

Received: 2012.04.25 Accepted: 2012.08.27 Published: 2013.01.18

High prevalence of Streptococcus pneumoniae in adenoids and nasopharynx in preschool children with recurrent upper respiratory tract infections in Poland - distribution of serotypes and drug resistance patterns

Authors' Contribution: Study Design A Data Collection B

Statistical Analysis C Data Interpretation D

Manuscript Preparation E Literature Search F Funds Collection G

ABDE 1 Artur Niedzielski

ABCDEF 2 Izabela Korona-Glowniak

ADE 2 Anna Malm

1 Department of Pediatric Otolaryngology, Phoniatrics and Audiology, Medical University of Lublin, Lublin, Poland / 2 Department of Pharmaceutical Microbiology, Medical University of Lublin, Lublin, Poland

Author's address: Izabela Korona-Glowniak, e-mail: iza.glowniak@umlub.pl

Source of support: Departmental sources

Background:

Streptococcus pneumoniae is one of the major bacterial pathogens colonizing nasopharynx, and often causes upper respiratory tract infections in children. We investigated the prevalence of S. pneumoniae in nasopharynx and adenoid core in 57 children aged 2-5 years who underwent adenoidectomy for recurrent pharyngotonsillitis, and we determined serotypes and antibiotic resistance patterns of the isolated pneumococci.

Material/Methods:

The nasopharyngeal specimens obtained before adenoidectomy and the adenoids after the surgery were cultured for pneumococci. All isolates were serotyped by means of Quellung reaction. Susceptibility to antibiotics was determined according to EUCAST recommendations.

Results:

S. pneumoniae colonization was observed in 40 (70.2%) children. From 29 (50.9%) children S. pneumoniae was isolated both from nasopharynx and adenoid core; 2 or 3 different isolates were identified in 8 (14.0%) children. In 8 (14.0%) children pneumococci were obtained from adenoid core only and in 3 (5.3%) children from nasopharynx only. Among the isolates, 35.3% were susceptible to all tested antimicrobials and 45.1% had decreased susceptibility to penicillin. Multidrug resistance was present in 52.9% of the isolates. The most frequent was serotype 19F (25.5%). The prevalence of serotypes included in pneumococcal conjugate vaccines PCV10 and PCV13 was 51.0% and 62.7%, respectively.

Conclusions:

The adenoids, like the nasopharynx, can be regarded as a reservoir of pneumococci, including multidrug resistant strains, especially in children with indication for adenoidectomy due to recurrent respiratory tract infections refractory to antibiotic therapy. Good vaccine coverage among the isolated pneumococci confirmed the validity of the routine immunization by PCVs in young children.

key words:

Streptococcus pneumoniae

serotypes

antibiotic resistance patterns

adenoids

full-text PDF:

ICID 883742

PMID 23328644

3097

1 2 4

35

54

Background

Upper respiratory tract infections (URTIs) can be regarded as a significant problem in childhood; they are the most frequent cause for pediatric visits in general practice, and are mainly reason for antibiotic prescriptions by pediatricians [1,2]. Streptococcus pneumoniae is one of the major bacterial pathogens colonizing the nasopharynx, and often cause URTIs in children. Adenoids can be regarded as a reservoir of pneumococci [3]. Contrary to common belief of many pediatricians, the most important pneumococcal infections are of the respiratory tract, not invasive diseases. However, despite the advances in the development of pneumococcal conjugate vaccines (PCVs) leading to a reduction of invasive disorders, eradication of pneumococcal diseases is not within easy reach. Of the 90 pneumococcal serotypes that have been identified so far, the most common ones that cause infections are 3, 6B, 9V, 14, 19F, and 23F. The morbidity of URTIs caused by antibiotic resistant pathogens has been increasing in children, creating a worldwide public health problem. The distribution of resistance among S. pneumoniae is of particular interest in the context of introduction of multivalent PCVs. The safety, efficacy and effectiveness in practice of heptavalent pneumococcal conjugate vaccine (PCV-7) and other PCV was established in multiple settings in many countries [4,5]. In 2007, World Health Organization (WHO) recommended that all countries incorporate PCV to their national infant immunization program [6]. PCV-7 was introduced in Poland in 2005 and recommended for children under 2 years old, but is not refunded. Since 2009, the PCV was recommended for children under 5 years old and is refunded for some risk groups.

In this study we investigated the prevalence of *S. pneumoniae* in the nasopharynx and adenoid core in children from Poland who underwent adenoidectomy for recurrent pharyngoton-sillitis and we determined serotypes and antibiotic resistance patterns of the isolated pneumococci.

Material and Methods

Patients

The study enrolled 57 children, aged between 2 and 5 years, undergoing adenoidectomy in the Department of Pediatric Otolaryngology, Phoniatrics and Audiology, Medical University of Lublin during May-June 2011. The indication for adenoidectomy was recurrent acute pharyngotonsillitis for at least 2 years, with 5 or more acute attacks per year. Patients did not receive any antibiotic therapy for at least 20 days before the operation. Informed content was obtained from all children's parents. The Ethics Committee of the Medical University of Lublin approved the study protocol (No. KE-0254/75/211).

Demographic data of studied children is shown in Table 1. Each patient received antibiotics 2 or 3 month before surgery. None of the children were immunized with a pneumococcal vaccine.

Laboratory procedures

Before adenoidectomy, the nasopharyngeal specimens were obtained with sterile alginate-tipped swabs on aluminium shafts. The surgeon removed the adenoids through the mouth by making several small incisions and cauterized the site once the adenoids were removed. Antiseptic and/or bactericidal agents were not used during the surgery. After the surgery, the adenoids were placed in a sterile container and with the nasopharyngeal swabs were transported 2 hours to the laboratory. One surface of the adenoid was cauterized with a heated scalpel and an incision was made through that cauterized area with a sterile scalpel, cutting the adenoid in half. The core was swabbed with sterile alginate-tipped applicator. Swabs were inoculated on Mueller-Hinton agar with 5% sheep blood and 0.5 mg/L of gentamicin for selective cultivation of streptococci. The streaked agar plates were incubated aerobically at 35°C in 5% CO2-enriched atmosphere for 24 to 48 hours. Pneumococci were identified by colony morphology, susceptibility to Optochin (5 µg), and bile solubility; identification was confirmed by the Slidex Pneumo-Kit (BioMerieux) slide agglutination test.

All isolates were serotyped by Quellung reaction using antisera provided by Statens Serum Institute (Copenhagen, Denmark). We used antisera for determination of serotypes belonging to the 23-valent pneumococcal polysaccharide vaccine (PPV23) (ie, 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F) and also serotypes 6A, 23A. The isolates negative to employed pooled sera but positive to omni serum were defined as nontypeable (NT). The untypeable isolates (rough –R) were confirmed by PCR analysis using primers for detecting the *lytA* gene encoding the autolysin enzyme specific to *S. pneumoniae* [7].

Susceptibility of the isolates to oxacillin, erythromycin (E), tetracycline (Te), chloramphenicol (C), clindamycin (Cc), Norfloxacin (Nor), rifampicin (Ra), teicoplanin (Tec), linezolid (Lzd), and trimethoprim-sulfamethoxazole (Sxt) was determined by the disk diffusion method of Bauer and Kirby. Results were interpreted according to the 2011 European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [8]. Isolates exhibiting a zone of ≥20 mm around a 1 µg oxacillin disk were reported as penicillin susceptible *S. pneumoniae* (PSSP); isolates exhibiting a zone of <20 mm were further tested by the E-test (AB Biodisk, Sweden), following the manufacturer's instruction, to determine minimal inhibitory concentration (MIC) for benzylpenicillin. Isolates with MIC ≤0.064 mg/L were considered as fully susceptible

Table 1. Demographic data of children undergoing adenoidectomy for recurrent URTIs.

Risk factor		SP colonized children (%)		SP uncolonized children (%)		Univariate analysis <i>p</i>	Multivariate analysis <i>p</i>	
Age (yr)	2	3	(75.0)	1	(25.0)	0.48		
	3	3	(42.9)	4	(57.1)			
	4	26	(72.2)	10	(27.8)			
	5	7	(70.0)	3	(30.0)			
Sex	Female	17	(85.0)	3	(15.0)	0.073	0.021	
	Male	22	(59.5)	15	(40.5)			
Sibling possessing	No	7	(70.0)	3	(30.0)	1.0		
	1	19	(67.9)	9	(32.1)			
	≥2	13	(68.4)	6	(31.6)			
Passive smoking		13	(59.1)	26	(74.3)	0.26		
DCC attendance		34	(66.7)	3	(75.0)	1.0		
Place of residence	Village	8	(50.0)	8	(50.0)	0.06	0.022	
	City	31	(75.6)	10	(24.4)			
Antibiotics taken for the last attack	Amoxicillin/ co-amoxiclav	22	(71.0)	9	(29.0)	0.72		
	Cephalosporins	9	(69.2)	4	(30.8)			
	Macrolides	7	(58.3)	5	(41.7)			

DCC - day care center; SP - Streptococcus pneumoniae.

to benzylpenicillin; isolates with MIC >0.064 mg/L were called penicillin non-susceptible *S. pneumoniae* (PNSSP). Multidrugresistant isolates of *S. pneumoniae* (MDR-SP) were defined as having resistance to at least 3 different classes of antibiotics. *S. pneumoniae* ATCC 49619 was used as a control strain in the antimicrobial susceptibility tests.

Statistical analysis

Data processing and analysis were performed using StatSoft, Inc. STATISTICA 10. The potential predictor variables were: age (years), gender, having siblings, passive smoking, day care center attendance, place of residence, and antibiotics taken for the last attack; these, were tested in separate univariate analyses (Chi-square or Fisher's exact test, as appropriate) for their association with nasopharyngeal and/or adenoid colonization by S. pneumoniae in general, and by PNSSP or MDR-SP. Significant univariate predictors (p<0.1) were tested for inclusion in the multivariate models, and nonsignificant variables were removed sequentially until only those significant at p<0.1 remained. Variables of particular interest based on previous studies (eg, child age, having siblings, and type of antibiotic used) were included even when were not statistically significant. Results of logistic regression analysis are reported as adjusted odds ratio (OR) with 95%CI. Statistical significance was set at p<0.05.

Results

S. pneumoniae colonization of nasopharynx and/or adenoid core were observed in 40 (70.2%) children who had undergone adenoidectomy for recurrent pharyngotonsillitis. From 29 (50.9%) children, S. pneumoniae was isolated both from nasopharynx and adenoid core, and 2 or 3 different isolates were identified in 8 (14.0%) children. In 8 (14.0%) children, S. pneumoniae isolates were obtained from adenoid core only and in 3 (5.3%) children from nasopharynx only.

Demographic data of studied children are shown in Table 1. According to multivariate analysis, female sex (p=0.02, OR 6.7, 95% CI 1.3–35.6) and city residence (p=0.02, OR 5.6, 95% CI 1.2–25.0) were the independent factors significantly increasing the carriage rates. When risk factors were analyzed for adenoid core colonization and nasopharyngeal colonization separately, no statistical significance was found.

A total of 51 isolates were recovered. Among the isolates, serotypes of PPV23 (82.4%) were identified; whereas 4 isolates (7.8%) were defined as NT-nontypeable, and 5 isolates (9.8%) were untypable (rough – R) (Table 2). Identification of all untypable, non-capsulated pneumococci was confirmed by detection of the *lytA* gene encoding the autolysin enzyme specific to *S. pneumoniae*. The most frequent was serotype 19F

Table 2. Antibiotic resistance in *Streptococcus pneumoniae* isolated from nasopharynx and/or adenoid core of children undergoing adenoidectomy for recurrent URTIs.

Antibiotic	No. of resistant isolates (%)	Serotypes (no. of isolates)
Penicillin	23 (45.1)	9V (1), 14 (3), 6B (3), 19F (6), 23F (5), R (5)
Erythromycin	27 (52.9)	6B (3), 14 (3), 15-nonB (1), 19F (10), 22F (1), 23F (5), R (4)
Clindamycin	26 (51.0)	6B (3), 14 (2), 15-nonB (1), 19F (10), 22F (1), 23F (5), R (4)
Tetracycline	22 (43.1)	6B (1), 14 (1), 10A (1), 15-nonB (1), 19F (11), 22F (1), 23F (2), R (4)
Chloramphenicol	22 (43.1)	6B (3), 10A (1), 19F (9), 23F (5), R (4)
Co-trimoxazole	27 (52.9)	9V (1), 6B (3), 14 (2), 19F (10), 23F (6), R (5)
Norfloxacin	None	None
Rifampicin	None	None
Teicoplanin	None	None
Linezolid	None	None
MDR	28 (54.9)	6B (3), 14 (2), 15-nonB (1), 19 F (11), 22F (1), 23F (5), R (5)

R - rough, untypeable strain; NT - nontypeable; MDR - multidrug resistant.

Table 3. Distribution of serotype and antibiotic resistance in *Streptococcus pneumoniae* isolated from nasopharynx (NP) and adenoid core (AD) samples of children undergoing adenoidectomy for recurrent URTIs.

Site of	Resistance to antibiotics No (%) of isolates						Serotypes No (%) of isolates												
isolation	P	E	Cc	Те	c	Sxt	3	6A	6B	10A	11A	9V	14	15- nonB	19F	22F	23F	NT	R
NP (n=35)	` '	` '	,	` '	10 (28.6)	` '	` '	0 (0)	1 (2.9)	1 (2.9)	2 (5.7)	0 (0)	3 (8.6)	4 (11.4)	(/	1 (2.9)	3 (8.6)	3 (8.6)	3 (8.6)
AD (n=41)	20	21	21	15	15 (36.6)	25	5	1 (2.4)	3 (7.3)	1 (2.4)	2 (4.9)	1 (2.4)	2 (4.9)	2 (4.9)	11 (26.8)	0 (0)	6 (14.6)	3 (7.3)	4 (9.8)

P – penicillin; E – erythromycin; Cc – clindamycin; Te – tetracycline; C – chloramphenicol; Sxt – co-trimoxazole; R – rough, untypeable strain; NT – nontypeable.

(25.5%). The prevalence of serotypes included in PCV10 (containing serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) and PCV13 (containing serotypes 3, 6A, 19A additionally to 10-valent vaccine) was 51.0% and 62.7%, respectively.

Among the pneumococcal strains, 35.3% were susceptible to all tested antimicrobial agents belonging to serotypes 3 (5 isolates), 6A (1 isolate), 10A (1 isolate), 11A (2 isolates), 15 non-B (1 isolate), 19F (1 isolate), and NT (4 isolates). Decreased susceptibility to penicillin was observed in 45.1% of strains (MIC range 0.12–2.0 mg/L, MIC $_{50}$ 0.5 mg/L and MIC $_{90}$ 2.0 mg/L). High rates of antibiotic resistance were found: co-trimoxazole – 52.9%, tetracycline – 43.1%, erythromycin – 52.9%, clindamycin – 51.0%, and chloramphenicol – 43.1% (Table 2). All isolates were susceptible to norfloxacin and. according to EUCAST

2011, they can be reported as susceptible to levofloxacin and moxifloxacin and intermediate to ciprofloxacin and ofloxacin.

None of the tested isolates was resistant to rifampicin, linezolid, or teicoplanin. Multidrug resistance (MDR) was present in 52.9% of all isolates. Among MDR-SP, 77.8% were non-susceptible to penicillin. PNSSP and MDR-SP strains were mostly distributed among PCV13 serotypes (78.3% and 77.8%, respectively). Neither PNSSP carriage (39.3%) nor MDR-SP carriage (46.4%) were associated with type of antibiotic therapy during the 3 months before adenoidectomy and other analyzed predictors. There were no significant discrepancies concerning serotype and antibiotic resistance distribution between groups of pneumococci isolated from the nasopharynx and adenoid core (Table 3).

Table 4. Characteristics of multiple co-colonization by Streptococcus pneumoniae isolated from nasopharynx and/or adenoid core of eight children undergoing adenoidectomy for recurrent URTIs.

No. of	Phenotypes of isolates from								
sample	Nasopharynx	Adenoid core							
9	3 S	3 S							
	NT S								
15	19F TeCSxt	19F TeCSxt							
	R PECcTeSxt								
30	11A S	11A S							
	R PECcTeSxt	R PECcTeSxt							
36	19F ECcTe	23F Sxt							
43	19F PeCcTeSxt	15-nonB S							
49	15-nonB S	19F PECcTeCSxt							
		R PECcTeSxt							
52	15-nonB ECcTe	19F S							
	3 S	3 S							
63	22F ECcTe	23F PECcCSxt							

S – susceptible to all tested antibiotics; P – penicillin;

A comparison of phenotypes of paired pneumococcal samples obtained from the nasopharynx and adenoid demonstrated that in 24 children (60% of colonized children) an identical phenotypically strain was present. Five children (12.5% of colonized children) were carriers of 2 or 3 phenotypically different strains in the adenoid and in nasopharynx (Table 4).

Discussion

We found a high frequency of *S. pneumoniae* colonization in the upper respiratory tracts of preschool children with recurrent upper respiratory infections. Because of the high rate of asymptomatic pneumococcal carriage in preschool children, especially those attending day care, it is difficult to indicate that *S. pneumoniae* is an important bacterial etiological agent of URTIs [2]. Jeong et al. [9] analyzed the differences between the bacterial pathogens of the tonsil core in recurrent tonsillitis and tonsillar hypertrophy with regard to age; *S. pneumoniae* was detected more often in recurrent tonsillitis in the patients 8–14 years, even though in both groups this pathogen was more common in younger patients (<8 years). However, it is well known that frequent RTIs, mainly of viral etiology, which may injure the mucous membrane and cause damage of the local immunological host response, facilitate adherence

of bacteria [10-13]. Syrjanen et al. [14] reported that nasopharyngeal carriage of pneumococci during RTIs (without otitis media) in children increased from 13-43% to 45-56%, depending on age. In contrast, Greenberg et al. [15] found no differences in the overall S. pneumoniae carriage between healthy and sick children in different age groups. Brook et al. [16] study showed that cores of "normal" adenoids of healthy children contain polymicrobial aerobic and anaerobic flora similar to the flora found in adenoids of patients with recurrent otitis media, recurrent adeno-tonsillitis, and obstructive adenoid hyperplasia. However, the concentration of most aerobic pathogens (S. pneumoniae, Staphylococcus aureus, Hemophilus influenzae, and Moraxella catarrhalis) was lower in the "normal" adenoids of the control group, as compared with all other groups. Karlidag et al. [17] demonstrated higher prevalence of bacterial pathogens in the adenoid tissue cultures in patients with otitis media with effusion (study group) in comparison to patients with adenotonsillar hyperthrophy (control group) - S. pneumoniae - 43.9% vs. 28%, H. influenzae - 36.6% vs. 16% and M. catarrhalis - 17.1% vs. 4%. The detection rate of S. pneumoniae in the adenoid core was higher than that in the nasopharynx (71.9% vs. 61.4%) in our study. This indicates that in children with recurrent upper respiratory infections, pneumococci mostly colonized the tissue of adenoids, which may be recognized as the main reservoir of S. pneumoniae. The presence of this pathogen can be one of the causes of chronically infected, swollen, and inflamed adenoids.

In our study, *S. pneumoniae* strains were isolated from the adenoid or nasopharynx in 27.5% of patients, and in 75% of patients from both sites (2 or 3 isolates). These data are comparable to those demonstrated by Tonnaer et al. [18], who collected pneumococcal cultures from middle ear fluid, oropharynx, and adenoid biopsy from the same patient. These authors [18] found genetic similarity in the majority of the paired pneumococcal samples obtained from the same patient (80–90%), which indicate clonal relatedness and suggest that pneumococci presenting at different sites in the upper respiratory region of a patient originate from a single source, most likely the nasopharynx or throat. In our study, a comparison of phenotypes of paired pneumococcal samples obtained from the nasopharynx and adenoid of 1 child demonstrated that in 24 (60%) children an identical phenotypically strain was present.

Longitudinal studies have demonstrated that children are successively colonized with multiple strains of pneumococci [19,20]. However, identification of simultaneous carriage of multiple serotypes is laborious, and the yield varies according to the method used [20,21]. In our study, 2 or 3 different phenotypically strains were identified in 8 (14.0%) children. Carriage of multiple pneumococcal serotypes is an important phenomenon concomitant with the increase in the carriage of non-vaccine serotypes. Eradication of the vaccine serotypes allows exposure

E – erythromycin; Cc – clindamycin; Te – tetracycline;

C – chloramphenicol; Sxt – co-trimoxazole; R – rough, untypeable strain; NT – nontypeable.

of the serotypes that are present, but in lower concentration relative to the vaccine serotypes [21]. Since *S. pneumoniae* is naturally competent for transformation and inter- and intraspecies horizontal gene transfer by homologous recombination, selection of non-vaccine serotypes could be due to subsequent serotype switching during simultaneous carriage of vaccine and non-vaccine pneumococcal strains under vaccination pressure or that lost the capsule. Among 8 children of our study with multiple strain carriage, in 3 children the second/third co-colonized strain was rough and multidrug resistant, probably after losing the capsule. In 6 (75%) children, from nasopharyngeal samples and in 3 (37.5%) children from adenoid core samples, co-colonized strains belonged to non-vaccine serotypes. These strains could be successful in colonization after vaccination.

Community-acquired respiratory tract infections remain the leading cause of physician office visits and use of antimicrobial agents, which are usually administered empirically. Reports of increasing resistance of S. pneumoniae to many antimicrobial agents have made treatment management more difficult. The percentage of PNSSP isolates has been reported to be 0%-60% among healthy children in Europe [14,20,22-24]. Data presented in this study confirmed that prevalence of PNSSP in Poland has been constantly increasing. In the recent Alexander Project, conducted from 1998 to 2000, the frequency of PNSSP was 12.3% [25], but a significant increase to 20.3% was observed among clinical isolates in 2002 [26]. However, at the same time in Poland, the percentage of PNSSP isolates from healthy children under 5 years old in settings such as day care centers (DCCs) and orphanages was 36.2% [23]. The prevalence of erythromycin resistance exceeded that of penicillin resistance in the majority of countries, as in our study [25]. The prevalence of both penicillin-resistant and erythromycin-resistant S. pneumoniae in our study was high (41.2%), consistent with findings from other surveillance studies [25,27]. An alarmingly high prevalence of MDR pneumococcal strains was observed in our study (55%), higher than was noted by other authors [19,25].

Infections cause by drug resistant pathogens in the nasopharynx or the adenoids seems to be harder to treat medically, which could result in the need for adenoidectomy, which seems to have a beneficial effect on the nasopharyngeal bacterial flora [28]. Aarts et al. [28] demonstrated that *S. pneumoniae* and *H. influenza* were most often detected in the nasopharynx of children with recurrent URTIs, and carriage of these bacteria decreased after adenoidectomy in the majority of studies.

Children with recurrent pharyngotonsillitis are usually treated with multiple courses of antibiotics before surgery, but many of them continue to carry pathogenic bacteria in the pharynx and the adenoids, including strains resistant to antibiotics [3]. Although all of the children from our studies were treated by antibiotics, a high prevalence of resistant pneumococci was

observed. On the other hand, the increase in antibiotic resistance of *S. pneumoniae* is generally attributed to the extensive use of antibiotics and the selective pressure on the bacterial strains of the nasopharyngeal flora [29]. There were no connections between presence of antibiotic-resistant strains and type of antibiotic taken by children in our study. Most children in our study attended day care centers (92.7%), which are recognized as places of successful transmission of pneumococci from person to person, including drug-resistant strains, irrespective of antibiotic therapy [22]. That may be the explanation of lack of association between the use of any antibiotic type and pneumococcal carriage, as well as recovery of resistant strains.

However, some studies demonstrated an association between the use of a specific antibiotic and selective colonization by pathogens resistant to that drug [22,30]; the number of courses of drugs to which pathogens are resistant has the utmost importance [22].

Worldwide, the clonal dissemination of a small group of MDR clones of S. pneumoniae produces the majority of clinical treatment failures [30]. The WHO considers immunization of infants and young children with pneumococcal vaccines a priority. Data published by other authors suggested that vaccination could potentially reduce the carriage rate of antibiotic-resistant pneumococci in Europe [23,31]. Currently, in Poland, the 23-valent polysaccharide vaccine for children ≥2 years old and adults (since 1996), and pneumococcal conjugate vaccines (PCV7 [since 2006], PCV10 [since 2009], and PCV13 [since 2010] for children ≤2 years old, have been recommended in the national immunization schedule. Since 2006, less than 5% of children ≤5 years were vaccinated per year, so currently we suppose that less than 15% of children in this group of age are immunized with PCV [32]. PCV7 is currently being replaced by PCV13 vaccine as manufacturing and supply are scaled up. An encouraging finding of our study was that a majority of PNSSP and MDR-SP belonged to serotypes included in PCV13 and PCV10.

According to studies performed in Poland [33–35] there were high invasive pneumococcal disease (IPD) incidence rates among children under 5 years of age. PCV10 and PCV13 covered 71.2–76.3%, and 86.3–92.3%, respectively, of cases involving children under 5 years of age [33,34]. The above data suggest that routine vaccination of infants with PCVs could effectively reduce the carriage rate of pneumococci, including drug-resistant strains, in children in Poland, as in other European countries [5].

Conclusions

A high prevalence of *S. pneumoniae* colonization in the nasopharynx and adenoids in children with recurrent URTIs was observed and a high rate of resistance to macrolides and β -lactams of

isolates was demonstrated. Over half of the isolates were classified as MDR-SP. There is a possibility that the adenoids, like the nasopharynx, are acting as a reservoir of pneumococci, including drug-resistant strains, especially in children with indication for adenoidectomy due to recurrent respiratory tract infections refractory to antibiotic therapy. Good vaccine coverage among the isolated pneumococci confirmed the validity of the introduction of PCVs in the national immunization programme for all children younger than 5 years of age. This procedure could significantly reduce pneumococcal related morbidity, especially that caused by pneumococci not susceptible to antibiotics.

References:

- 1. Brook I: Microbial factors leading to recurrent upper respiratory tract infections. Pediatr Infect Dis J. 1998: 17: S62-67
- Gunnarsson RK, Holm SE, Soderstrom M: The prevalence of potential pathogenic bacteria in nasopharyngeal samples from individuals with a respiratory tract infection and sore throat – implications for the diagnosis of pharyngotonsillitis. Family Practice, 2001; 18: 266–71
- 3. McCay JE: Resistant bacteria in the adenoids: a preliminary report. Arch Otolaryngol Head Neck Surg, 2000; 126: 625–29
- Johnson HL, Deloria-Knoll M, Levine OS et al: Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: The pneumococcal global serotype project. PLOS Medicine, 2010; 7: e10000348
- O'Brien KL, Wolfson LJ, Watt JP et al: Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet, 2009; 374: 893–902
- World Health Organization: Pneumococcal conjugate vaccine for childhood immunization – WHO position paper. Wkly Epidemiol Rec, 2001; 12: 93–104
- Simoes AS, Valente C, de Lencastre H, Sa-Leao R: Rapid identification of noncapsulated Streptococcus pneumoniae in nasopharyngeal samples allowing detection of co-colonization and reevaluation of prevalence. Diagn Microbiol Infect Dis, 2011; 71: 208–16
- 8. http://www.eucast.org/clinical_breakpoints/
- 9. Jeong JH, Lee DW, Ryu RA: Bacteriologic comparison of tonsil core in recurrent tonsillitis and tonsillar hypertrophy. Laryngoscope, 2007; 117: 2146–51
- Yagupsky P, Porat N, Fraser D et al: Acquisition, carriage, and transmission of pneumococci with decreased antibiotic susceptibility in young children attending a day care facility in southern Israel. J Infect Dis, 1998; 177: 1003–12
- Borer A, Meirson H, Peled N et al: Antibiotic-resistant pneumococci carried by young children do not appear to disseminate to adult members of a closed community. Clin Infect Dis, 2001; 33: 436–44
- Katsarolis I, Poulakou G, Anatitis A et al: Risk factors for nasopharyngeal carriage of drug-resistant Streptococcus pneumoniae: data from a nationwide surveillance study in Greece. BMC Infect Dis, 2009; 9: 120
- Raymond J, Le Thomas I, Moulin F et al: Sequential colonization by Streptococcus pneumoniae of healthy children living in a orophange. J Infect Dis, 2000; 181: 1983–88
- Syrjänen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK: Nasopharyngeal carriage of Streptococcus pneumoniae in Finnish children younger than 2 years old. J Infect Dis, 2001; 184: 451–59
- Greenberg D, Broides A, Blancovich I et al: Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of Streptococcus pneumoniae and Haemophilus influenzae from healthy and sick individuals varies with age. J Clin Microbiol, 2004; 42: 4604–9
- Brook I, Shah K, Jackson W: Microbiology of healthy and diseased adenoids. Laryngoscope, 2000, 110: 994–99
- Karlidag T, Demirdag K, Kaygusuz I et al: Resistant bacteria in the adenoid tissues of children with otitis media with effusion. Int J Pediatr Otorhinolaryngol, 2002; 64: 35–40
- Tonnaer EL, Rijkers GT, Meis JF et al: Genetic relatedness between pneumococcal populations originating from the nasopharynx, adenoid, and tympanic cavity of children with otitis media. J Clin Microbiol, 2005; 43: 3140–44

Conflicts of interest

None of the authors have any conflicts of interest pertaining to this article.

Acknowledgements

The paper was developed using the equipment purchased within the agreement No. POPW.01.03.00-06-010/09-00 Operational Program Development of Eastern Poland 2007–2013, Priority Axis I, Modern Economy, Operations 1.3, Innovations Promotion.

- Korona-Glowniak I, Niedzielski A, Malm A: Upper respiratory colonization by Streptococcus pneumoniae In healthy pre-school children in south-east Poland. Int J Pediatr Otorhinolaryngol, 2011; 75: 1529–34
- Vestrheim DF, Hoiby EA, Aaberge IS, Caugant DA: Phenotypic and genotypic characterization of Streptococcus pneumoniae strains colonizing children attending day-care centers in Norway. J Cin Microbiol, 2008; 46(8): 2508–18
- Huebner RE, Dagan R, Porath N et al: Lack of utility of serotyping multiple colonies for detection of simultaneous nasopharyngeal carriage of different pneumococcal serotypes. Pediatr Infect Dis J, 2000; 19: 1017–19
- Principi N, Marchisio P, Schito GP, Mannelli S: Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Pediatr Infect Dis J. 1999; 18: 517–23
- Sulikowska A, Grzesiowki P, Sadowy E et al: Characteristics of Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis isolated from the nasopharynges of asymptomatic children and molecular analysis of S. pneumoniae and H. influenzae strain replacement in nasopharynx. J Clin Microbiol, 2004; 42: 3942–49
- Sa-Leao R, Nunes S, Brito-Avo A et al: High rates of transmission of and colonization by Streptococcus pneumoniae and Haemophilus influenzae within a day care center revealed in a longitudinal study. J Clin Microbiol, 2008; 46: 225–34
- Jacobs MR, Felmingham D, Appelbaum PC, Grüneberg RN, Alexander Project Group: The Alexander Project 1998–2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents. J Antimicrob Chemother, 2003; 52: 229–46
- Sadowy E, Izdebski R, Skoczynska A et al: Phenotypic and molecular analysis
 of penicillin-nonsusceptible Streptococcus pneumoniae isolates in Poland.
 Antimicrob Agents Chemother, 2007; 51: 40–47
- Johnson DM, Stilwell MG, Fritssche TR, Jones RN: Emergence of multidrugresistant Streptococcus pneumoniae: report from the SENTRY antimicrobial surveillance program (1999–2003). Diagn Microbiol Infect Dis, 2006; 56: 69–74
- Aarts JW, van der Aardweg MT, Rovers MM et al: Alterations in the nasopharyngeal bacterial flora after adenoidectomy in children: a systematic review. Otolaryngol Head Neck Surg, 2010; 142: 15–20
- Cohen R, Bingen E, Varon E et al: Change in nasopharyngeal carriage of Streptococcus pneumoniae resulting from antibiotic therapy for acute otitis media in children. Pediatr Infect Dis J, 1997; 16: 555–60
- Appelbaum PC: Resistance among Streptococcus pneumoniae: implications for drug selection. Clin Infect Dis, 2002; 34: 1613–20
- 31. McIntosch ED, Fritzell B, Fletcher MA: Burden of paediatric invasive pneumococcal disease in Europe, 2005. Epidemiol Infect, 2007; 135: 644–56
- Czarkowski MP, Cielebak E, Kondej B, Staszewska E: Vaccinations in Poland in 2010. National Institute of Public Health – National Institute of Hygiene – Department of Epidemiology. Warsaw 2010; http://www.pzh.gov.pl/oldpage/epimeld/2010/Sz_2010.pdf
- Skoczyńska A, Kuch A, Gołebiewska A et a: [Invasive pneumococcal disease in Poland in 2010]. Pol Merkur Lekarski, 2011; 31: 80–85 [in Polish]
- Skoczyńska A, Sadowy E, Bojarska K et al: Participants of laboratory-based surveillance of community acquired invasive bacterial infections (BINet). The current status of invasive pneumococcal disease in Poland. Vaccine, 2011; 29: 2199–205
- Konior R, Skoczyńska A, Bojarska K et al: [Invasive pneumococcal disease in the Malopolska region of Poland, in the year 2002–2008. Is introduction of mass vaccination with conjugated pneumococcal vaccine justified?] Med Wieku Rozwoj, 2009; 13: 317–23 [in Polish]