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Resistance Patterns of *Streptococcus pneumoniae* Strains Isolated in the West Pomerania Province in 2001–2003

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Abstract

An abrupt antimicrobial resistance increase among *Streptococcus pneumoniae* strains has become a serious therapeutic problem in the recent years. The aim of this study was to describe the resistance increase of *S. pneumoniae* strains isolated in the West Pomerania Province over three years (2001–2003). Using E-tests method and NCCLS criteria for 80 pneumococal resistant strains the resistance degrees and patterns have been determined and analyzed in connection with their clinical origin. The majority of specimens of resistant strains isolated came from nasopharynx (80% strains) of infected ambulatory patients (81.3%), from children at nursery school age (65.7%), suffering from chronic upper respiratory tracts infection (86.7%). However, strains originated from older patients, hospitalized, in serious health condition showed higher resistance degrees. The greatest number of isolates (27.5%) showed resistance to 3 out of 9 tested drugs and over a half (53.8%) of the tested strains belonged to MDR strains, with increasing percentage over time: from 62.5% in 2001 to 69.8% in 2003. Resistance to 8 out of 9 determined antibiotics (except vancomycin) has occurred and domination of 4 resistance patterns: ELTS, S, TSH, PSI, present in 50.1% of the tested strains was observed. The phenomena observed in the study: growing resistance degree, increasing amount of MDR strains, emergence of new resistance patterns, testify to gradual pneumococcal resistance increase and give a picture of local trends in antibiotic therapy. Also the epidemiological data concerning patients, from whom the tested strains were isolated are adequate to risk factors of infection with resistant pneumococci.

Key words: *Streptococcus pneumoniae,* Poland, multidrug resistance, risk factors

Introduction

S. pneumoniae causes a lot of serious diseases such as lobar pneumonia, meningoecephalitis and septicaemia belonging to main reasons of disease incidence and mortality regardless of age and part of the world (Feldman and Klugman, 1997; Appelbaum, 1992; Caputo *et al.*, 1993). It is also the most frequent etiological factor of upper respiratory tracts infections, especially sinusitis and otitis media (Appelbaum, 1992), which being not so life-threatening but are the most often found in ambulatory diagnoses. Antibiotic therapy is not always supported with microbiological diagnostics and antimicrobial susceptibility determination, what more and more often leads to empirical therapy failures, especially in recent years. The source of these failures lies in abrupt antibiotic resistance increase among *S. pneumoniae* strains (Kaplan and Mason, 1998; Feikin *et al.*, 2000; Lister, 1995; Dagan *et al.*, 1996).

The first penicillin resistant strain was described in Australia in 1967 (Hansman and Bullen, 1967). Since that time in many countries there have been reported pneumococci resistant to penicillin and later also to nearly all known groups of antibiotics (other than penicillin betalactams, macrolides, lincosamids, tetracyclines, cotrimoxasoles, chloramphenicol, fluorochinolons, aminoglycosides) and even strains showing tolerance to vancomycin in animals (Feldman and Klugman, 1997; Appelbaum, 1992; Dowson *et al.*, 1994; Pallares *et al.*, 1987; Carbon and Poole, 1999; Baguero, 1997; Appelbaum *et al.*, 1996; Appelbaum, 1996; Baquero, 1997; Novak *et al.*, 1999). The fact of co-occurrence of resistance to many antibiotics is especially distressing. Pneumococci resistant to 3 or more drug-groups are defined as multidrug resistant (MDR).

In the recent years we could observe not only appearance and increase of pneumococcal resistance, but also abrupt spreading of multidrug resistant strains in many countries, including Poland (Trzciński and Hryniewicz, 1997). National range of many isolated resistant clones has been described. A few of them have gained the name of pandemic strains: Spanish^{23F}-1, Spanish^{6B}- and Spanish^{14}-5. The presence of the Spanish^{23F}-1 clone and other its capsular variants has been documentary proved in 24 countries from all the inhabited continents excluding Australia (Appelbaum, 1992; Dowson and Trzciński, 2001; Hermans *et al.*, 1997; Reichmann *et al.*, 1995; Caputo *et al.*, 1993). In Poland in the 90s two of them (Spain 23F-1 and France9V-3) were detected and also two other epidemic national clones of Poland^{23F}-16 and Poland^{6B}-20 serotypes were described (Overweg *et al.*, 1999).

Microbial resistance is variable and unstable. It depends mainly on antibiotic policy in particular region. In certain countries multiresistance is very high *e.g.* in Korea it reaches 80% (Kim *et al.*, 1996), in Spain and Hungary 40%, in France 20% (Grzesiowski *et al.*, 1999). Also geographic variability of penicillin "nonsusceptibility" ranges from over 40% in 16 out of 60 countries analyzed by Dowson to below 5% in only three of them (Dowson and Trzciński, 2001). Hence, there is a justified need for continuous and multicenter monitoring of local antibiotic resistance in order to build a more effective strategy of pneumococcal therapy.

The aim of this study is to describe the changeability of the degree and patterns of resistance of *S. pneumoniae* strains isolated in the West Pomerania Province during three years (2001–2003) in connection with their clinical origin.

Experimental

Materials and Methods

Bacterial strains. In the research there has been applied a collection of 132 strains of *S. pneumoniae* showing lowered antibiotic sensitivity in routine testing, isolated from various specimens from infected patients in the Department of Microbiology and Immunology at the Pomeranian Medical Academy and in 5 microbiological laboratories in the West Pomerania Province from 2001 to 2003. Also data concerning patients' sex and age, disease symptoms and kinds of specimens from which particular bacterial strains came were collected. The strains were preserved in Tryptic Soy Broth with addition of 15% glycerol at the temperature of – 70°C.

Antimicrobial susceptibility testing. Initial determination of antibiotic sensitivity was carried out with disc-diffusion method according to NCCLS (NCCLS, 2000). The incubation was performed on Miller-Hinton agar with addition of 5% of sheep blood at the temperature of 35°C in air supplemented with 5% CO_2. With disc-diffusion method using discs with oxacillin (1 μg), erythromycin (15 μg), clindamycin (2 μg), tetracycline (30 μg), cotrimoxazole (1.25/23.75 μg) (Beckton Dikinson) resistant strains and in case of oxacillin "nonsusceptible" strains (*i.e.* with growth inhibition zone below 20 mm) were determined. The macrolide resistance phenotype was defined with application of double-disc method with erythromycin and clindamycin discs. Then strains resistance was verified and subjected to extended antimicrobial susceptibility analysis with determination of the minimum inhibitory concentration (MIC) using E-tests for the following antimicrobial agents: benzylpenicillin (P), erythromycin (E), clindamycin (L), tetracycline (T), cotrimoxazole (S), ceftriaxone (C), chloramphenicol (H), vankomycin (W), imipenem (I) according to the producer's directions (AB Biodisk, Solna, Sweden Jacobs *et al.*, 1992). The obtained MICs were interpreted according the NCCLS criteria as resistant: for P≥2 μg/ml, E≥2 μg/ml, L≥1 μg/ml, T≥8 μg/ml, S≥4 μg/ml, C≥2 μg/ml, H≥8 μg/ml, I≥1 μg/ml, and as intermediate: for P 0.12–1 μg/ml, E 1 μg/ml, L 0,5 μg/ml, T 4 μg/ml, S 1–2 μg/ml, C 1 μg/ml, I 0.25–0.5 μg/ml (NCCLS, 2000). Subsequently, for every strain the resistance pattern was determined by qualifying intermediate and high resistance to particular antibiotics. The degree of their resistance (from 1- to 7-drug resistant strains) was determined and strains resistant to at least one of the antibiotics were subjected to further analysis. Percentage share of particular resistance patterns and degrees in years covered by study (2001–2003) were analyzed with relation to strain epidemiological data.

Results

From previously collected 132 strains, after initial selection with disc-diffusion method and subsequent verification with E-tests in accordance with the above mentioned criteria, 80 strains resistant to at least one antibiotic were qualified for further analysis.

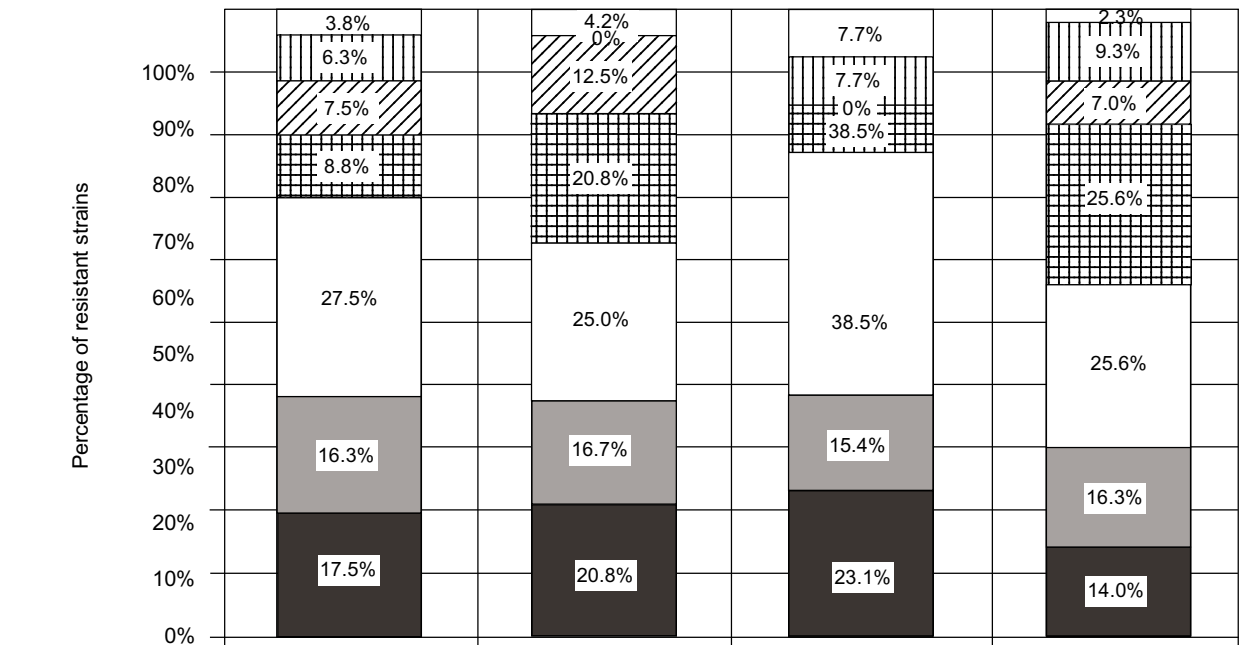
These strains came from patients of different age: from 3 months to 68 years (average – 10.3 years, median: 5 years); in 54.7% from men. The patients suffered mainly form upper respiratory tract infections (59 out of 68 *i.e.* 86.7%): chronic pyogenic rhinitis (42), sinusitis (8), otitis media (6) or bronchitis (4). Single isolates came from people with conjunctivitis (2), vaginitis (1), fever of unknown origin (1). Other 5 strains were isolated from patients with serious infections: 3 from people with pneumonia and 2 from patients staying in intensive care units. These 5 strains were characterized by high degree of multidrug resistance (7-, 6-, 6-, 5-, 4- drug resistant). The majority of strains came from nosopharynx: from nose – in

44 patients being tested (55%), from pharynx – in 8 (10%); both from nose and pharynx of the same patient in 12 cases (15%). The other materials from which mainly high resistance degree strains were isolated are the following: BAL (broncho – alveolar lavage) – two strains: 6-drug resistant and 7-drug resistant, sputum (5-drug resistant strain), swab from external acoustic duct (4-drug resistant strain), 2 swabs from conjunctival sac (4-drug resistant strains) and one swab from a five-year girl's vagina (6-drug resistant strain). Two strains taken from sinus punctuates were resistant to three of tested antibiotics: highly cotrimoxazole resistant and intermediately penicillin and imipenem resistant.

It is worth mentioning that strains of high resistance degree were isolated mainly in hospital wards: 2 out of 3 (66.7%) 7-drug resistant strains, 3 out of 5 (60%) 6-drug resistant strains and half (3 out of 6) 5-drug resistant strains. Whereas 13 out of 14 strains (92.9%) resistant to one antibiotic and all of two-drug resistant strains were obtained from ambulatory patients. Detailed description of epidemiological data together with the degree and patterns of resistance are presented in Table I.

Among tested strains over a half (53.8%) constituted multidrug resistant strains MDR (43 out of 80), with growing tendency of their percentage in time: 62.5% in 2001, 61.5% in 2002, and 69.8% in 2003. The greatest number of isolates (22 out of 80 *i.e.* 27,5%) showed resistance to 3 out of 9 tested antibiotics, the smallest number showed resistance to 7 drugs (3 out of 80 *i.e.* 3,8%). The other percentage shares constituted 17.5% of strains 1-drug resistant, 16.3% of 2-drug resistant, 8.8% of 4-drug resistant, 7.5% of 5-drug resistant and 6.3% of 6-drug resistant (Fig. 1).

The resistance degree analysis of isolates in given years and especially comparison of the most numerous occurrences in 2001 and 2003 points to the fact that the percentage of low degree resistance strains decreased: 1-drug resistant strains from 20.8% in 2001 to 14% in 2003 and 2-drug resistant strains from 16.7% to 16.3%. Instead, the percentage of multidrug resistant strains increased. From five 6-drug resistant strains four were isolated in 2003 and one in 2002; in reference to all isolates in a particular year it amounted



	TOTAL	2001	2002	2003
7-drug resistant	3.8%	4.2%	7.7%	2.3%
6-drug resistant	6.3%	0.0%	7.7%	9,3%
5-drug resistant	7.5%	12.5%	0.0%	7.0%
4-drug resistant	8.8%	20.8%	7.7%	25.6%
3-drug resistant	27.5%	25.0%	38.5%	25.6%
1-drug resistant	16.3%	16.7%	15.4%	16.3%
1-drug resistant	17.5%	20.8%	23.1%	14.9%

Fig. 1. Percentage share of different resistance degrees of *Streptococcus pneumoniae* resistant strains isolated in 2001–2003 period and in each year

Table I
Epidemiological characterisic and resistance patterns of *Streptococcus pneumoniae* resistant strains isolated in West Pomerania Province in 2001–2003

Strain no	Origin / hospital ward	The year of strain isolation	Patient sex	Patient age	Specimen	Patient clinical diagnosis	Resistance pattern	Resistance degree
	(a)*		M/K	years	(b)*	(c)*	(d)*	(e)*
80	ICU	2003	K	8	BAL	P	PELTSCiIi	7
128	NEPH	2002	M	X	C	C	PiELTSHIi	7
93	AMB	2001	K	6	P	U	PiELTSiHIi	7
29	ICU	2003	K	52	BAL	Rean	PiELSHIi	6
32	ICU	2003	K	52	N	Rean	PiELSHIi	6
56	AMB	2003	K	27	P	RU	PiELSHIi	6
125	AMB	2002	K	60	N	R	PTSCiHIi	6
129	GIN	2003	K	5	V	V	PiELTSiIi	6
27	HEM	2003	M	68	S	P	PiELSH	5
81	AMB	2003	M	8	N	R	PiELTS	5
24	AMB	2003	K	6	NP	OR	PESCiIi	5
98	PED	2001	M	2	P	RB	PiTSHIi	5
106	GIN	2001	X	X	X	X	PTSHIi	5
69	AMB	2001	K	10	N	R	PTSiHIi	5
15	AMB	2003	X	X	X	X	ELTS	4
23	LAR	2003	M	4	N	S	ELTS	4
41	AMB	2003	M	5	N	RS	ELTS	4
72	AMB	2003	M	4	N	RS	ELTS	4
78	AMB	2001	M	4	E	O	ELTS	4
1	AMB	2003	M	59	P	S	ELTSi	4
10	PED	2003	K	1	N	F	ELTSi	4
11	AMB	2003	M	8	N	B	ELTSi	4
30	AMB	2003	M	9	N	X	ELTSi	4
44	AMB	2003	M	15	P	U	ELTSi	4
49	AMB	2003	K	1	N	R	PiELT	4
66	GIN	2001	K	X	N	X	ELTSi	4
82	AMB	2003	M	1	N	RU	PiELT	4
95	AMB	2001	K	39	N	R	ELTSi	4
110	AMB	2001	K	10	N	RO	PiELT	4
111	PED	2001	M	X	N	P	ELTSi	4
120	AMB	2002	M	3	N	R	ELTSi	4
46	AMB	2003	M	0	NP	OR	ELT	3
68	AMB	2001	K	5	N	X	TSH	3
83	AMB	2001	X	X	X	X	TSH	3
87	AMB	2001	K	15	N	R	TSH	3
99	AMB	2001	M	5	N	R	TSH	3
114	AMB	2002	K	4	N	RB	TSH	3
124	AMB	2002	M	12	C	C	ELT	3
127	ICU	2002	K	4	N	U	ELT	3
86	AMB	2003	M	6	P	U	TSH	3

(a)* AMB – ambulatory strain, ICU – intensive care unit NEPH – nephrology department, PED – pediatric department, HEM – hematology department, GIN – gynecology department, LAR – laryngology department SRG – surgery department;

(b)* BAL-broncho-alveolar lavage C-conjunctival sac swab N-nasal swab S- sputum P- pharyngeal swab E- external acoustic duct swab NP- nasopharyngeal swab V- vaginal swab M-sinus punctuate X- unknown;

Table I continued

Strain no	Origin / hospital ward	The year of strain isolation	Patient sex	Patient age	Specimen	Patient clinical diagnosis	Resistance pattern	Resistance degree
	(a)*		M/K	years	(b)*	(c)*	(d)*	(e)*
5	AMB	2003	K	2	N	U	PiSH	3
13	AMB	2003	X	X	X	X	TSiH	3
14	AMB	2003	K	1	N	R	TSiH	3
112	AMB	2001	K	26	P	X	TSiH	3
115	AMB	2002	K	4	N	R	ELSi	3
123	AMB	2002	M	4	N	R	ELSi	3
18	AMB	2003	M	5	M	S	PiSIi	3
19	AMB	2003	M	2	N	R	PiSIi	3
52	AMB	2003	M	7	N	R	PiSIi	3
55	AMB	2003	K	7	N	R	PiSIi	3
61	PED	2001	M	5	M	S	PiSIi	3
79	AMB	2003	M	7	N	R	PiSIi	3
3	AMB	2003	M	5	N	R	PiSiIi	3
28	AMB	2003	M	3	NP	SUi	SH	2
48	AMB	2003	M	9	NP	U	SH	2
59	AMB	2001	M	2	N	R	TS	2
67	AMB	2003	M	2	NP	RU	TS	2
91	AMB	2001	X	X	X	X	TH	2
101	AMB	2001	M	7	N	R	SH	2
107	AMB	2003	K	2	N	R	TS	2
118	AMB	2002	M	6	P	U	TH	2
119	AMB	2002	K	4	NP	RU	PiS	2
134	AMB	2003	M	3	N	R	TS	2
105	AMB	2003	K	7	NP	R	PiS	2
109	AMB	2001	X	X	X	X	PiS	2
133	AMB	2003	M	6	N	R	PiSi	2
2	AMB	2003	K	2	N	R	E	1
26	AMB	2003	K	5	NP	RSO	S	1
51	AMB	2003	K	9	N	R	T	1
54	AMB	2003	K	4	NP	R	S	1
62	SRG	2001	M	5	N	U	S	1
71	AMB	2001	M	5	N	X	S	1
73	AMB	2001	K	6	N	RB	T	1
74	AMB	2001	K	6	N	RU	T	1
96	AMB	2001	X	X	X	X	S	1
121	AMB	2002	M	6	NP	R	S	1
122	AMB	2002	M	4	NP	R	S	1
126	AMB	2002	M	2	N	RU	S	1
34	AMB	2003	K	3	NP	OR	Si	1
57	AMB	2003	M	4	N	U	Si	1

(c)* Rean- state after resuscitation P- pneumonia C- conjunctivitis O- otitis R-rhinitis S- sinusitis B- bronchitis U- upper respiratory tract infection V- vaginitis G- fever of unknown origin X- unknown;

(d)* P- penicyllin E-erythromycin L-clindamycin T-tetracycline S-cotrimoxazole C-ceftriaxon H-chloramphenicol I-imipenem i-intermediate strain;

(e)* 7- 7-drug resistant strain etc.

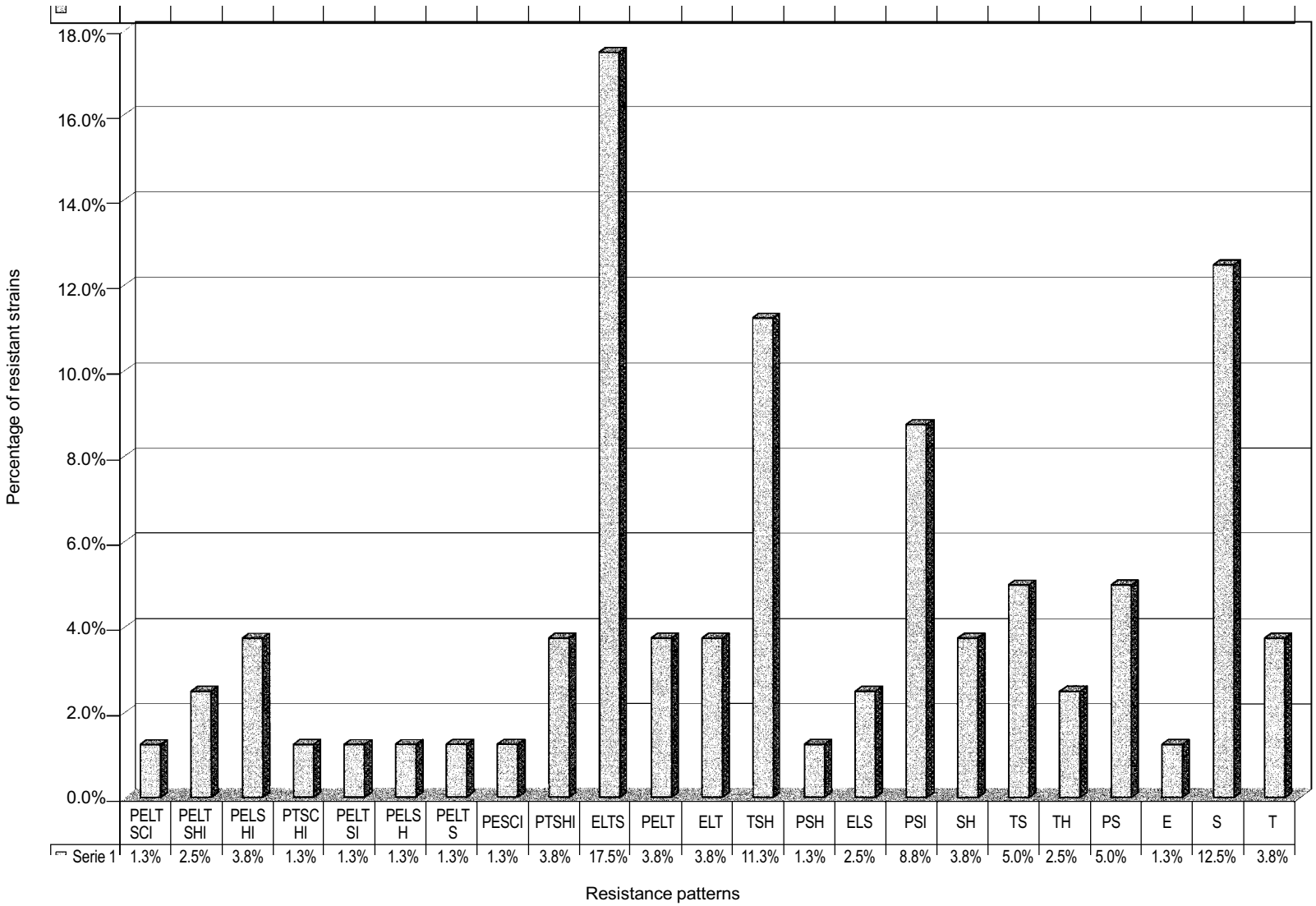
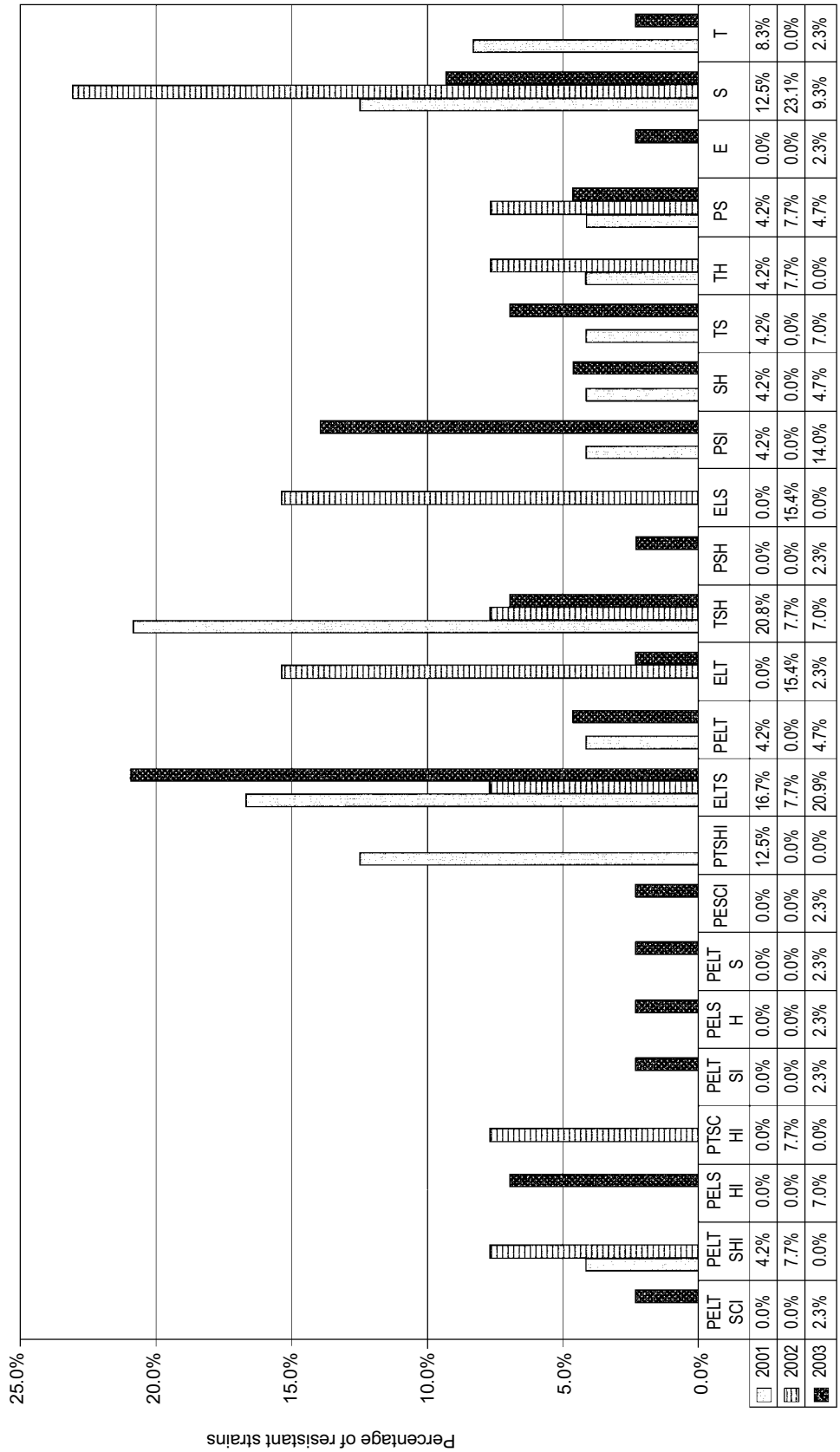


Fig. 2. Resistance patterns of resistant *Streptococcus pneumoniae* strains isolated in West Pomerania Province in 2001–2003



	PELT SCI	PELT SHI	PELS HI	PTSC HI	PELT SI	PELS H	PELT S	PESCI	PTSHI	ELTS	PELT	ELT	TSH	PSH	ELS	PSI	SH	TS	TH	PS	E	S	T
2001	0.0%	4.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	12.5%	16.7%	4.2%	0.0%	20.8%	0.0%	0.0%	4.2%	4.2%	4.2%	4.2%	4.2%	0.0%	12.5%	8.3%
2002	0.0%	7.7%	0.0%	7.7%	0.0%	0.0%	0.0%	0.0%	0.0%	7.7%	0.0%	15.4%	7.7%	0.0%	15.4%	0.0%	0.0%	0,0%	7.7%	7.7%	0.0%	23.1%	0.0%
2003	2.3%	0.0%	7.0%	0.0%	2.3%	2.3%	2.3%	2.3%	0.0%	20.9%	4.7%	2.3%	7.0%	2.3%	0.0%	14.0%	4.7%	7.0%	0.0%	4.7%	2.3%	9.3%	2.3%

Fig. 3. Resistance patterns analysis in individual years.

9.3% and 7.7% respectively. A similar growing tendency can be observed in case of percentage of 4-drug resistant strains: from 20.8% in 2001 to 25.6% in 2003, and 3-drug resistant strains: 25% in 2001, 38.5% in 2002 to 25.6% in 2003. One 7-drug resistant strain was found every year.

All of the 80 tested resistant strains showed 23 various resistance patterns (Fig. 2). It is worth mentioning that resistance to 8 out of 9 tested antibiotics was noticed among tested strains: penicillin (P), erythromycin (E), clindamycin (L), tetracycline (T), cotrimoxazole (S), chloramphenicol (H), ceftriaxone (C) and imipenem (I). No vancomycin (W) resistant strains were observed. Resistance to ceftriaxone (Ci) and imipenem (Ii) was intermediate.

Four resistance patterns: ELTS, S, TSH, PSI, clearly dominated and were present in half (50.1%) of the tested strains. The ELTS resistance pattern was most frequent, present in 14 resistant strains out of 80 being tested (17.5%), the other patterns occurred in the following percentages: S – 12.5%, TSH – 11.3% a PSI – 8.8%. More rarely occurring TS resistance pattern was traced in 4 strains; PELSHI, PTSHI, PELT, ELT, SH, T resistance patterns in 3; PELTSHI, ELS and TH in 2 strains. The other 8 resistance patterns: PELTSCI, PTSCHI, PELTSI, PELSH, PELTS, PESCI, PSH, E were represented by single isolates. To this group belong mainly multidrug, 5-and-more-drug resistant strains out of which nearly all, with exception to the PTSCHI resistance pattern strain, appeared only in 2003. The other two strains of this group with rarely occurring M-phenotype of macrolide resistance – PESCI and E, were also determined for the first time in strains isolated only in 2003. The percentage of particular resistance patterns in years 2001–2003 is presented in Table I and Fig. 3.

Discussion

The study presented here aimed at analysis of resistance patterns and resistance degree of resistant strains collected in West Pomerania Province of Poland in years 2001–2003 with special attention paid to their clinical origin. The study covered only resistant strains, so the results cannot form a basis neither for conclusions concerning antimicrobial resistance of *S. pneumoniae* population in our region nor for comparison with epidemiological data from other regions. These results, however, enable us to follow the dynamics of changes in antimicrobial susceptibility.

The epidemiological data concerning patients, from whom resistant *S. pneumoniae* strains were isolated, are consistent with risk factors of pneumococcal infection presented by others. The most important are: extreme age groups, staying in large communities, chronic diseases and dysfunction of local and systemic immunological system (Ussery *et al.*, 1996; Block *et al.*, 1995; Klugmann, 1996). Whereas protracting infections and long lasting antibiotic therapy are the factors predisposing to infection with multidrug resistant strain (Klugmann, 1996; Kristinsson, 1997; Cohen and Tartasky, 1997; Zenni *et al.*, 1995; Dowell and Schwartz, 1997; Nava *et al.*, 1994; Castillo *et al.*, 1998; Jacobs and Appelbaum, 1995).

In our study majority of strains were isolated from ambulatory patients (65 in 80; 81.3%), from children at nursery school age – below 7 years (46 in 70, 65.7%), patients suffering from chronic upper respiratory tracts infection. In such cases microbiological diagnostics is carried out only after a long-term antimicrobial therapy or therapy failures. This explains the fact that the resistance degree of certain strains reached even 7 drugs. The greatest number of resistant strains was isolated from nose or pharynx swabs, as it is the easiest, least invasive and therefore the most often performed testing in case of ambulatory diagnosed upper respiratory tracts infections. Majority of pneumococcal infections originate from either carriage that is present in nasopharynx in 20–40% of children population and 5–10% (Grzesiowski *et al.*, 1999) of adults, or droplet transmitted infection (Arnold *et al.*, 1996).

Due to their prevalence in our study, the strains isolated from ambulatory patients could be found in each group with differing resistance degree. While strains isolated from hospitalized patients belonged mainly to groups of the highest resistance degree: 2 out of 3 (67%) of 7-drug resistant, 3 out of 5 (60%) of 6-drug resistant, 3 out of 6 (50%) of 5-drug resistant, 4 out of 17 (23%) 4-drug resistant, 3 out of 22 (13.6%) of 3-drug resistant, none of 2-drug resistant and only one out of 14 (7.1%) 1-drug resistant strains came from hospital wards. The above mentioned regularity especially concerns the strains from patients with serious diseases like pneumonia or treated in intensive care unit, immunocompromised, exposed to various drugs including antimicrobial ones and therefore predisposed to multidrug resistant strains infection.

The other group of patients susceptible to pneumococcal infections is constituted by people of old age. Insignificant number of 6 strains from patients of mature age (39–68 years of age) also showed high resistance degree: 3 of them are 6-drug resistant (60% of 6-drug resistant strains), 1 is 5-drug resistant (16,7% of

5-drug resistant strains) and 2 are 4-drug resistant (11.7% of 4-drug resistant strains). Half of the patients of this group belonged also to before mentioned group of people hospitalized because of serious health state. A similar percentage of resistant pneumococci was isolated from women (45.2%) and from men (54.7%). Slight prevalence of pneumococcal infections in men, though not clear, is often reported (Fenoll *et al.*, 1998; Bennett *et al.*, 2003; McKenzie *et al.*, 2000).

The analysis of resistance patterns presented here also demonstrates the gradual increase of resistance among *S. pneumoniae* strains. It is proved by increase in amount of MDR strains and resistance degree as well as by formation of new resistance patterns. Half of the isolates are multidrug resistant (53.8%). The percentage of MDR amounted 62.5% in 2001 and reached 69.8% in 2003. Abrupt growth in the amount of MDR strains was noted in many long-term studies of pneumoccoci resistance. In the USA the MDR percentage of all isolates grew from 9.1% in 1994–1995 to 22.4% in 1999–2000 (Doern *et al.*, 2001).

The only one among antibiotics tested in our study to which no resistance has been reported is vancomycin at maximum MIC 0.5 mg/ml.

Occurrence of new resistance patterns over time, especially among the multidrug resistant strains, can also indirectly prove the increase of pneumococcal resistance. Practically all of singularly represented resistance patterns: PELTSCI, PELTSI, PELSH, PELTS, PESCI, PSH were identified in 2003 for the first time. Similarly, with reference to strains with high resistance degree – the first 6-drug resistant strain was reported in 2002 and other 4 strains of 2 new patterns appeared next year. Also 3 out of 4 patterns of resistance to 5 antibiotics were first reported in 2003. Though 7-drug resistant strains were present individually in every of the tested years, yet the isolate from 2003 showed different pattern. However, almost all other resistance patterns maintained throughout the analyzed years, patterns with resistance to tetracycline (T) and chloramphenicol (H) were an exception. The TH resistance pattern disappeared in 2003. Likewise, all other patterns with the TH resistance combination: PELTSHI, PTSCHI, PTSHI, TSH either disappeared till 2003, or, as in case of the TSH pattern, their percentage significantly decreased from 2001 to 2003 (from 28%, to 7.7% and finally to 7%). This fact can be connected with significant limitation of tetracycline and chloramphenicol consumption in the recent years, particularly in pediatrics. Besides, molecular testing proved that genes coding resistance to chloramphenicol (*cat* gene) and tetracycline (*tetM* gen) are carried by the same highly mobile transpozones: Tn5253 and Tn1545 and they tend to appear together (Ayoubi *et al.*, 1991; Clewell *et al.*, 1995).

The another phenomenon observed in our study is the appearance of another macrolide resistance phenotype. There has been described two macrolide resistance phenotypes: cross MLSb resistance to macrolides of MIC>64 mg/ml for erythromycin(CO_2), lincosamides and streptogramines determined by the *ermB* gene and methylation of the drug target, ribosome, and M phenotype with only 14- and 15-membered-ring macrolides resistance with MIC within 1–32 mg/ml for erythromycin(CO_2) resulting from active drug elimination mediated mainly by the product of *mefA* gene (Johnston *et al.*, 1998; Roberts *et al.*, 1999; Leclercq and Courvalin, 1991; Tait-Kamradt *et al.*, 1997; Pihlajamaki *et al.*, 2003). Our strains showed distinct prevalence of the MLSb phenotype, which is typical for Europe (Johnston *et al.*, 1998; Pihlajamaki *et al.*, 2003) and for the majority of multiresistant clones (McGee *et al.*, 2001), though Polish clone – Poland^{23F}-16 is of an M phenotype and harbored neither the *ermB* nor the *mefA* gene (Overweg *et al.*, 1999; McGee *et al.*, 2001). The first strain showing resistance to macrolides with susceptibility to lincosamides noted in our study appeared in January 2003 as E-pattern. In February resistance to erythromycin in strain of PESCiIi pattern was reported.

The lack of P, I or C resistance pattern despite numerous representation of multiresistant strains with these antibiotics (patterns: PELTSHI, PELSHI, PTSCHI, PESCI, PELST, PSI, PSH, PSH, PS) can be explained by phenomenon of coexistence of penicillin resistance with resistance to other antibiotics of betalactam group described in numerous reports (Bruggemann *et al.*, 2001; Munoz *et al.*, 1992) and reports about increased frequency of antimicrobial resistance to drugs from other groups among penicillin resistant strains (Baquero 1996; Doern *et al.*, 2001; Markiewicz and Tomasz, 1989; Allen, 1991; Lister, 1995). Cross antibiotic resistance to drugs from betalactam group is connected with common grip point-penicillin binding protein (PBP) (Markiewicz and Tomasz, 1989; Barcus *et al.*, 1995; Grebe and Hakenbeck, 1996). In Poland about 14% of *S. pneumoniae* strains are "nonsusceptible" to penincillin and half of them (6,8%) is highly resistant to penicillin and also to cephalosporines of the 2nd and 3rd generation (Trzciński and Hryniewicz, 1997; Hryniewicz *et al.*, 2000). In our study 5 strains (6.2%) showed high resistance to penicillin and only one of them isolated only in 2003 revealed MIC over 2 μg/ml (4 μg/ml). All these strains were multidrug resistant: 5–7-drug resistant and showed cross intermediate resistance to other betalactams: ceftriaxone and imipenem. Much higher amount of strains – 24 (30%) indicated intermediate resistance to

penicillin. In case of ceftriaxone only 3 strains with intermediate resistance and no highly resistant strains were described. Susceptibility to ceftriaxone among collected strains was relatively high in contradiction to data from other regions (Doern *et al.*, 2001).

Strains from the much more numerous group of 19 strains intermediately resistant to imipenem presented both high and intermediate resistance to penicillin. They were being isolated throughout the whole time of study and were already present in strains resistant to only one non-betalactam antibiotic. Such a significant amount of strains intermediately resistant to imipenem is surprising especially after consideration of the fact that this antibiotic is not used in ambulatory treatment and is not listed in any guidelines for treatment of pneumococcal diseases. Among few studies of pneumococcal resistance to imipenem that have been carried out in Europe a similar phenomenon was observed in Belgium, where the percentage of intermediately resistant strains amounted to 3.8% (Vanhoof *et al.*, 2003) and in France where MIC for imipenem ranged from 0.03 to 0.25 (Barakett *et al.*, 1992). In 304 strains from Hungary, 18.1% intermediately resistant and even 9.6% high resistant strains have been noted (Dobay *et al.*, 2003). Whereas, reports from Island, Norway and even Italy present 100% susceptibility to imipenem (Michault and Simac, 2000; Marchese *et al.*, 2000; Bergan *et al.*, 1998). More reports about lowered susceptibility to imipenem come from Far East (Satoh *et al.*, 2002; Hsueh *et al.*, 1999) and the USA (Pallares *etal.*, 1995; Frick *et al.*, 1998) though no such prevalence of strains intermediately resistant to imipenem in relation to strains "nonsusceptible" to cephalosporines of 3rd generation. In Taiwan, where there is one of the highest degree of antimicrobial resistance, among isolates from intensive care units the percentage of strains intermediately resistant to imipenem amounted to 21% and to cefotaxime to 33% (Hsueh *et al.*, 2001). The percentage of strains "nonsusceptible" to ceftriaxone and imipenem in Japan was 28,9% and 8,9% respectively (Satoh *et al.*, 2002). The tests carried out in the Washington State between 1995 and 1997 proved that among strains "nonsusceptible" to penicillin 28.6% were at the same time "nonsusceptible" to imipenem and 23,8% to ceftriaxone (Frick *et al.*, 1998). In our study this percentage amounted to 79.1% for imipenem (19 out of 24 penicillin resistant) and to 12.5% for ceftriaxone (3 out of 24). The phenomenon observed here is worth of deeper insight, the more so because imipenem is a drug of final choice in many serious diseases.

Coexistence of penicillin resistance with "nonsusceptibility" to antibiotics of other groups has been described since long time throughout the world. In collection of 10 000 strains tested from 1990 to 1996 in Spain, 72% of penicillin resistant strains were also resistant to other antibiotics, while among penicillin susceptible strains this percentage amounted 21.5% (Fenoll *et al.*, 1998). In Poland the presence of strains "nonsusceptible" to penicillin and resistant to other antibiotics (tetracycline, cotrimoxazole, macrolides and cephalosporines of the 1st and 2nd generation) was already reported in 1996 (Trzciński and Hryniewicz, 1997; Vanhoof *et al.*, 2003). In our study we have noted 41 (out of 80 *i.e.* 51,2%) strains susceptible to penicillin and resistant to other antibiotics, whereas all the 29 penicillin "nonsusceptible" strains showed resistance to other groups antibiotics.

Analysis of resistance patterns is of substantial practical meaning. Apart from providing directions for rational treatment it can also serve as an index of tendencies in antibiotic therapy applied in a given region. In our region the ELTS, S, TSH and PSI patterns occurred the most often. The amount of strains of the ELTS pattern during the period covered by study increased from 16.7% in 2001 to 20.9% in 2003. It can be connected with macrolide overdosing in ambulatory treatment not only in our country. Many sources, also studies within the Alexander project report alarming increase of macrolide resistance both among penicillin resistant and susceptible strains (16.5% and 10.4%, respectively, in 1996; while in 1997 it was 21.9% and 14.1%) (Schito *et al.*, 2000). In our country the percentage of macrolide resistant pneumococci increased from 1,9% in 1992 to 12.3% in 1996 (Trzciński and Hryniewicz, 1997).

Similarly, cotrimoxasole resistance that as the S pattern is present in 12,5% strains also occurs almost in all of the described resistance patterns (with exception of two: ELT and PELT). Thereby it additionally covers 60 out of 66 (90.9%) of 2- and more- drug resistant strains. It can be explained by the fact that in our country cotrimoxasole belongs to relatively often applied drug, though it is not always justifiable.

The phenomena presented in this study: growing resistance degree, increasing amount of multidrug resistant strains, emergence of new resistance patterns, prove gradual increasing resistance among *S. pneumoniae* strains in our region. The above changes have taken place in a relatively short time of 3 years. This testifies dynamics of this disturbing process and it more strongly justifies the necessity of constant monitoring pneumococcal resistance. We should be conscious that frequent therapeutic failures and exposition to further antibiotics can induce acquiring of resistance by bacteria and are a risk factor of infection with multidrug resistant strain.

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