TPS system distribution by cohort, serogroup and clonal complex



Supplementary Figure 1. TPS system distribution by cohort, serogroup and clonal complex. The proportion of TPS system distribution by cohort (A), serogroup (B) and cloncal complex (cc; C) in both cohorts is shown. ND = not determined. Each isolate could only contribute to one of the 6 TPS distribution categories.



Supplementary Figure 2. Western Blot analysis of TpsA proteins. Immunoblots of whole cell lysates (P) and concentrated culture supernatants (S) of cultures of H44/76, H44/76 Δ tpsB2, H44/76 Δ tpsB2/tpsA3, H44/76 Δ tpsB2 with pEN-TpsB2 and H44/76 Δ tpsB2/tpsA3 with pEN-TpsB2, the latter grown in the presence or absence of 0.5 mM IPTG to induce expression of the tpsB2 gene, as indicated above the lanes. The blots were incubated with α TPS2 (top), α TpsB2 (middle), and α TPS1 (bottom) as indicated on the left. The blots show secretion of the TPS1 system in all strains, indicating that it was not affected by the knock-out mutations in systems 2 and 3. Furthermore, the TpsB2 is only present in the wild-type and complemented strains. When present it resulted in the secretion of ~145 and ~130 kDa TpsA protein variants of systems 2 and 3.



Supplementary Figure 3. N. meningitidis growth curve in RPMI. Growth of N. meningitidis H44/76 wild type (WT) and several TPS system 2&3 knock-out and complemented knock-out strains in RPMI medium.

Supplementary table 1. TPS system primers

Primer name	Target gene	DNA sequence
LR1	tpsB1 AATTTTTTCCTGCTCCATGTCTGT	
LR2		TTAGAAACTGTAATTCAAGTTGAAGCCGTAA
tps1	tpsA1	ATGAATAAAGGTTTACATCGCATTATCTTTAGT
tps2		AAGCATTTAATACCGTGGTAGCTGGGCGA
LR6	tpsB2	TCCCCCAACCCTGCCGAAATCCG
LR8		TTAAAACGTATAGCCTACCTGAAAACCGCT
tps4	tpsA2a	ATGGAATAAAACTCTCTATCGTGTAATTTTCAA
tps5		AGTGTTTTGAATCGACTGACTGTGAATT
tps9	tpsA2b	TATGAACCGCACCCTGTACAAAGTTGT
tps10		GCCGGCAGATACCAAATCCAATGCT
tps7	tpsA3	ATGAACAAGCGATGCTACAAGGTTATCT
tps8		GCGTCCGGCTTCAATATCGCGT

Supplementary table 3. Summary of TPS system distribution in all available cohorts

	Isolates with TPS1	Isolates with TPS system 2
		and/or 3
Van Ulsen et al. (invasive disease isolates)(6) ^a	72/88 (82%)	41/88 (47%)
Schmitt et al. (carrier isolates)(7) ^b	485/632 (77%)	328/822 (40%)
Current study		
1998-2002 cohort	246/254 (97%)	162/254 (64%)
2006-2014 cohort	89/115 (77%)	84/115 (73%)

^aPositive isolates are redefined as stated in the material and methods section of this article.

^bPositive isolates are defined as giving a positive result in the dot plot with specific probes for *tpsA1*, *tpsA2a*, *tpsA2B* or *tpsA3* genes (the corresponding information on the *tpsB* status of each strain was not reported).