



The presence of pilus and clade type among invasive pneumococci recovered in the Czech Republic.

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Acknowledgements. This work was supported by a research grant A/CZ0046/2/0007 from Iceland, Liechtenstein and Norway through the EEA financial mechanism and by a research grant from the Internal Grant Agency, Ministry of Health (CZ) IGA 9643-4.

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Presented at the 20th ECCMID (10 – 13 April, 2010, Vienna, Austria).

OBJECTIVES

Streptococcus pneumoniae is major cause of bacterial meningitis and community acquired pneumonia among children and adults. Although there is at least 91 different serotypes, only limited number of them demonstrated higher frequency in invasive pneumococcal disease. The effective spread of given serotypes represented by a few invasive clones was not fully elucidated yet and number of virulence factors has been suggested to influence the success of particular clones. One of the identified virulence factor is pneumococcal adhesive pilus encoded by *rliA* islet, which can be organised into 3 clades. The aim of the study was to determine the prevalence of the pilus and a clade type among pilus positive isolates in a collection of invasive strains of serotypes 4, 6B, 9V, 14, and 19F.

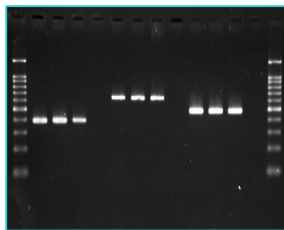


Fig. 3. The clade type assessment. Marker; lines 1-2, clade type I positive isolates; line 3, positive control *S. pneumoniae* ATCC 700905; line 4, negative control; lines 5-6, clade type II positive isolates; line 7, positive control *S. pneumoniae* ATCC 700670; line 8, negative control; lines 9-10, clade type III positive isolates; line 11, positive control *S. pneumoniae* ATCC 700906; line 12, negative control; marker.

CONCLUSIONS

The expression of the pilus could constitute a selective advantage in competition with other serotypes and could contribute to the increase of serotype 4 among invasive pneumococcal isolates and the expansion of Spain^{9V-3} clone among PNSP isolates.

RESULTS

The pilus was found in all serotypes, although the proportion of pilus positive isolates among serotypes varied from 1.7% (1) in serotype 14 to 100% of pilated isolates in serotype 6B, Figure 1. Out of 88 pilus positive isolates, 25% (22) were penicillin non-susceptible (MIC of penicillin > 0.06 mg/l). The ST's were homogenous for presence of the pilus, Table 1. The pilus has been found in all isolates of serotypes 9V, 6B, and 4 representing Spain^{9V-3} (ST156), Poland^{6B-20} (ST315), and Sweden⁴⁻³⁸ (ST205) clones, respectively. Among pilated isolates, 59 (67%) were defined as clade type I, 17 (19.3%) as clade type II, and 12 (13.6%) as clade type III, Figure 2. The clade I was found in serotypes 4 (ST205, 247, and 2342), serotype 14 (ST143), 9V (ST156 and 162), and serotype 19F (ST380); clade II in serotype 6B (ST176 and 1770); clade III in serotypes 4 (ST2341) and 6B (ST315). During the study period, the prevalence of serotype 4 in invasive isolates increased from 1.7% in 1996 to 11.1% in 2003, the analogical pattern was also seen in serotype 9V (from 3.3% to 5.3%), Figure 3.

MATERIALS AND METHODS

A total of 223 invasive isolates of serotypes 4 (55), 6B (20), 14(55), 9V (46), and 19F (47) recovered from 1996 to 2003 were studied. Serotyping was performed by the Quellung reaction and all isolates were analyzed by MLST. MICs of penicillin, erythromycin, clindamycin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole were determined by broth microdilution method. The presence of pilus was performed by PCR of the sortase B, C, and D genes which are a structural part of the *rliA* pathogenicity islet. Confirmation of pneumococcal pilus absence was done by amplification of the entire pilus islet with primers PFL-up and PFL-dn and comparison of the obtained PCR product with R6 DNA as non-piliated control. For pilus positive strains, the clade type (I, II, and III) was determined using PCR.

Table 1. Allelic profiles of studied strains.

Serotype	ST	No. of pilus negative isolates	No. of pilus positive isolates	Clade type	antE	gdh	glt	ncp	opt	opt	sdh	Comments
4	2341	9	51	II	7	138	4	1	15	1	20	
	1022	10			8	8	4	1	15	1	20	
	2361	2			8	8	4	1	15	1	20	
	800	2			8	41	4	1	15	1	20	
	801	8			8	70	4	1	15	1	20	
	2017	8			8	70	4	16	8	116	6	
	205	13	1	I	10	5	4	5	13	10	18	Sweden ⁴⁻³⁸ clone
	247	2	1	II	16	13	4	5	6	10	14	
	2342	4	1	II	16	141	4	5	6	10	14	
	6B	176	6	5	I	7	13	8	6	10	6	14
1770		11	6	I	7	13	41	8	10	6	14	
315		3	81	II	20	28	1	1	15	14	14	Poland ^{6B-20} clone
9V	156	20	1	I	7	11	10	1	6	8	1	Spain ^{9V-3} clone
	162	17	1	I	7	11	10	1	6	8	14	
	1770	1	15	I	15	17	4	16	6	19	14	
	239	8			15	17	4	16	6	19	17	
14	124	54			7	5	1	8	14	11	14	Netherlands ¹⁴⁻³³ clone
	143				7	5	10	18	6	4	1	DVJ of Spain ^{9V-3} clone
	1960	1	1	I	1	5	4	6	5	3	5	
19F	103	35			1	5	4	12	5	3	8	
	1815	1			1	5	4	12	5	3	159	
	2024	1			1	5	141	12	5	3	8	
	380	2	1	I	7	11	19	1	6	20	1	DVJ of Spain ^{9V-3} clone
	261	6			18	2	22	1	9	23	14	
2023	1			87	5	4	12	5	3	8		
		135	88									

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Fig. 1. Frequency of pilus positive isolates in studied serotypes.

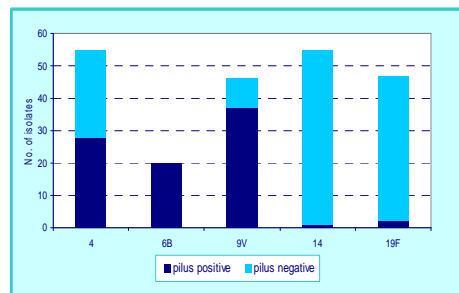


Fig. 2. Frequency of clade types in pilus positive isolates.

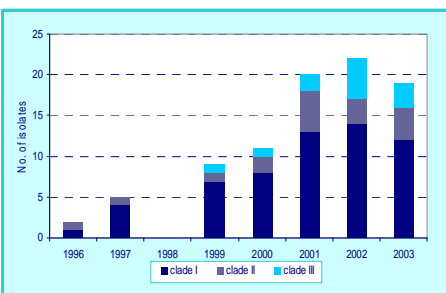


Fig. 3. Change in proportion of serotypes 4 and 9V among invasive isolates.

