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ORIGINAL PAPER

Serotypes and Antibiotic Resistance of *Streptococcus pneumoniae* from Adenoids in Preschool Children with Recurrent Upper Respiratory Tract Infections

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Abstract

We investigated children aged 2–5, who had gone adenoidectomy for recurrent and/or persistent symptoms of upper respiratory tract infections for prevalence of pneumococci in adenoid tissue. Serotypes and antibiotic resistance patterns of the isolated pneumococci were determined and also risk factors of pneumococcal colonization were defined. *S. pneumoniae* colonization in adenoids was found in 62 (60.2%) children. Serotypes belonged to 10-valent and 13-valent pneumococcal conjugated vaccines (PCVs) constituted 56.1% and 68.2% of the isolates, respectively. Decreased susceptibility to penicillin was found in 45.5% of isolates; pneumococci were resistant to cotrimoxazole (62.1%), tetracycline (43.9%), erythromycin (54.5%), clindamycin (54.5%) and chloramphenicol (31.8%). Multidrug resistant *S. pneumoniae* comprised 57.6% of the isolates. Antibiotic resistant pneumococci were mostly distributed among serotypes belonged to 10-valent and 13-valent PCVs. Good vaccine coverage among the isolated pneumococci confirmed that the introduction of PCVs in the national immunization programme may reduce the pool of resistant and multidrug resistant pneumococci in a community.

Key words: *Streptococcus pneumoniae*, adenoids, antibiotic resistance patterns, serotypes

Introduction

Upper respiratory tract infections (URTIs) are the most common childhood illnesses but remains clinically indistinguishable whether it is of viral or bacterial etiology. However, URTIs are crucial cause of visits in family doctor practice, and the major cause of antibiotic prescriptions, especially by pediatricians. *Streptococcus pneumoniae* is an important etiologic agent of meningitis, pneumonia, bacteriemia and acute otitis media in both children and adults and one of the major bacterial pathogens colonizing nasopharynx, mainly asymptomatically (Bogaert *et al.,* 2004). Young children are colonized the most frequently and they have been found to be main reservoir of pneumococci, paying the key role in spreading and selecting of multidrug resistant strains (De Lencastre and Tomasz, 2002). Recently, there has been an increasing rate of antibiotic resistance in the pneumococcal serotypes that are responsible for the infections of middle ear, nasal cavity and pharynx in children and are causing difficulties in the treatment (Appelbaum, 2002). In fact, a fundamental pneumococcal infections are of the respiratory tract and not invasive diseases in spite of the common belief by many pediatricians.

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. The first introduced conjugate vaccine contains seven pneumococcal capsular antigens (PCV7) covering serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Its impact on the decrease of invasive pneumococcal disease has been significant both in infants, older children and adults. Additional benefit of the vaccine was a decrease in rates of antimicrobial resistance among pneumococcal isolates resulting from association resistance to penicillin, macrolides and multidrug resistance with serotypes 6B, 9V, 14, 19F and 23F included to PCV7 (O'Brien *et al*., 2009). In 2007, World Health Organization (WHO) recommended all countries to incorporate PCV to their national infant immunization program. Only in countries with routine effective use of PCV7, the proportion of current coverage of serotypes belonged to PCV7 is lower than in the pre-vaccine era (Isaacman *et al*., 2010). PCV-7 was introduced in Poland in 2005 and recommended for

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children under 2 years old but not refunded by health authorities. Since 2009, the PCV was recommended for children under 5 years old and refunded for some risk groups. According to our previous study, the adenoids like the nasopharynx can be regarded as a reservoir of *S. pneumoniae*, including resistant and/or multiresistant strains. As a continuation of that study we investigated pneumococci isolated from adenoid tissue of children aged 2–5, who had gone adenoidectomy for recurrent and/or persistent symptoms of upper respiratory tract infections. Serotypes and antibiotic resistance patterns of the isolated pneumococci were determined and also risk factors of adenoid colonization by pneumococci were defined.

Experimental

Material and Methods

Patients. The study enrolled 103 children, aged between 2 and 5, undergoing adenoidectomy in Department of Pediatric Otolaryngology, Phoniatrics and Audiology, Medical University of Lublin during May-June and November-December 2011. The indication for adenoidectomy was recurrent acute pharyngotonsilitis for at least 2 years with 5 or more acute attacks per year. Patients didn't received any antibiotic therapy for at least 20 days before the operation. From all children's parents, the informed content were obtained. The Ethical Committee of the Medical University of Lublin approved the study protocol (No. KE-0254/75/211).

Demographic data of studied children was shown in Table I. None of the children were immunized by a pneumococcal vaccine.

Laboratory procedures. After the surgery, the adenoid were placed in the sterile container and were transported to laboratory then the adenoid was swabbed with sterile alginate-tipped applicator. Swabs were inoculated on selective 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% CO_2 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 a slide agglutination test Slidex Pneumo-Kit (BioMerieux).

All isolates were serotyped by means of Quellung reaction using antisera provided by Statens Serum Institute (Copenhagen, Denmark). We applied antisera for determination of serotypes belonging to the 23-valent pneumococcal polysaccharide vaccine (PPV23), *i.e.* – 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 Others, serotypes not present in PPV23. The isolates negative to slide agglutination test and negative to omni serum were defined as untypeable (rough – R); their belonging to pneumococci was confirmed by PCR analysis using primers for detecting the *lytA* gene encoding the autolysin enzyme specific to *S. pneumoniae* (Simoes *et al.*, 2011).

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

Risk factor		Total number of children (%)	SP colonized children (% in group)
Age (yr)	2	11 (10.7)	6 (54.5)
	3	24 (23.3)	11 (45.8)
	4	51 (49.5)	33 (64.7)
	5	17 (16.5)	12 (70.6)
Sex	female	43 (41.7)	26 (60.5)
	male	60 (58.3)	36 (60.0)
Sibling possessing	no	17 (16.5)	11 (64.7)
	1	46 (44.6)	28 (60.9)
	≥2	40 (38.8)	23 (57.5)
Passive smoking		32 (31.1)	19 (59.4)
DCC attendance		83 (80.6)	49 (59.0)
Place of residence	village	41 (39.8)	23 (56.1)
	city	62 (60.2)	39 (62.9)
Antibiotics taken for the last attack	amoxicillin/co-amoxiclav	55 (54.5)	34 (61.8)
	cephalosporins	24 (23.8)	16 (66.7)
	macrolides	22 (21.8)	10 (45.5)

DCC, day care center; SP, *Streptococcus pneumoniae*

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 European Committee on Antimicrobial Susceptibility Testing recommendations (EUCAST, 2011). 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 to benzylpenicillin; isolates with MIC > 0.064 mg/L were called penicillin non-susceptible *S. pneumoniae* (PNSSP). Multidrug-resistant 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 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 tested in separate univariate analyses (Chi-squared or the Fisher exact test, as appropriate) for their association with upper respiratory 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, such as children age, having siblings, passive smoking and type of antibiotic used, were included even when were not statistically significant. Statistical significance was set at $p < 0.05$.

Results

S. pneumoniae colonization was observed in 62 (60.2%) children who had undergone adenoidectomy for recurrent and/or persistent symptoms of upper respiratory tract infections. A total of 66 isolates were recovered; 4 (3.9%) children were colonized by two different in colony morphology isolates, which were identified by serotyping and antimicrobial resistance tests as different pneumococcal strains. Among the isolates, serotypes belonged to PPV23 (89.4%) were identified, and 4 isolates (6.1%) were untypeable (rough – R) (Table II).

The most frequent was serotype 19F (24.2%). Serotypes belonged to pneumococcal conjugated vaccines

Table II
Serotypes and antibiotic resistance patterns of *Streptococcus pneumoniae* isolated from children undergoing adenoidectomy for recurrent URTIs.

Serotypes	No. (%) of isolates	Antibiotic resistance pattern (no. of isolates)
3	5 (7.6)	S (5)
6A	2 (3.0)	ECcTeSxt (1), S (1)
6B	9 (13.6)	PECcCTeSxt (2), PECcCSxt (4), PECcTeSxt (1), S (2)
9V	2 (3.0)	PSxt (2)
10A	1 (1.5)	CTe (1)
11A	3 (4.5)	S (3)
14	2 (3.0)	PECcTeSxt (1), PECcSxt (1)
15B	2 (3.0)	Sxt (1), S (1)
15 (nonB)	4 (6.1)	Sxt (2), S (2)
18C	1 (1.5)	S (1)
19A	1 (1.5)	PECcTeSxt (1)
19F	16 (24.2)	PECcCTeSxt (4), PECcTeSxt (4), ECcCTeSxt (1), ECcTeSxt (2), ECcTe (1), CTeSxt (1), CSxt (1), S (2)
23A	1 (1.5)	ECcCTe (1)
23B	1 (1.5)	S (1)
23F	7 (10.6)	PECcCTeSxt (1), PECcCSxt (4), PECcTeSxt (1), Sxt (1)
33F	2 (3.0)	ECcTe (2)
Others	3 (4.5)	PECcTeSxt (1), S (2)
R	4 (6.1)	PECcTeSxt (3), PCSxt (1)

P, penicillin; E, erythromycin; Cc, clindamycin; Te, tetracycline; C, chloramphenicol; Sxt, co-trimoxazol; S, sensitive to all tested antibiotics; R, rough, untypeable strain; Others, serotypes not present in PPV23.

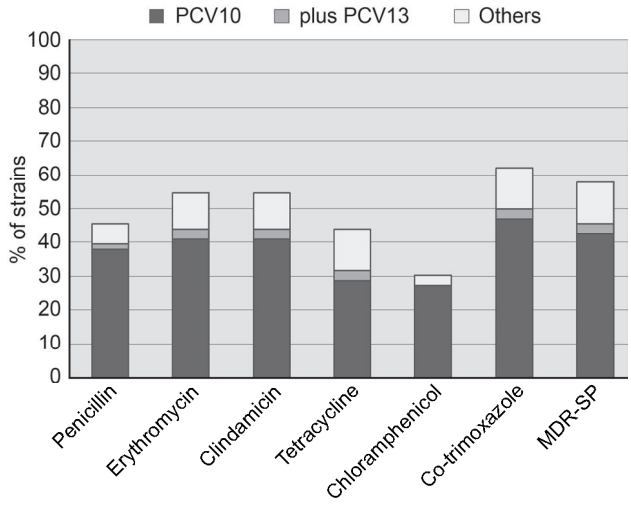

Fig. 1. Resistance rate to antibiotics of *Streptococcus pneumoniae* isolated from children undergoing adenoidectomy for recurrent URTIs.

Dark blocks, samples containing PCV10 type pneumococci; grey blocks, samples containing serotypes in PCV13 additional to PCV10 types; white blocks, samples containing non-vaccine type pneumococci.

– 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) constituted 56.1% and 68.2% of the isolates, respectively.

The pneumococcal isolates were susceptible to all tested antimicrobial agents in 30.3%. These strains belonged to serotypes 3 (5 isolates), 11A (3 isolates), 6B, 19F, 15 non-B, NT (2 isolates per each serotype), and 6A, 10A, 15B, 18C (1 isolate per each serotype). Among all of the strains, 45.5% had decreased susceptibility to penicillin (MIC range 0.12–2.0 mg/L, MIC_{50} 0.5 mg/L and MIC_{90} 2.0 mg/L). *S. pneumoniae* isolates were resistant to co-trimoxazole (62.1%), tetracycline (43.9%), erythromycin (54.5%), clindamycin (54.5%) and chloramphenicol (31.8%) (Fig. 1). All isolates were susceptible to norfloxacin and according to EUCAST 2011, they should be reported as susceptible to levofloxacin and moxifloxacin and intermediate to ciprofloxacin and ofloxacin. None of the tested isolates was resistant to rifampicin, linezolid and teicoplanin. Each strain was characterized phenotypically by serotype and antibiotic resistance pattern (Table II). Multidrug resistance was present in 57.6% of isolates. Among MDR-SP 76.3% were non-susceptible to penicillin. Antibiotic resistant pneumococci were mostly distributed among serotypes belonged to PCV10 and PCV13 (Fig. 1). PNSSP and MDR-SP strains represented PCV10 serotypes in 83.3% and 73.6%, respectively and PCV13 serotypes in 86.7% and 78.9%, respectively. Colonization with PNSSP and MDR-SP strains was found in 30 (29.1%) and 35 (34.0%) children, respectively.

During the analysis of risk factors predisposing to pneumococcal colonization, including PNSSP and MDR strains, no predictors were found in the total population of children. However, when analysis in group of 32 children exposed to passive smoking was done, two predictors turned out to be significant for pneumococcal colonization: female gender ($p = 0.049$, OR 1.59, 98%CI 1.1–2.4) and type of antibiotics taken for the last attack ($p = 0.019$). Moreover, in these children consumption of β-lactams increased the risk of pneumococcal colonization in comparison to macrolide consumption ($p = 0.015$, RR 5.1, 95%CI 1.2–20.8). Type of taken antibiotics was a predictor of MDR-SP colonization in children exposed to passive smoking ($p = 0.02$). Macrolide consumption in these children decreased rate of MDR-SP colonization in comparison to children with consumption of β-lactams ($p = 0.012$, RR 0.55, 95%CI 0.4–0.8).

Discussion

The adenoids are involved in the pathology of adenotonsilitis, rhinosinusitis, otitis media and chronic nasal obstruction (Brook and Shah, 2001; Karlidag *et al.*, 2002). High frequency of adenoid colonization by *S. pneumoniae* in preschool children with recurrent upper respiratory infections was found in our study, which is in agreement with the previous observations (Niedzielski *et al.*, 2013). However, high rate of asymptomatic pneumococcal carriage in preschool children, especially attending day care center is common so it is difficult to indicate that *S. pneumoniae* is an important bacterial etiological agent of URTIs even though it is isolated from children with URTI symptoms (Gunnarsson *et al.*, 2001). Jeong *et al.* (2007) analyzed the differences between the bacterial pathogens of tonsil core in recurrent tonsillitis and tonsillar hypertrophy with regard the age; *S. pneumoniae* was detected with high frequency in recurrent tonsillitis in the patients between 8–14 years, even though this pathogen was more common in younger patients (< 8 years) in both groups. Syrjanen *et al.* (2001) reported that nasopharyngeal carriage of pneumococci during RTIs (without otitis media) in children increased from 13–43% to 45–56%, depending on age. Contrary Greenberg *et al.* (2004) found no differences in the overall *S. pneumoniae* carriage between healthy and sick children in different age groups.

Resistant pneumococci are common among young children, especially attending day care centers. Transmission of these resistant strains in the community is constantly investigated (De Lencastre and Tomasz, 2002; Borer *et al.*, 2001). Resistance to antimicrobial agents among pneumococcal isolates in this study was much higher than data concerning pneumococcal isolates described in Poland (Jacobs *et al.*, 2003; Riedel

et al., 2007). Children with recurrent pharyngotonsilitis are usually treated with multiple courses of antibiotics before surgery but many of them continue to carriage of pathogenic bacteria in the pharynx and the adenoids, including strains resistant to antibiotic (McCay, 2000) and all of children from our studies were treated by antibiotics before surgery. Poland belong to a part of Europe with high level of antibiotic consumption and above 20% rate of penicillin and macrolide resistance among the pneumococcal isolates (Riedel *et al*., 2007, van de Sande-Bruinsma *et al*., 2008).

The passive smoking increases colonization rate by pathogens in children and their risk for respiratory tract infections (Gryczynska *et al*., 1999; Kosikowska *et al*., 2011). Principi's *et al*. (1999) observation with healthy children indicated that exposure to tobacco smoke did not influence the *S. pneumoniae* carriage in the upper respiratory tract in children aged <5 years. Bakhshaee *et al*. (2012) found a significant difference in carriage rates between children who lives in smoking families compare to those with nonsmoking families for *M. catarrhalis*, but not for *S. pneumoniae* and *H. influenzae*. However, Greenberg *et al*. (2006) showed that exposure to tobacco smoke increased *S. pneumoniae* carriage rates particular in children. Data obtained in the present study indicated no influence of passive smoking on pneumococcal colonization in children with recurrent URTIs. However, contrary to our previous data (Niedzielski *et al*., 2013), in present paper no risk factor for pneumococcal colonization in total population of children undergoing adenoidectomy for recurrent URTIs was found, but in group of children exposed to passive smoking some predictors of the adenoid colonization by pneumococci were revealed – female gender as a predictor of pneumococcal colonization and type of antibiotics taken for last attack as a predictor of pneumococcal colonization and MDR-SP colonization. These observations suggest that the effect of passive smoking on pneumococcal prevalence in the upper respiratory tract, including adenoids, may be correlated with other predisposing factors, *e.g.* age, gender, season, staying in a close population (*e.g.* day care center), chronic respiratory infections, previous antibiotic treatment, allergy or geographic region.

Our data revealed that consumption of β-lactams increased the rate of pneumococcal colonization in adenoids but only in children exposed to tobacco smoke. On the other side the decrease of MDR-SP colonization was observed in this group of children who received macrolides. Some studies demonstrated an association between the use of a specific antibiotic and selective colonization with strains resistant to this drug as well as that number of courses of drugs to which pathogens are resistant has the utmost importance (Appelbaum, 2002). Borer *et al*. (2001) described statistically significant impact of antimicrobial drug use on nasopharyngeal carriage of *S. pneumoniae*, whereas Principi *et al*. (1999) showed macrolide therapy increased nasopharyngeal pneumococcal carriage. Findings from southern Israel strongly suggest that azithromycin affects increased multidrug resistance in *S. pneumoniae* (Barkai *et al*., 2005).

Widespread use of PCVs resulted in decreased in invasive pneumococcal disease and pneumonia among children and elderly persons in countries that have introduced conjugate vaccines (Center, 2008; Lopalco, 2009). In Poland PCV is already included to routine immunization program: vaccination is recommended universally for children ≤2 years old and since 2009 is mandatory and refunded for some risk groups. However, low vaccination rate was observed, especially in south-east region of Poland. Our data showed that the *S. pneumoniae* serotype coverage of isolates colonizing adenoids in pre-school children with recurrent/persistent URTIs from Poland by the currently available PCVs is high (56.1–68.2%) and similar to that reported in other European countries (McIntosch *et al*., 2007). According to studies performed by Skoczynska *et al*. (2011), in children aged less than 5 years in Poland, serotypes 14, 6B, and 19F were most prevalent, comprising 52.7% of the invasive pneumococcal disease (IPD) cases. The PCV10, and PCV13 covered 54.8%, and 68.8% of all IPD cases, and 76.3%, and 86.3% of cases involving children under 5 years of age. An encouraging finding of our present data was also that a majority of PNSSP and MDR-SP belonged to serotypes included in PCV13 and PCV10. The above data suggest that routine vaccination of infants with PCVs could effectively reduce the reservoir of pneumococci within upper respiratory tract, including resistant and/or multidrug resistant strains, in children in Poland, similarly to that in other European countries (O'Brien *et al*., 2009).

High carriage rate of *S. pneumoniae* in adenoids, including multidrug resistant strains, was observed in our study in children with an indication for adenoidectomy due to recurrent upper respiratory tract infections refractory to antibiotic therapy. Good vaccine coverage among the isolated pneumococci allows the suggestion that the introduction of PCVs in the national immunization program in young children may reduce the high carriage rate of pneumococci, colonizing not only nasopharynx but also adenoids.

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