaunu (9,6 proc., p = 0,011) ir Klaipėda (8,3 proc., p = 0,045). 19F serotipas dažniau nustatytas Klaipėdoje (33,3 proc.), palyginti su Vilniumi (13,5 proc., p = 0,018) ir Panevėžiu (9,8 proc., p = 0,009). 23F serotipas dažnesnis Kaune (23,1 proc.) nei Panevėžyje (9,8 proc., p = 0,035). Alytus neįtrauktas į šį palyginimą dėl nedidelio išaugintų ir serotipuotų pneumokokų kultūrų kiekio (n = 2).

S.pneumoniae serotipų pasiskirstymas priklausė nuo amžiaus. Serotipas 6A ir 11 serogrupė dažniau rasti vaikų iki 2 metų amžiaus nosiaryklėje, o 3 serogrupė ir 18C serotipas buvo dažnesni vyresniųjų, 4–5 metų amžiaus vaikų, nosiaryklėje. Kiti serotipai pasiskirstė tolygiau. Tarp lyčių statistiškai reikšmingų skirtumų nenustatyta, išskyrus 6B serotipą, kuris buvo dažnesnis mergaičių (ŠS (95 proc. PI) – 1,616 (1,003–2,604), p = 0,049).

S.pneumoniae serotipų pasiskirstymas skyrėsi skirtingu metų laiku. 6B serotipas buvo dažnesnis rudenį (22,0 proc.) ir žiemą (19,4 proc.), palyginti su pavasariu (11,0

proc., atitinkamai p = 0,018 ir p = 0,108) ir vasara (3,1 proc., p = 0,014 ir p = 0,030). Atvirkščiai, 19F serotipas buvo dažnesnis vasarą (25,0 proc.), palyginti su žiema (6,0 proc., p = 0,017), o 23F serotipas – pavasarį (21,3 proc.), palyginti su rudeniu (7,6 proc., p = 0,004). Kitų serotipų pasiskirstymas skirtingu metų laiku statistiškai reikšmingai nesiskyrė. Nedideli skaičiai riboja tikslų *S.pneumoniae* serotipų palyginimą atsižvelgiant į metų laiką.

Vaikų, lankančių kolektyvą, nosiaryklėje daugiau aptikta 6B serotipo pneumokokų (18,9 proc.) nei nelankančiųjų (7,4 proc., p = 0,009), o 6A serotipo ir 11 serogrupės pneumokokų daugiau rasta kolektyvo nelankančių vaikų nosiaryklėje (atitinkamai 14,9 proc., *vs* 7,4 proc., p = 0,032 ir 10,6 proc. *vs* 2,6 proc., p = 0,003). Tai, kad turima brolių ir seserų (iki 6 metų amžiaus) ar buvo vartoti antibakteriniai preparatai (1–6 mėn. laikotarpiu iki tyrimo), *S.pneumoniae* serotipų pasiskirstymui neturėjo įtakos.

S.pneumoniae nešiojimas nosiaryklėje turėjo neigiamą įtaką ŪKTI eigai. Ilgiau (t. y. 3–4 sav.) sirgo tie vaikai, kurie nešiojo *S.pneumoniae* nosiaryklėje, palyginti su tais, kurių nosiaryklėje pneumokokų nebuvo, atitinkamai 34,0 proc. *vs* 19,9 proc. (ŠS (95 proc. PI) – 1,496 (1,194–1,876), p = 0,000). Serotipai 6A ir 19F bei 14, 15, 23 (išskyrus 23F serotipą) serogrupės buvo susijusios su ilgesne pasveikimo trukme. Pasveikimo trukmė nesiskyrė palyginus serotipus, esančius KPV-10 ar KPV-13 sudėtyje, su tais, kurių nėra KPV sudėtyje.

Vaikai, kurie nosiaryklėje nešiojo *S.pneumoniae*, 1,5 dienos ilgiau nelankė kolektyvo nei tie, kurių nosiaryklėje pneumokokų nebuvo (95 proc. PI: 0,138–2,867, p = 0,031). Mergaitės, nosiaryklėje nešiojančios *S.pneumoniae,* kolektyvo nelankė 1,9 dienos ilgiau (95 proc. PI: 0,042–3,764, p = 0,045), o berniukai – 1,0 dieną ilgiau (95 proc. PI: 0,937–3,052, p = 0,298), palyginti su tais, kurių nosiaryklėje pneumokokų neaptikta.

Tyrimo metu nerasta *S.pneumoniae* nešiojimo nosiaryklėje ir ŪKTI simptomų (karščiavimo, slogos, kosulio) bei fizinio ištyrimo duomenų (ryklės, tonzilių pakitimų, plaučių auskultacinių duomenų) ryšio pirmojo vizito pas gydytoją metu.

Ūminis vidurinės ausies uždegimas, bronchitas ir plaučių uždegimas buvo dažniau diagnozuoti *S.pneumoniae* nešiojantiems tiriamiesiems (30,2 proc. *vs* 19,1 proc., p = 0,000). Ūminė viršutinių kvėpavimo takų infekcija dažniau diagnozuota tiems

tiriamiesiems, kurių nosiaryklėje *S.pneumoniae* nebuvo aptikta (74,5 proc. *vs* 62,9 proc., p = 0,000).

Sergančiųjų ūmine viršutinių kvėpavimo takų infekcija nosiaryklėje vyravo 23F, 6B, 19F serotipai, sergančiųjų ūminiu vidurinės ausies uždegimu – 19F, 6A ir 6B serotipai, sergančiųjų ūminiu bronchitu – 6B, 19F ir 23F serotipai bei 14 serogrupė, sergančiųjų ūminiu plaučių uždegimu – 14 serogrupė ir 6B, 23F serotipai.

Apie pusė (56,7 proc.) *S.pneumoniae* padermių buvo jautrios visiems tirtiems antibakteriniams preparatams. Tiriant *S.pneumoniae* jautrumą penicilinui, didžiausia MSK buvo 2 mg/l. Pagal 2012 EUCAST gaires, nė vienas tirtas pneumokokas nebuvo atsparus penicilinui, tačiau 15,8 proc. buvo tik vidutiniškai jautrūs penicilinui (VJP).

Tyrimo metu rasta 16,9 proc. (n = 62) klindamicinui atsparių *S.pneumoniae* padermių. Atsparumas eritromicinui didesnis: 21,0 proc. (n = 77) pneumokokų atsparūs eritromicinui, o 0,3 proc. (n = 1) – vidutiniškai jautrūs. Didžiausias *S.pneumoniae* atsparumas rastas trimetoprimui-sulfametoksazoliui: 21,0 proc. (n = 77) pneumokokų buvo atsparūs, 6,3 proc. (n = 23) – vidutiniškai jautrūs. Norfloksacinui ar vankomicinui atsparių *S.pneumoniae* padermių nebuvo.

Nejautrumas vienam antibakteriniam preparatui nustatytas 24,0 proc. *S.pneumoniae* padermių, dominuojant nejautrumui trimetoprimui-sulfametoksazoliui (19,3 proc.). Atsparumas dviem antibakteriniams preparatams rastas 5,2 proc. pneumokokų. Tarp jų dažniausiai nustatytas atsparumas eritromicinui ir klindamicinui (3,0 proc.). Trims antibakteriniams preparatams atsparūs buvo 9,8 proc. pneumokokų. Tarp jų dažniausiai (7,9 proc.). nustatytas atsparumo derinys buvo nejautrumas penicilinui, eritromicinui, klindamicinui Keturiems antibakteriniams vaistams (penicilinui, eritromicinui, klindamicinui, trimetoprimui-sulfametoksazoliui) buvo nejautrūs 4,4 proc. pneumokokų. Dauginis atsparumas antibakteriniams vaistams rastas 12,5 proc. pneumokokų.

S.pneumoniae jautrumas antibakteriniams preparatams tirtuose Lietuvos miestuose buvo skirtingas. Trimetoprimui-sulfametoksazoliui nejautrių *S.pneumoniae* padermių daugiausia nustatyta Vilniuje, o nejautrių penicilinui, eritromicinui ir klindamicinui bei DAV pneumokokų – Panevėžyje. Alytus neįtrauktas į šį palyginimą dėl nedidelio išaugintų *S.pneumoniae* padermių kiekio (n = 2).

S.pneumoniae padermių jautrumas tirtiems antibakteriniams preparatams nuo amžiaus, lyties, metų laiko, kolektyvo lankomumo ar antibakterinių preparatų vartojimo (1–6 mėn. laikotarpiu iki tyrimo) nepriklausė.

Palyginus pagal diagnozes, didžiausias nosiaryklėje nešiojamų *S.pneumoniae* nejautrumas tirtiems antibakteriniams preparatams buvo tų vaikų, kuriems buvo diagnozuotas plaučių uždegimas (n = 14): penicilinui nejautrių – 50,0 proc., eritromicinui nejautrių – 57,1 proc., klindamicinui atsparių – 57,1 proc., trimetoprimui-sulfametoksazoliui nejautrių – 21,4 proc. *S.pneumoniae* padermių.

Iš nejautrių penicilinui, eritromicinui, klindamicinui ir DAV pneumokokų dominavo 14 serotipas, iš nejautrių trimetoprimui-sulfametoksazoliui – 19F serotipas.

Tirto ŪKTI epizodo metu antibakteriniai preparatai skirti 40,1 proc. sirgusiųjų. Dažniau jie buvo skirti *S.pneumoniae* nešiotojams (57,5 proc. *vs* 30,5 proc., ŠS (95 proc. PI) – 1,901 (1,540–2,346), p = 0,000). Dažniausiai preparatai skirti sirgusiems ūminiu plaučių uždegimu (95,8 proc.), ūminiu vidurinės ausies uždegimu (72,4 proc.) ar ūminiu bronchitu (55,5 proc.), rečiau sirgusiems ūmine viršutinių kvėpavimo takų infekcija (32,9 proc.).

Šiuo metu rinkoje esančių KPV-10 ir KPV-13 teorinis veiksmingumas būtų atitinkamai 58,3 proc. ir 72,8 proc. Didžiausias KPV-10 veiksmingumas (62,4 proc. ir 68,4 proc.) būtų 2 ir 3 metų vaikų. KPV-13 teorinis veiksmingumas siektų daugiau nei 65 proc. bet kuriais tirtų vaikų gyvenimo metais, o trečiaisiais metais jis siektų net 81,6 proc.

Be to, 67,9 proc. ir 82,4 proc. antibakteriniams preparatams nejautrių *S.pneumoniae* serotipų buvo atitinkamai KPV-10 ir KPV-13 sudėtyje.

Išvados

1. *S.pneumoniae* kolonizacija buvo dažna (40,8 proc.) ikimokyklinio amžiaus vaikų, sergančių ūminėmis kvėpavimo takų infekcijomis, nosiaryklėje.
2. Lietuvoje ikimokyklinio amžiaus vaikų nosiaryklėje vyravo tie patys *S.pneumoniae* serotipai (6A, 6B, 14, 15, 19F ir 23F), kurie dominavo kitose Europos šalyse iki pneumokokinių vakcinų įtraukimo į nacionalinę imunizacijos programą.

3. *S.pneumoniae* kolonizacija turėjo neigiamą įtaką ūminių kvėpavimo takų infekcijų eigai:
    - ilgesnė ligos trukmė,
    - ilgesnis kolektyvo nelankymo laikas,
    - dažnesnis ūminis vidurinės ausies uždegimas, bronchitas, plaučių uždegimas,
    - dažnesnis gydymas antibakteriniais preparatais.
4. Dauguma *S.pneumoniae* buvo jautrūs dažnai vartojamiems antibakteriniams preparatams (penicilinams, makrolidams).
5. Šiuo metu rinkoje esančios konjuguotosios pneumokokinės vakcinos galėtų būti veiksmingos, skiepijant Lietuvos vaikus.

Praktinės rekomendacijos

S.pneumoniae dažnai randamas ikimokyklinio amžiaus vaikų, sergančių ŪKTI, nosiaryklėje. Gydytojams, įtarus bakterinę viršutinių ar apatinių kvėpavimo takų infekciją, svarbu nepamiršti pagalvoti ir apie galimą pneumokokinę infekciją.

Empiriniam pneumokokinės infekcijos gydymui pirmojo pasirinkimo antibakteriniai preparatai turėtų būti penicilinų grupės antibakteriniai preparatai, tik esant alergijai penicilinui – makrolidų grupės antibakteriniai preparatai. Pneumokokų atsparumas didėja, todėl labai svarbu vengti nereikalingo antibakterinių preparatų vartojimo.

Svarbu informuoti gydytojus ir tėvus apie dažną *S.pneumoniae* nešiojimą nosiaryklėje, vyraujančius serotipus ir teorinį vakcinų veiksmingumą Lietuvoje, tokiu būdu motyvuoti skiepyti vaikus konjuguotąja pneumokokine vakcina. Šiuo metu tai yra veiksmingiausia profilaktikos priemonė.

Būtų vertinga sukurti ir nuolat atnaujinti Lietuvos bei Europos duomenų bazę ne tik apie invazines *S.pneumoniae* ligas, kurios yra tik ledkalnio viršūnė, bet ir pateikti duomenų apie kitas pneumokokų sukeltas ligas ir kolonizaciją, nešiojamų *S.pneumoniae* serotipų pasiskirstymą ir atsparumą antibakteriniams preparatams. Tada būtų galima efektyviau stebėti kitimų dinamiką bei gerinti vakcinacijos ir antibakterinių preparatų vartojimo politiką.

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9. PUBLICATIONS AND CONFERENCE REPORTS

Publications

1. Usonis V, **Stacevičienė I,** Petraitienė S, Vaičiūnienė D, Alasevičius T, Kirslienė J. *Streptococcus pneumoniae nasopharyngeal colonisation in children aged under six years with acute respiratory tract infection in Lithuania, February 2012 to March 2013*. Euro Surveill 2015; 20(13): 34-41.

2. Petraitienė S, Alasevičius T, **Stacevičienė I,** Vaičiūnienė D, Kačergius T, Usonis V. *The influence of Streptococcus pneumoniae nasopharyngeal colonization on the clinical outcome of the respiratory tract infections in preschool children*. BMC Infect Dis 2015; 15:403.

3. **Stacevičienė I,** Petraitienė S, Vaičiūnienė D, Alasevičius T, Kirslienė J, Usonis V. *Antibiotic resistance of Streptococcus pneumoniae, isolated from nasopharynx of preschool children with acute respiratory tract infection in Lithuania*. BMC Infect Dis. 2016; 16(1):216.

4. Petraitienė **S**, Rimkutė D, Vaičiūnienė D, **Stacevičienė I,** Usonis V. *Streptococcus pneumoniae* nasopharyngeal colonisation and acute otitis media. Vaikų pulmonologija ir alergologija 2013; XVI (2): 5379-5385.

Conference reports and presentations

1. Usonis V, Vaičiūnienė D, Petraitienė S, Alasevičius T, **Stacevičienė I.** Nasopharyngeal carriage of *Streptococcus pneumoniae* serotypes in 0-6 years old children with respiratory disease in Lithuania. First results. Oral presentation at the 31st Annual Meeting of the European Society for Paediatric Infectious Diseases in Milan, Italy, May 28-June 1, 2013

2. Petraitienė S, Alasevičius T, **Stacevičienė I,** Vaičiūnienė D, Usonis V. *Streptococcus pneumoniae* nasopharyngeal colonisation in children with acute respiratory infections before introduction of universal conjugated pneumococcal vaccine in Lithuania. Abstract and poster were presented at the 33rd Annual

Meeting of the European Society for Paediatric Infectious Diseases in Leipzig, Germany, May 12-16, 2015.

3. **Stacevičienė I,** Petraitienė S, Alasevičius T, Vaičiūnienė D, Usonis V. The influence of *S.pneumoniae* nasopharyngeal colonisation on the course of acute respiratory tract infections. Oral presentation at the 10th European Respiratory Society Lithuanian division conference "Interventional pulmonology and allergology" in Rusnė, Lithuania, June 17-18, 2016.

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Education

Since 2016 – present – residency of paediatrics and paediatric infectious diseases at Vilnius University, Lithuania.

2009–2016 – residency of paediatrics and paediatric pulmonology at Vilnius University, Lithuania.

2008–2009 – the internship at Klaipėda Seamen's Hospital, Lithuania.

2002–2008 – studies at Vilnius University, Faculty of Medicine, Lithuania. Completion of the integral studies of Medicine Studies at Vilnius University. Awarded the qualification of a medical doctor.

Work experience

Since Oct 2011 – present – a junior research fellow in the Clinic of Children Diseases, Faculty of Medicine, Vilnius University.

Training observerships

July 2007 – participation in a 4 week international medicine student exchange programme in Turkey, Baskent's University Hospital.

July 2011 – September 2011 – participation in the Erasmus Programme as a trainee at the Bristol Royal Hospital for Children in the United Kingdom.

March 2013 – ESPID (European Society for Paediatric Infectious Diseases) training fellowship at Paediatric Clinic I, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy.

Professional memberships

European Society for Paediatric Infectious Diseases
Lithuanian Paediatric Society
Lithuanian Paediatric Respiratory Society
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