Pneumococcal populations assessed by MLST and MLVA

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Introduction

In the era of global pneumococcal vaccination, selective pressure on the pneumococcal population may lead to changes in its composition. To monitor these alterations in the population, genotyping tools for large-scale applications are essential. For this, we compared the multiple-locus variable number tandem repeat analysis (MLVA) with multilocus sequence typing (MLST) using 2 different pneumococcal populations, isolated from patients with invasive disease from Catalonia and the Netherlands.

Method

The MLST and MLVA were performed as previously described (Enright & Spratt, Microbiology 1998 and Elberse et al. PLoS One 2011, www.MLVA.net). Pneumococcal populations of 163 and 166 strains obtained in Catalan region in Spain and in the Netherlands, respectively, were genotyped. The population included consecutive pediatric strains from Catalan pediatric patients (n=78) and all Dutch pediatric strains (n=71) of 2009-2012 and consecutive strains in the age group >5 year from Catalonia (n=85) and the Netherlands (n=95) of 2009-2012.

Figure 1. Schematic representation of the MLVA and MLST. **a)** Schematic overview of the MLVA, of 8 loci the number of tandem repeats are determined. **b)** Schematic overview of the MLST, partial sequences of 7 housekeeping genes are determined.

Results

In total, the MLST and MLVA yielded 126 and 176 types, respectively. The MLST and MLVA performed on the Catalan strains yielded 73 and 92 types, respectively, and on the strains from the Netherland, 70 and 99 types, respectively. The discriminatory power of both methods, calculated using the Simpson's diversity index (SID), was high (Table 1). Congruence, calculated using Wallace coefficient, differed per region (Table 2).

	SID	[95% CI]	No. of types
Serotype	0.934	[0.923 - 0.946]	39
MLST	0.969	[0.960 - 0.978]	126
MLVA	0.978	[0.970 - 0.986]	176

Table 1. Simpons Index of diversity (SID) of the different typing methods for all samples. The Simpsons indices of diversity were high for both methods and comparable between the countries.

Results - continued

Catalonia, Spain:

	Serotype	MLST_ST	MLVA_MT
Serotype	-	0.458	0.314
MLST	0.960	-	0.623
MLVA	0.961	0.935	-

The Netherlands:

	Serotype	MLST_ST	MLVA_MT
Serotype	-	0.512	0.415
MLST	0.985	-	0.482
MLVA	0.997	0.597	-

Table 2. Difference per region in congruence between typing methods by Wallace coefficient. The probability of 2 Catalan strains having the same MLVA type also sharing the same sequence type was 93.5%. However, in the Dutch strains this probability was only 59.7%.

Major clones detected with MLST were ST306 (n=22; 13.5%), ST191 (n=15; 9.2%) and the multiresistant clone ST320 (n=10; 6.1%) in Catalonia and ST191 (n=17; 10.2%, ST306 (n=15; 9.0%) and ST53 (n=11; 6.6%) in the Netherlands (Figure 2). Difference in clonality of the pneumococcal populations in both regions may provide an explanation for the difference in Wallace coefficient (Table 3).

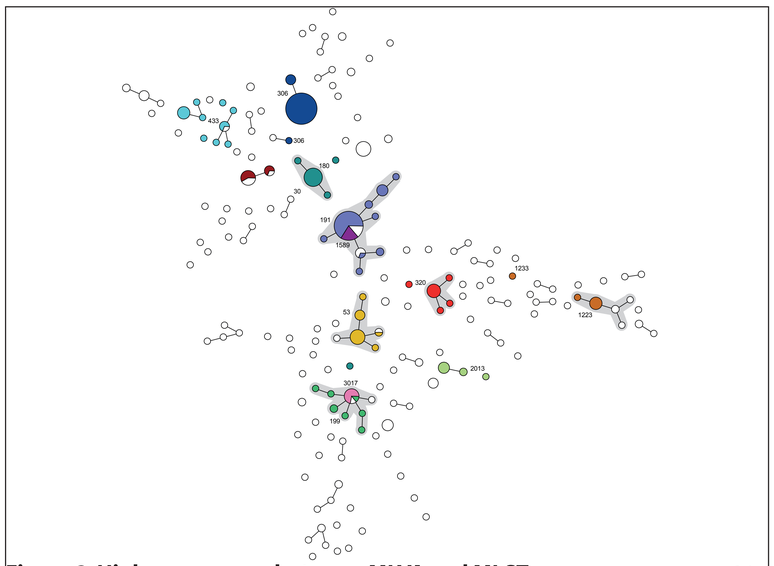

Figure 2. High congruence between MLVA and MLST. Minimum spanning tree of the results of MLVA (n=329). The colors and text indicate the prevalent Sequence Types within this study.

Results - continued

Serotype 19A, Penicillin non-susceptible MIC >0.06 (n=19):

	SID	[95% CI]	No. of types
MLST	0.684	[0.561 - 0.808]	4
MLVA	0.869	[0.790 - 0.948]	8

	Serotype	MLST	MLVA
Serotype	-	0.316	0.131
MLST	1.000	-	0.435
MLVA	1.000	1.000	-

Serotype 19A, Penicillin susceptible (MIC ≤0.06) (n=44):

	SID	[95% CI]	No. of types
MLST	0.890	[0.809 - 0.971]	13
MLVA	0.920	[0.829 - 1.000]	17

	Serotype	MLST	MLVA
Serotype	-	0.110	0.080
MLST	1.000	-	0.344
MLVA	1.000	0.500	-

Table 3. Difference in congruence between typing methods is dependent on the high clonality of antibiotic non-susceptible strains. The probability of 2 non-susceptible serotype 19A strains having the same MLVA type also sharing the same sequence type was 100%. However, for susceptible serotype 19A strains this probability was only 50%. The same holds true for all susceptible and non-susceptible serotypes analysed together, but less pronounced. An explanation could be that the housekeeping genes in non-susceptible strains are more conserved compared to BOX loci in those strains.

Conclusions

Both methods yield a high diversity index and congruence between the methods was high, but differed between . Using MLVA, we could further distinguish highly clonal isolates, such as the penicillin non-susceptible clones, when grouped by MLST. Although MLST is globally used as gold standard in genotyping of the pneumococcus, the MLVA yield comparable results, may be preferable for typing highly clonal populations and is the cheaper alternative.

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