The presence of piliated strains among penicillin-non susceptible pneumococci of serotypes 9V and 19A recovered in the Czech Republic.

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OBJECTIVES

Only limited number of serotypes demonstrated decreased susceptibility to antibiotics and the effective spread of certain antibiotic resistant clones has been shown. A number of virulence factors have been suggested to play a role in the success of particular clones. One of the identified virulence factor is pneumococcal adhesive pilus encoded by *rlrA* islet, which can be organised into 3 clades.

Since 1986, penicillin non-susceptible pneumococci (PNSP) have been found in the Czech Republic. Although the level of resistance to penicillin remains stable, the contribution of different capsular serotypes among the PNSP population varies. Since its first isolation in 1994, serotype 9V has gradually replaced serotype 19A that was highly prevalent in the Czech Republic until 1997. Among non-invasive PNSP strains, the proportion of serotype 9V increased from 0 % in 1992 to 44,4% in 2002 while that of serotype 19A decreased from 42,8% to 8,6% during the same period, Figure 1. Since 1998, serotype 9V has become the most predominant PNSP serotype in the Czech Republic.

The aim of the study was to determine the prevalence of the pilus and a clade type among pilus positive isolates in a set of PNSP strains of serotypes 9V and 19A.

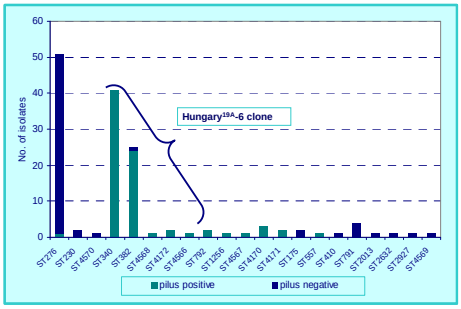

Fig. 1. Change in serotype distribution among PNSP non-invasive strains.

CONCLUSIONS

The higher presence of piliated strains and the expresion of the pilus belonging to the different clade family could contribute to the expansion of Spain9V-3 clone and decline of 19A serotype among PNSP isolates.

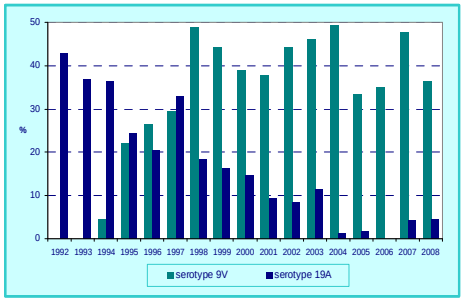

Fig. 2. Frequency of pilus positive isolates in 19A sequence types

MATERIALS AND METHODS

A total of 211 PNSP isolates of serotypes 19A (n = 145) and 9V (n = 66) recovered from 1991 to 2008 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 *rlrA* 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.

RESULTS

STs identical or related to those of two globally spread penicillin resistant *S. pneumoniae* clones, Spain9V-3 and Hungary19A-6, were detected among penicillin non-susceptible isolates of serotypes 9V (100%; n = 66) and 19A (48,3%; n = 70), respectively. The ST's were almost homogenous for presence of the pilus, Table 1. The pilus has been found in all isolates of serotype 9V representing Spain9V-3 clone and only one isolate of Hungary19A-6 clone was pilus negative. Eleven isolates unrelated to Hungary19A-6 clone were also found pilus positive. Except of one strain, isolates of ST276 (n = 50), ST230 (n = 2) and ST4570 (n = 1) belonging to the second major 19A cluster, were pilus negative, Figure 2. Isolates of serotype 9V belonged to clade type I, all pilus positive isolates of serotype 19A were assigned into clade type II.

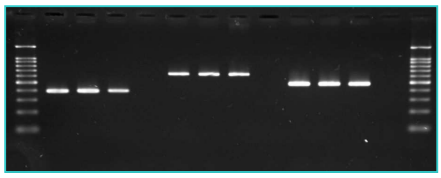

Fig. 3. The clade type assesment. Marker; lines 1-2, clade type I positive isolates; line 3, positive control *S. pneumoniae* ATCC 700905; line 4, negative control; lines 5-6, clade type II positive isolates; line 7, positive control *S. pneumoniae* ATCC 700670; line 8, negative control; lines 9-10,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

Serotype	ST	No. of pilus positive isolates	No. of pilus negative isolates	*aroE*	*gdh*	*gki*	*recP*	*spi*	*xpt*	*ddl*	Comments
19A	ST2632		1	1	12	5	5	148	217	8	
	ST276	1	50	2	19	2	17	6	22	14	
	ST4570		1	2	19	2	17	222	22	14	
	ST230		2	12	19	2	17	6	22	14	
	ST2013		1	12	19	36	17	6	20	14	
	ST175		2	7	5	4	26	10	3	55	CSR19A-11 clone
	ST557	1		7	11	10	1	6	58	1	
	ST4171	2		7	13	8	4	10	6	14	
	ST4170	3		7	13	8	5	10	1	14	
	ST1256	1		7	13	8	6	10	1	14	
	ST792	2		7	13	8	6	10	1	115	
	ST4567	1		7	13	8	6	10	1	370	
	ST791		4	7	13	8	6	13	6	8	
	ST340	41		7	13	42	6	10	6	58	SLV of Hungary19A-6 clone
	ST382	24	1	7	13	42	6	10	6	92	SLV of Hungary19A-6 clone
	ST4172	2		7	13	63	6	10	6	92	
	ST4566	1		7	13	153	6	10	6	58	
	ST4568	1		7	13	153	6	10	6	92	
	ST4569		1	7	234	8	6	13	1	2	
	ST410		1	8	10	2	16	7	26	1	
	ST2927		1	16	10	2	16	1	26	1	
9V	ST156	65		7	11	10	1	6	8	1	Spain9V-3 clone
	ST2025	1		88	11	10	1	6	8	1	SLV of Spain9V-3 clone
		146	65								

Table 1. Allelic profiles of isolated strains

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