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NASOPHARYNGEAL CARRIAGE OF *STREPTOCOCCUS PNEUMONIAE* AND SEROTYPES INDENTIFIED AMONG NURSING HOME RESIDENTS IN COMPARISON TO THE ELDERLY AND PATIENTS YOUNGER THAN 65 YEARS LIVING IN DOMESTIC ENVIRONMENT

NOSILSTVO PNEVMOKOKA V NOSNEM ŽRELU IN IDENTIFIKACIJA SEROTIPOV MED OSKRBOVANCI DOMA STAREJŠIH OBČANOV V PRIMERJAVI S STAROSTNIKI IN MLAJŠIMI OD 65 LET, KI ŽIVIJO V DOMAČEM OKOLJU

Maja KOLŠEK-ŠUŠTERŠIČ[1*], Andreja BEG KRASNIČ[1], Verica MIOČ[2], Metka PARAGI[2], Janez RIFEL[3]

[1]Community Health Centre Ljubljana, Metelkova 9, 1000 Ljubljana, Slovenia
[2]National Laboratory of Health, Environment and Food, Centre for Medical Microbiology, Department for Public Health Microbiology, Grablovičeva 44, 1000 Ljubljana, Slovenia
[3]University of Ljubljana, Faculty of Medicine, Department of Family Medicine, Poljanski nasip 58, 1000 Ljubljana, Slovenia

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ABSTRACT

Introduction. In Slovenia, there is little data available on pneumococcal vaccination rates and no data on asymptomatic NPCR and serotypes in the population of nursing home residents in comparison to the elderly living in domestic environment, therefore the goal was to gain these data.

Methods. A cross sectional epidemiological study was performed. Nasopharyngeal swabs from 151 nursing home residents, 150 elderly living in domestic environment, and 38 adults less than 65 years old were collected twice (in two consecutive years). The swabs were analysed for pneumococcal identification and serotyping. Patient data were collected from medical files and medical history.

Results. No statistically significant differences in NPCR were seen between compared groups in two consecutive years. An average NPCR in two consecutive years in nursing home residents was 1.45%, in the elderly living in domestic environment 0.85%, and in adults less than 65 years old 7.05%. Serotypes identified among nursing home residents were 6B and 9N, among the group of elderly living in domestic environment, 6A and among adults less than 65 years old, 35F, 18C and 3. Pneumococcal vaccination rates were low (3.3% in nursing home residents, 6% in the elderly from domestic environment and 0% in the group of adults less than 65 years old).

Conclusions. Our data suggests that NPCR and the proportion of people vaccinated with pneumococcal vaccine among the elderly are low. We identified different serotypes in all groups, only one person was a chronic carrier (serotype 35F).

Keywords:
S. pneumoniae, nasopharyngeal carriage, serotype identification, retirement home residents, vaccination

IZVLEČEK

***Izhodišča**. V Sloveniji je malo podatkov o stopnji precepljenosti s pnevmokoknimi cepivi in ni dostopnih podatkov o nosilstvu pnevmokoka v nosnem žrelu ter o serotipih med oskrbovanci doma starejših občanov (DSO) v primerjavi s starostniki, ki živijo v domačem okolju, zato je bil namen raziskave pridobiti te podatke.*

***Metode**. Izvedli smo presečno epidemiološko raziskavo. Dvakrat (v dveh zaporednih letih) smo odvzeli brise 151 oskrbovancem DSO, 150 starostnikom, ki živijo v domačem okolju, in 38 odraslim, mlajših od 65 let. Z analizo brisov smo identificirali pnevmokoke in jih serotipizirali. Podatke o bolnikih smo pridobili iz medicinske dokumentacije ter iz anamneze.*

***Rezultati**. V nobeni od primerjanih skupin nismo ugotovili razlike v deležu nosilstva pnevmokoka v dveh zaporednih letih. Povprečni delež nosilstva v dveh zaporednih letih skupaj je bil med oskrbovanci DSO 1,45%, med starostniki, ki živijo v domačem okolju, 0,85% ter med odraslimi, mlajšimi od 65 let, 7,05%. Med oskrbovanci DSO smo identificirali serotipa 6B in 9N, med starostniki, ki živijo v domačem okolju, serotip 6A ter med odraslimi, mlajšimi od 65 let, stereotipe 35F, 18C in 3. Delež cepljenih s pnevmokoknim cepivom je bil nizek (3,3% med oskrbovanci DSO, 6% med starostniki, ki živijo v domačem okolju, in 0% med odraslimi, mlajšimi od 65 let).*

***Zaključki**. Glede na naše izsledke sklepamo, da sta delež nosilcev pnevmokoka v nosnem žrelu ter delež oseb, cepljenih s pnevmokoknimi cepivi, med starostniki nizka. Identificirani serotipi v vseh skupinah so bili različni, odkrili smo le enega kroničnega nosilca (serotip 35F).*

Ključne besede:
pnevmokok, nosilstvo v nosnem žrelu, identifikacija serotipov, oskrbovanci doma starejših občanov, cepljenje

***Corresponding author:** Tel: ++ 386 41 519 962; E-mail: maja.kolsek@gmail.com

1 INTRODUCTION

Elderly living in nursing homes represent 5% of the total elderly population in Slovenia (1). Among them respiratory tract infections are the second most prevalent and represent one of the most frequent causes of morbidity, mortality and hospital admissions (1-3). In this group, pneumonia is regarded a special category due to advanced age, numerous co-morbidities and non-specific, but often severe disease course, which all contribute to a high (30%) mortality rate (2). One of the main pathogens causing pneumonia in the group of elderly is *S. pneumoniae* (*Streptococcus pneumoniae*; 4-7).

S. pneumoniae is part of the normal nasopharyngeal microbial flora (8). Colonization occurs early in life, with prevalence of 40% in children and 15% in adults (9), transmission between individuals is via aerosol (8). Nasopharyngeal colonization is a risk factor for pneumococcal diseases: otitis media, pneumonia, sepsis, meningitis. Asymptomatic carriers are the main reservoir of pneumococci and the principal source of spread among new hosts (10). More than 20% of deaths associated with pneumococcal infection are supposed to occur among people older than 80 years, although nasopharyngeal pneumococcal carriage rate (NPCR) in this group is low (11, 12).

The estimated incidence of pneumonia among nursing home residents in the United States is 365/1000 residents per year and it is significantly higher in comparison to elderly living in domestic environment (20-40/1000) (13). Due to frequent and sometimes unjustified use of antibiotics, numerous antibiotic resistant pneumococcal strains develop (4, 13), representing an increasing problem worldwide (5, 14).

The incidence of invasive pneumococcal disease in Slovenia was 11.9/100,000 in year 2012 (most frequently pneumonia) (15). The highest rates were among children under 5 years of age (46.2/100,000) and among elderly over 65 years of age (27/100,000) (15). Among adults most prevalent serotypes, causing invasive pneumococcal disease, were 3, 9V, 14, 4, 1, 23F and 19F (15). Similar data was found in United States, German and Czech studies (16-18).

Although pneumococcal pneumonia is an important cause of morbidity and mortality among nursing home residents and represents great cost for the society (19), pneumococcal vaccination rates remain low (6, 13, 20). In situations where vaccination rates within a closed population, such as in a nursing home, are less than 5%, there is a greater risk for pneumococcal infection outbreak (6). Pneumococcal vaccination represents effective protection from pneumococcal pneumonia for an individual and vaccination of critical number of individuals can lead to herd immunity effect (6, 21). So far, there is little data available on pneumococcal vaccination rates (20) and no data on asymptomatic NPCR and serotypes in the population of nursing home residents in comparison to adults living in domestic environment in Slovenia. Therefore the goal of this research is to gain these data.

2 METHODS

2.1 Type of a Study and Study Population

We performed a cross sectional epidemiological study, in which two swabs were taken from all the participants (one swab every year) in two consecutive years (February 2013 – November 2013 and February 2014 – November 2014).

The study included 151 nursing home residents from "Dom upokojencev center" in Ljubljana, 152 elderly living in domestic environment and 38 adults less than 65 years, all visiting General practitioners in the same region ("Zdravstveni dom Ljubljana Center"). Due to lack of data, 2 of the participants in the first year, both from the group of elderly living in domestic environment, and one participant in the second year, from the group of nursing home residents, were excluded from the analysis.

Inclusion criteria were i) no sign of acute respiratory infection, ii) living in a defined geographical region in a domestic environment or retirement home and iii) informed consent form signed by the patient or patient's legal representative.

Exclusion criteria were i) ongoing acute respiratory infection, ii) living outside defined geographical region, iii) refusal to sign informed consent form by patient or patient's legal representative, iv) the use of antibiotics in the two weeks prior to taking the swab, and v) immunocompromised patients (hematological malignancies, inherited immunodeficiency, HIV, post splenectomy status, cancer chemotherapy).

2.2 Data Collection

Recruitment of participants started in the nursing home. We asked all the residents from "unit Poljane" to participate. On-site recruitment approach, with the possibility to turn down the participation was used. This was previously identified as one of more successful approaches (22). 95 out of 168 decided to join the study. We continued in "unit Tabor", where the random number method was used until we included the expected number of participants. The same method was used to select the participants among elderly patients and adults less than 65 years from different General practitioner offices at "Zdravstveni dom Ljubljana Center". These were contacted by telephone.

Case report form was filled in for each participant. It included patient's identification number, gender,

age, preceding vaccination data against influenza and pneumococcal infections, preceding antibacterial therapy (from three months up to two weeks prior to taking the swab), data on preschool children contacts, corticosteroid or immunosuppressant treatment and data on chronic diseases. This data was collected by reviewing patient medical files and by taking patient history.

2.3 Nasopharyngeal Swab Collection

We used R Sterile Copan swab (Copan, Brescia, Italy) with a universal transport medium to take a sample from nasopharynx. Nasopharyngeal space is currently defined as the most appropriate spot for *S. pneumoniae* detection (23). The swab was inserted through a nostril about 10 cm deep into the nasopharynx, rotated there and removed after 5 seconds and inserted directly into the transport medium.

2.4 *Streptococcus Pneumoniae* Identification and Serotyping

The swabs were transferred to the National Laboratory of Health, Environment and Food, Centre for Medical Microbiology, Department for Public Health Microbiology, Laboratory for Public Health Bacteriology, Ljubljana, Slovenia no later than 24h after specimen collection, where the identification and serotyping of S. pneumonia was performed.

The cultures were grown on Columbia blood agar with 5% sheep blood and Blood agar with bovine blood, both manufactured at the laboratory, and incubated on 35±2 °C in the 5% CO2 for 48h. The plates were checked for the presence of suspected *S. pneumoniae* colonies after 24 and 48h of incubation. All suspected colonies based on colony morphology and hemolysis were cultured on Columbia agar for 24h and then stained by Gram, tested for the presence of catalase, optochin sensitivity and bile solubility. An isolate was considered to be *S. pneumoniae* if the Gram stain morphology of the isolate showed gram-positive diplococci, cocci, single or in chains, was catalase negative, optochin sensitive and bile soluble. Serotyping was performed with the Neufeld-Quellung reaction, using specific pneumococcal typing antisera (Omni, pooled, group, type and factor) containing specific antibodies against pneumococcal capsular polysaccharide type antigens (Statens Serum Institute, Copenhagen, Denmark). To avoid misidentification of *S. pneumoniae* like viridians group streptococci, a Real-Time PCR Assay targeting Lyt A (autolysin) a gen specific for S. pneumoniae, has been used (24). The DNA isolation was done using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

2.5 Statistical Analysis

The collected data was analyzed with SPSS, version 20.0.0. Pearson Chi Square test was used to test the differences between different groups of participants. A value of $p<0.05$ was used as a limit to describe significant scientific differences between groups.

3 RESULTS

3.1 Description of the Groups

In the group of nursing home residents, the average age was 85.24 years (range 67-105 years), 120 (79.4%) were females and 31 (20.6%) were males.

In the group of elderly from domestic environment, the average age was 82.07 years (range 65-97 years), 115 (76.7%) were females and 35 (23.3%) were males.

In the group of adults less than 65 years the average age was 55.34 years (range 47-64 years), 19 (50%) were females and 19 (50%) were males.

3.2 Swab Results

NPCR were low in groups of elderly studied in two consecutive years. In the group of adults less than 65 years NPCR were higher, in the second year NPCR in this group was significantly higher, compared to others ($p<0.05$) (Table 1).

71 participants were eliminated from the study after the first year; 42 participants turned down the second swab (1 from nursing home residents, 38 elderly from domestic environment and 3 adults less than 65 years) and 29 due to death (20 nursing home residents, 8 elderly from domestic environment, 1 adult less than 65 years).

Table 1. Number of positive swabs in different groups in two consecutive years.

	Nursing home residents	Elderly domestic environment	Adults < 65 years
First year	2 (1.3%) n=151	1 (0.7%) n=150	2 (5.3%) n=38
Second year	2 (1.6%) n=129	1 (1%) n=104	3 (8.8%) n=34*

* $p<0.05$

3.2.1 Characteristics of Participants with Positive Swab Results

There were five positive swab results in the first year (Table 2) and six in the second (Table 3). Serotype 6B and a non-typeable *S. pneumoniae* was identified in nursing home residents in the first year (Table 2), in the second year serotypes 6B and 9N were identified (Table 3). In the group of elderly from domestic environment a non-typeable

S. Pneumoniae was identified in the first year (Table 2) and 6A serotype in the second year (Table 3). In adults less than 65 years a non-typeable *S. pneumoniae* and 35F serotype were identified in the first year (Table 2) and 35F serotype (chronic carrier) and 18C and 3 in the second year (Table 3).

Table 2. Characteristics of participants with positive swab result and *S. pneumoniae* serotypes in the first year.

	Participant Nr. 1	Participant Nr. 2	Participant Nr. 3	Participant Nr. 4	Participant Nr. 5
Age	88 years	78 years	80 years	47 years	58 years
Gender	Female	Male	Female	Male	Male
Group	Nursing Home	Nursing Home	Domestic Environment	Adults <65 years	Adults <65 years
Influenza vaccination[a]	No	Yes	No	No	No
Pneumococcal vaccination[b]	No	Yes	No	No	No
Antibiotics 3 months prior to swab	No	No	No	No	Yes
Preschool children contacts	No	No	No	No	Yes
Corticosteroid treatment[c]	No	Yes	No	No	Yes
1 chronic illness	/	/	/	No	Yes
2 chronic illnesses	/	/	Yes	No	/
≥ 3 chronic illnesses	Yes	Yes	/	No	/
***S. pneumoniae* serotype**	**NT**	**6B**	**NT**	**35F**	**NT**
Death after the first year	No	Yes	No	No	No
Second swab refusal	No	/	No	No	No

[a] in the previous season, [b] in the last 5 years, [c] in the last month, NT, non-typeable; /, not applicable

Table 3. Characteristics of participants with positive swab result and *S. pneumoniae* serotypes in the first year.

	Participant Nr. 1'	Participant Nr. 2'	Participant Nr. 3'	Participant Nr. 4'	Participant Nr. 5'	Participant Nr. 6'
Age	86 years	77 years	80 years	47 years	58 years	60 years
Gender	Male	Female	Female	Male	Male	Female
Group	Nursing Home	Nursing Home	Domestic Environment	Adults <65 years	Adults <65 years	Adults <65 years
Influenza vaccination[a]	No	No	No	No	No	No
Pneumococcal vaccination[b]	No	No	No	No	No	No
Antibiotics 3 months prior to swab	No	No	No	No	Yes	No
Preschool children contacts	No	No	No	No	Yes	Yes
Corticosteroid treatment[c]	No	No	No	No	Yes	No
1 chronic illness	/	/	/	No	Yes	No
2 chronic illnesses	Yes	Yes	Yes	No	/	No
≥ 3 chronic illnesses	/	/	/	No	/	No
***S. pneumoniae* serotype**	**6B**	**9N**	**6A**	**35F**	**18C**	**3**

[a] in the previous season, [b] in the last 5 years, [c] in the last month; /, not applicable

3.2.2 Characteristics of Participants with Negative Swab Results

The rate of influenza vaccination was significantly lower in the group of adults less than 65 years, compared to both groups of elderly, the rate of influenza vaccination was significantly higher in the group of nursing home residents, compared to elderly from domestic environment (Table 4). The rate of pneumococcal vaccination was low in all the groups, there was no statistically significant difference (Table 4). There was also no difference considering preschool children contacts and immunosuppressant therapy. Antibiotic consumption rate was significantly higher in both groups of elderly, compared to adults less than 65 years and the number of comorbidities (≥3) was significantly higher in the group of nursing home residents, compared to others. Death incidence was significantly higher among nursing home residents, compared to the other two groups, whereas the rate of second swab refusal, was significantly higher in the group of elderly living in domestic environment, compared to others.

Table 4. Characteristics of participants with negative swab results in two consecutive years.

		Nursing home residents		Elderly domestic environment		Adults <65 years	
		First year	Second year	First year	Second year	First year	Second year
Influenza vaccinationa	Yes	50 (33.6%)*	23 (18.1%)	31 (20.8%)	20 (19.4%)	2 (5.6%)	2 (6.4%)
	No	99 (66.4%)	63 (49.6%)	118 (79.2%)	56 (54.4%)	34 (94.4%)*	28 (90.3%)
	Unknown	/	41 (32.3%)	/	27 (26.2%)	/	1 (3.3%)
Pneumococcal vaccinationb	Yes	4 (2.7%)	4 (3.1%)	9 (6%)	8 (7.8%)	0	/
	No	145 (97.3%)	123 (96.9%)	139 (93.3%)	94 (91.2%)	36 (100%)	31 (100%)
	Unknown	/	/	1 (0.7%)	1 (1%)	/	/
Antibiotics 3 months	Yes	43 (28.9%)*	37 (29.1%)	19 (12.8%)*	12 (11.7%)	5 (13.9%)	4 (12.9%)
prior to swab	No	106 (71.1%)	90 (70.9%)	130 (87.2%)	91 (88.3%)	31 (86.1%)	27 (87.1%)
	Unknown	/	/	/	/	/	/
Preschool children contacts	Yes	29 (19.5%)	26 (20.5%)	38 (25.5%)	28 (27.2%)	14 (38.9%)	12 (38.7%)
	No	119 (79.9%)	100 (78.7%)	111 (74.5%)	75 (72.8%)	22 (61.1%)	19 (61.3%)
	Unknown	1 (0.6%)	1 (0.8%)	/	/	/	/
Corticosteroid treatmentc	Yes	9 (6%)	6 (4.7%)	6 (4%)	4 (3.9%)	0	/
	No	140 (94%)	121 (95.3%)	143 (96%)	99 (96.1%)	36 (100%)	31 (100%)
	Unknown	/	/	/	/	/	/
1 chronic illness	Yes	38 (25.5%)	35 (27.6%)	64 (43%)	43 (41.7%)	11 (30.6%)	10 (32.3%)
2 chronic Illnesses	Yes	50 (33.6%)	42 (33.1%)	43 (28.9%)	28 (27.2%)	5 (13.9%)	4 (12.9%)
≥ 3 chronic illnesses	Yes	47 (31.6%)*	38 (29.9%)	21 (14.1%)	14 (13.6%)	4 (11.1%)	3 (9.7%)
Death after the first year	Yes	19 (12.8%)*		8 (5.4%)		1 (2.8%)	
	No	130 (87.2%)		141 (94.6%)		35 (97.2%)	
Second swab refusal	Yes	1 (0.7%)		38 (25.6%)*		3 (11.2%)	
	No	148 (99.3%)		111 (74.4%)		32 (88.8%)	

[a] in the previous season, [b] in the last 5 years, [c] in the last month, *, p<0.05 – see discussion

4 DISCUSSION

Swab analysis in two consecutive years in nursing home residents showed a NPCR of 1.45% on average, which is similar to the data found in the United States study among retirement community residents (25). Some characteristics of nursing home residents in our study, such as a higher number of patients with two or more comorbidities in the group of nursing home residents and a more common use of antibiotics in this group, were similar to the data found in studies by Lee and Sundvall (26, 27). An average NPCR in the group of elderly living in domestic environment in two consecutive years was 0.85%, which also correlates to some studies conducted among the elderly, where NPCR were 0.32% and 5.3%, respectively (28, 12). Most of the pneumococci isolated were serotyped, some of them were non-typeable, which means they did not have the polysaccharide capsule.

6B and 9N serotypes are present, along with other serotypes, in the 23-valent polysaccharide pneumococcal vaccine (PPV23); moreover, 6B is also present in the 13-valent conjugate pneumococcal vaccine (PCV13). The one *S. pneumoniae* carrier, who was vaccinated with PPV23 vaccine, carried a 6B serotype. Vaccination with this type of vaccine only prevents invasive pneumococcal disease, whereas vaccination with PCV13 vaccine also prevents nasopharyngeal colonization (29). A 6A serotype, which was identified in the participant from the group of the elderly from domestic environment, may have a potential to cause micro-epidemics in closed communities; it is present in the PCV13 vaccine (30).

Although NPCR among the elderly are low, this group represents a high risk population for invasive pneumococcal disease. This might implicate that long-term pneumococcal carriage among the elderly is rare, and that newly acquired pneumococci are responsible for invasive pneumococcal disease (31, 32). Seasonality also affects pneumococcal carriage rates - pneumococci are transmitted better during cooler and drier months (33).

The nasopharyngeal swab analysis among adults less than 65 years old showed that pneumococcal carriage rates were higher in this group in comparison to the elderly population – 5.3% in the first year and 8.8% in the second year. This may be because the participants from this group have more preschool children contacts (39.5%), compared to the groups of the elderly, although statistically significant difference was not confirmed in this case. Adults less than 65 years old carried 35 F (chronic carrier), 18C, 3 and a non-typeable serotype. None of the participants were vaccinated with either of pneumococcal vaccines.

The strong side of this study is that it is the first study addressing the topic of pneumococcal carriage and serotype identification among nursing home residents and the elderly living in domestic environment in Slovenia, and that the results of this study (low NPCR and serotype diversity) are consistent with foreign studies (34). There are also some limitations to this study. The first is that the sample is not representative, since it includes only participants from one nursing home and one community health center in the same region, and as the number of participants is not very high. The second limitation is that the study was not conducted in the coolest and driest months of the year, which might have influenced our results.

Although the NPCR among nursing home residents are low, the incidence of pneumonia (3) and the mortality rate in this population are significantly higher in comparison to the group of the elderly living in domestic environment (30% vs.16.7%) (2). In 2012, 81% of invasive pneumococcal infections in the population >65 years of age in Slovenia were caused with serotypes included in the PCV13 vaccine and 95% with serotypes included in the PPV23 vaccine (15). Pneumococcal vaccines are important means of invasive disease prevention, and are related to a lower mortality rate from pneumococcal pneumonia (6); nevertheless, the vaccination rates remain low also according to our data (3.3% in nursing home residents and 6% in the elderly from domestic environment). It is necessary to increase the number of vaccinated elderly.

5 CONCLUSIONS

Our data suggests that NPCR among the elderly are low and not significantly different in nursing home residents, compared to the elderly living in domestic environment. We identified different serotypes in all groups, only one person was a chronic carrier (serotype 35F). Our data also shows that pneumococcal vaccination rates are low in all compared groups, despite the benefits the vaccination brings.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

The Committee of the Republic of Slovenia for medical ethics approved the study on 19.12.2011, Nr. 72/11/11.

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