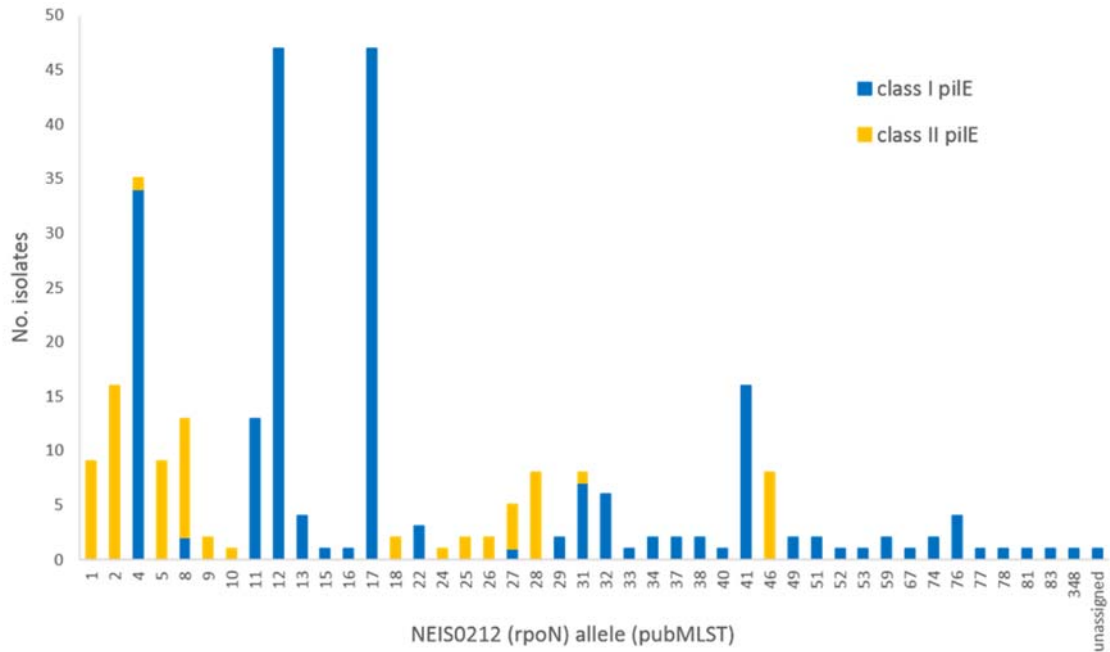


**A**



**Fig. S1. Prevalence of *rpoN* alleles in meningococcal genomes analysed in this study.**

A total of 290 meningococcal genomes were analysed. We identified 44 unique *rpoN* (NEIS0212) alleles among the 290 isolates. Isolates with class I *pilE* are indicated by blue bars, isolates with class II *pilE* locus are indicated by yellow bars. Of the 44 different alleles, 11 are exclusive to isolates with class II *pilE* and 29 are found only in isolates with class I *pilE*. Alleles 4, 8, 27 and 31 were found in isolates with class I *pilE* or class II *pilE*.

**SUPPLEMENTARY TABLES**

**Table S2: Bacterial strains used in this study**

Strain	Genotype/Description	Source
<b><i>E. coli</i></b>		
Dh5α	F <sup>-</sup> <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20</i> φ80d <i>lacZ</i> ΔM15 Δ( <i>lacZYA-argF</i> )U169, hsdR17( <i>r<sub>K</sub><sup>-</sup>m<sub>K</sub><sup>+</sup></i> ), λ <sup>-</sup>	Invitrogen
<b><i>N. meningitidis</i></b>		
S4	Serogroup C, ST-11 complex/ET-37, class II <i>pilE</i>	(1)
S4Δ <i>rpoN</i>	<i>rpoN</i> replaced with kanamycin or erythromycin resistance marker	This study
S4Δ <i>rpoE</i>	<i>rpoE</i> replaced with kanamycin resistance marker	This study
S4φP <sub><i>pilE</i></sub> <i>lacZ</i>	Reporter strain for class II <i>pilE</i> promoter activity. S4 with <i>lacZ</i> in place of <i>pilE</i> ORF at native locus. Kanamycin resistant.	This study
S4Δ <i>rpoN</i> φP <sub><i>pilE</i></sub> <i>lacZ</i>	Reporter strain for class II <i>pilE</i> promoter activity. S4Δ <i>rpoN</i> ( <i>erm(C)</i> ) with <i>lacZ</i> in place of <i>pilE</i> ORF at native locus. Kanamycin and erythromycin resistant.	This study
S4P <sub><i>lac</i></sub> - <i>rpoN</i> <sup>Nm</sup>	S4 with gene encoding FLAG-tagged σ <sup>N</sup> from <i>N. meningitidis</i> strain S4 integrated at <i>trpB-iga</i> locus under control of IPTG inducible promoter. Erythromycin resistant.	This study
S4P <sub><i>lac</i></sub> - <i>rpoN</i> <sup>Nel</sup>	S4 with gene encoding FLAG-tagged σ <sup>N</sup> from <i>N. elongata</i> strain 29315 integrated at <i>trpB-iga</i> locus under control of IPTG inducible promoter. Erythromycin resistant.	This study
S4 <i>ery</i>	S4 with empty vector integrated at <i>trpB-iga</i> locus. Erythromycin resistant.	This study
S4P <sub><i>lac</i></sub> - <i>rpoE</i>	S4 with gene encoding FLAG-tagged σ <sup>E</sup> integrated at <i>trpB-iga</i> locus under control of IPTG inducible promoter. Erythromycin resistant.	This study
S4P <sub><i>lac</i></sub> - <i>rpoH</i>	S4 with gene encoding FLAG-tagged σ <sup>H</sup> integrated at <i>trpB-iga</i> locus under control of IPTG inducible promoter. Erythromycin resistant.	This study

1. **Uria MJ, Zhang Q, Li Y, Chan A, Exley RM, Gollan B, Chan H, Feavers I, Yarwood A, Abad R, Borrow R, Fleck RA, Mulloy B, Vazquez JA, Tang CM.** 2008. A generic mechanism in *Neisseria meningitidis* for enhanced resistance against bactericidal antibodies. *J Exp Med* **205**:1423-1434.

**Table S3: Primers used in this study**

Name	Description/Purpose	SEQUENCE 5'-3'
ML20	Forward primer for upstream fragment for <i>rpoN</i> deletion in S4	<u>CACGAATTCTTTTCATTCAGCCACAAAT</u>
ML21	Reverse primer for upstream fragment for <i>rpoN</i> deletion in S4	<u>CCAGGTACCGATAATGTGGGAGAAATTGT</u>
ML22	Forward primer for kanamycin resistance marker for <i>rpoN</i> deletion in S4	<u>CCAGGTACCAATTGTGTCTCAAAATCTCTGA</u>
ML23	Reverse primer for kanamycin resistance marker for <i>rpoN</i> deletion in S4	<u>CCAGGTACCCGATGCATGCCAACAGATAA</u>
ML24	Forward primer for downstream fragment for <i>rpoN</i> deletion in S4	<u>CCAGGTACCTTGCTGAATAATCTTATAAAGAC</u>
ML25	Reverse primer for downstream fragment for <i>rpoN</i> deletion in S4	<u>CCAGGATCCTTTTCGGTCATTTCTGATAAAC</u>
ML284	Forward primer for upstream fragment for <i>rpoE</i> deletion in S4	<u>GATCCTCTAGAGTCGACCTGCAGGCATGCACTTTTAGACGGCATT</u> TGGCACTG
ML285	Reverse primer for upstream fragment for <i>rpoE</i> deletion in S4	<u>CCCGTTGAATATGGCTCATAGTATCGGGAACAAGGTAAT</u>
ML288	Forward primer for downstream fragment for <i>rpoE</i> deletion in S4	<u>GCTCGATGAGTTTTTCTAAAACACCGCACGGGTATTAC</u>
ML289	Reverse primer for downstream fragment for <i>rpoE</i> deletion in S4	<u>CAGGAAACAGCTATGACCATGATTACGCCATGCTGCTTTTGAAGCG</u> TG
ML286	Forward primer for kanamycin fragment for <i>rpoE</i> deletion in S4	<u>AATTACCTTGTTCCCGATACTATGAGCCATATTCAACGGG</u>
ML287	Reverse primer for kanamycin fragment for <i>rpoE</i> deletion in S4	<u>GTAATACCCGTGCGGTGTTTTAGAAAACTCATCGAGCATC</u>
ML46	Reverse primer for upstream fragment for <i>rpoN</i> deletion in S4 $\phi$ <sub><i>pilE</i></sub> <i>lacZ</i> strains	<u>GGATCCAAGGGCGATAATGTGGGAGAA</u>
ML52	Forward primer for downstream fragment for <i>rpoN</i> deletion in S4 $\phi$ <sub><i>pilE</i></sub> <i>lacZ</i> strains	<u>TCCCCGGGAAGGGCTCAGAATTGGTTAAT</u>
ML37	Forward primer for upstream (5') fragment for replacement of class II <i>pilE</i> ORF with <i>lacZ</i> in S4 $\phi$ <sub><i>pilE</i></sub> <i>lacZ</i> strains	<u>ATCCCACCAACCCACTTTTCCGC</u>
ML34	Reverse primer for upstream (5') fragment for replacement of class II <i>pilE</i> ORF with <i>lacZ</i> in S4 $\phi$ <sub><i>pilE</i></sub> <i>lacZ</i> strains	<u>TGAATCCGTAATCATGGTCATTTGGATGACTCCTG</u>
ML31	Forward primer for downstream (3') fragment for replacement of class II <i>pilE</i> ORF with <i>lacZ</i> in S4 $\phi$ <sub><i>pilE</i></sub> <i>lacZ</i> strains	<u>TGGTGTCAAAAATAACACCAAATAAGGACA</u>

ML30	Reverse primer for downstream (3') fragment for replacement of class II <i>pilE</i> ORF with <i>lacZ</i> in $S4\phi P_{pilE}/lacZ$ strains	TCACGATAGGCGAGGCGCA
ML33	Forward primer amplification of <i>lacZ</i> from pRS415 fragment for replacement of class II <i>pilE</i> ORF with <i>lacZ</i> in $S4\phi P_{pilE}/lacZ$ strains	CAGGAGTCATCCAAATGACCATGATTACGGATTCA
ML32	Reverse primer amplification of <i>lacZ</i> from pRS415 fragment for replacement of class II <i>pilE</i> ORF with <i>lacZ</i> in $S4\phi P_{pilE}/lacZ$ strains	TGTCCTTATTGGTGTATTTTTGACACCA
ML151	Forward primer for amplification of 3' end of <i>iga</i> gene for pNMC2 construction	<u>GAGATTTTCGATTCCACCGCCGCTTATGCAGTCAGCACCAATAC</u>
ML149	Reverse primer for amplification of 3' end of <i>iga</i> gene for pNMC2 construction	<u>GAACATGATGAGTGATCGTTAAATTTGTTAATCCACTATAAAAAT</u> GCC
ML148	Forward primer for amplification of <i>erm(C)</i> and <i>lac</i> regulatory region from pGCC4, for pNMC2 construction	TTATTTTGCTATGAGGGATCCGCTAGCACTAG
ML150	Reverse primer for amplification of <i>erm(C)</i> and <i>lac</i> regulatory region from pGCC4, for pNMC2 construction	AACA <u>AAATTTAACGATCACTCATCATGTTC</u>
ML152	Forward primer for amplification of plasmid backbone from pNCC1, for pNMC2 construction	<u>AAGGCGGCGGTGGAATCG</u>
ML146	Forward primer for amplification of 3' end of <i>trpB</i> gene for pNMC2 construction	<u>CGCGGAATTCTCATGTTTGACAGCTTATAAGCGGAAGACTTGAAC</u> CAC
ML147	Reverse primer for amplification of 3' end of <i>trpB</i> gene for pNMC2 construction	<u>CTAGTGCTAGCGGATCCCTCATAGCAAAATAAAATGCCGT</u>
ML153	Reverse primer for amplification of plasmid backbone from pNCC1, for pNMC2 construction	<u>ATAAGCTGTCAAACATGAGAATTCCGC</u>
ML154	Forward primer for amplification of <i>rpoN</i> from <i>S4</i> . For cloning into pNMC2	<u>CCCAAGCTTGAGTAATTTTATGACCTTACTCGGAATAAAGCT</u>
ML155	Reverse primer for amplification of <i>rpoN</i> from <i>S4</i> . For cloning into pNMC2. Primer includes sequence for in frame triple FLAG TAG	<u>GTGGTTTAAACTCACTTGTCGTCGTCGTCCTTGTAGTCGATGTCGT</u> <u>GGTCCTTGTAGTCACCGTCGTCGTCCTTGTAGTCTTCTGCGGTTTT</u> GCGTTT
ML173	Forward primer for amplification of <i>rpoN</i> from <i>N. elongata</i> . For pNMC2 <i>rpoN<sup>Nel</sup></i> construction	<u>TTACGAATCCCGGATTAATTAAGCTTGGAGTAATTTTATGACA</u> TTGCTCGGATTAAACT

ML175	Reverse primer for amplification of <i>rpoN</i> from <i>N. elongata</i> . For pNMC2 <i>rpoN</i> <sup>NeI</sup> construction	<u>CTTGTAGTCACCGTCGTGGTCTTGTAGTCGATCCTGCGTTGGTGT</u> GC
ML172	Forward primer for amplification of pNMC2 fragment for pNMC2 <i>rpoN</i> <sup>NeI</sup> construction.	<u>GACTACAAGGACCACGACGGTGAC</u>
ML174	Reverse primer for amplification of pNMC2 fragment for pNMC2 <i>rpoN</i> <sup>NeI</sup> construction.	<u>AAGCTTTTAATTAATCCGGGAA</u>
ML280	Forward primer for amplification of <i>rpoE</i> from S4. For cloning into pNMC2	<u>CCATGATTACGAATCCCGGATTAATTAAGGAGTAATTTATGCC</u> GCTACCCGACCT
ML282	Reverse primer for amplification of <i>rpoE</i> from S4. For cloning into pNMC2. Primer includes sequence for in frame triple FLAG TAG	<u>TCACTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCTTGTAGT</u> <u>CACCGTCGTGGTCTTGTAGTCCTTCGGGTTTTCTTGGTTGAAC</u>
ML184	Forward primer for amplification of <i>rpoH</i> from S4. For cloning into pNMC2	<u>ACCATGATTACGAATCCCGGATTAATTAAGGAGTAATTTATGA</u> ATAACGCTTTCGC
ML188	Reverse primer for amplification of <i>rpoH</i> from S4. Primer includes sequence for in frame triple FLAG TAG	<u>TCGTTAAATTTTCACTTGTGTCGTCGTCCTTGTAGTCGATGTCGTG</u> <u>GTCCTTGTAGTCACCGTCGTGGTCTTGTAGTCAACCGCTTCGGCT</u> TCTTC
ML66	cDNA synthesis for <i>rpoN</i>	<u>CCGTCTAGCTCTCTAATCGATGCTGTCGAGGGCGTTTCG</u>
ML67	Strand-specific RT-PCR for <i>rpoN</i>	CAAGCCCTGACTGCATTGC
ML69	cDNA synthesis for <i>pilE</i>	<u>CCGTCTAGCTCTCTAATCGCCTTCAACCTTAACCGATGC</u>
ML70	Strand-specific RT-PCR for <i>pilE</i>	AAGGTCAAAAATCCGCAGTG
ML71	cDNA synthesis for tmRNA	<u>CCGTCTAGCTCTCTAATCGAACCCGGTAGGAAACCGATC</u>
ML72	Strand-specific RT-PCR for tmRNA	AGTCGCAAACGACGAACTT
ML68	Strand-specific RT-PCR tag primer	<u>CCGTCTAGCTCTCTAATCG</u>
P1	Primer extension to identify class II <i>pilE</i> TSS	CCTTCTTACCAGCAAGCGGC
P2	Primer extension to identify class II <i>pilE</i> TSS	CGTAATGTATTTGACCGTGG

Underlined overlap sequences that are not present in genomic sequence added for Gibson cloning. FLAG tag is double underlined.