

In the current study, examination of 1157 S2 sequences extracted from invasive *Streptococcus pneumoniae* whole genomes revealed that except for one, all S2-sequences grouped together. The exception was an S2-sequence from a pneumococcal isolate serotype 35B (2060880) from CSF, which was identical to that of *S. mitis* SK575. The blood isolate of the same patient had the same serotype and S2-type. To confirm the species of this strain, we performed *in silico* DNA hybridisation using the recommended formula and the Genome-To-Genome Distance Calculator (<http://ggdc.dsmz.de/distcalc2.php>) (1).

DNA-DNA hybridisation (DDH) estimate (in silico) (GLM-based) (identities / High-scoring Segment Pair (HSP) length)

Strain	2060880	2070268	2100466	2120989	<i>S. mitis</i> SK575
<i>S. pneumoniae</i> TIGR4	87.30% [84.7 - 89.5%]	87.40% [84.9 - 89.6%]	87.40% [84.8 - 89.6%]	87.70% [85.1 - 89.8%]	47.80% [45.2 - 50.4%]
<i>S. pneumoniae</i> D39	92.90% [90.1 - 94.9%]	88.80% [86.3 - 90.8%]	88.70% [86.3 - 90.8%]	88.90% [86.5 - 90.9%]	43.00% [40.5 - 45.6%]
<i>S. pneumoniae</i> 35B 2060880	100.00% [100 - NaN%]	88.90% [86.4 - 90.9%]	88.70% [86.3 - 90.8%]	89.00% [86.6 - 91%]	39.50% [37.1 - 42.1%]
<i>S. pneumoniae</i> 35B 2070268		100.00% [100 - NaN%]	99.00% [98.5 - 99.4%]	99.40% [99 - 99.6%]	47.20% [44.6 - 49.8%]
<i>S. pneumoniae</i> 35B 2100466			100.00% [100 - NaN%]	99.10% [98.6 - 99.4%]	47.00% [44.5 - 49.6%]
<i>S. pneumoniae</i> 35B 2120989				100.00% [100- %]	47.00% [44.5 - 49.6%]
<i>S. mitis</i> SK575					100.00% [100 - %]

Probability of being the same species (identities / HSP length)

Strain	2060880	2070268	2100466	2120989	<i>S. mitis</i> SK575
<i>S. pneumoniae</i> TIGR4	94.78%	94.84%	94.81%	94.93%	13.54%
<i>S. pneumoniae</i> D39	98.16%	95.36%	95.34%	95.41%	5.47%
<i>S. pneumoniae</i> 35B 2060880	98.30%	95.39%	95.34%	95.45%	2.41%
<i>S. pneumoniae</i> 35B 2070268		99.60%	98.07%	98.14%	27.20%
<i>S. pneumoniae</i> 35B 2100466			98.30%	98.08%	11.94%
<i>S. pneumoniae</i> 35B 2120989				98.30%	11.95%
<i>S. mitis</i> SK575					98.30%

Reference

1. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics. 2013;14(1):60.