

## Supplementary Information

### **Heteroresistance to the model antimicrobial peptide polymyxin B in the emerging *Neisseria meningitidis* lineage11.2 urethritis clade: mutations in the *pilMNOPQ* operon**

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**Supplemental table S1.** Polymyxin B E-test results

**Supplemental table S2.** Comparison of antibiotic resistance levels of the PmB heteroresistant mutant by disc diffusion

**Supplemental table S3.** Sequences of primers used in this study

**Supplemental figure 1.** Polymyxin B E-test results of *N. meningitidis* isolates and mutants

**Supplemental Table S1.** Polymyxin B E-test results

Name	ID	E Test, PmB ( $\mu\text{g/ml}$ )	Note
CNM1	COL-201501-07	192	US_NmUC
CNM2	COL-201501-15	192	US_NmUC
CNM3	COL-201501-27	256	US_NmUC
CNM4	COL-201501-29	192	US_NmUC
CNM5	COL-201502-20	128	US_NmUC
CNM6	COL-201503-05	192	US_NmUC
CNM7	COL-201503-27	96	US_NmUC
CNM8	COL-201504-01	256	US_NmUC
CNM9	COL-201504-04	128	US_NmUC
CNM10	COL-201504-11	128	US_NmUC
CNM11	COL-201504-14	192	US_NmUC
CNM12	COL-201504-15	192	US_NmUC
CNM13	COL-201504-18	128	US_NmUC
CNM14	COL-201504-20	192	US_NmUC
CNM15	COL-201504-22	192	US_NmUC
CNM16	COL-201505-02	192	US_NmUC
CNM17	COL-201505-03	192	US_NmUC
CNM18	COL-201505-05	192	US_NmUC
CNM19	COL-201505-12	192	US_NmUC
CNG20	COL-201505-15	48	<i>N. gonorrhoeae</i>
CNM21	COL-201505-17	192	US_NmUC
CNM22	COL-201505-21	192	US_NmUC
CNM23	COL-201505-25	128	US_NmUC
CNM24	COL-201505-28	96	US_NmUC
CNM25	COL-201505-29	128	US_NmUC
CNM26	COL-201505-31	192	US_NmUC
CNM27	COL-201505-34	192	US_NmUC
CNM28	COL-201506-02	128	US_NmUC
CNM29	COL-201506-03	128	US_NmUC
CNM30	COL-201506-04	96	US_NmUC
CNM32	COL-201506-27	128	US_NmUC
CNM33	COL-201506-31	96	US_NmUC
CNM34	COL-201507-10	16	US_NmUC
CNM35	COL-201507-26	192	US_NmUC
CNM36	COL-201507-33	192	US_NmUC
CNM37	COL-201507-37	96	US_NmUC
CNM38	COL-201508-02	128	US_NmUC
CNM39	COL-201508-07	96	US_NmUC

CNM40	COL-201508-16	128	US_NmUC
CNM41	COL-201508-17	192	US_NmUC
CNM42	COL-201508-18	128	US_NmUC
CNM43	COL-201508-19	128	US_NmUC
CNM44	COL-201508-20	128	US_NmUC
CNM45	COL-201508-26	192	US_NmUC
CNM46	COL-201508-31	96	US_NmUC
CNM47	COL-201508-41	128	US_NmUC
CNM48	COL-201508-49	64	US_NmUC
CNM49	COL-201509-03	96	US_NmUC
CNM50	COL-201509-11	128	US_NmUC
CNM51	COL-201509-13	192	US_NmUC
CNM52	COL-201509-23	64	US_NmUC
CNM53	COL-201509-25	128	US_NmUC
CNM54	COL-201509-28	128	US_NmUC
ATL#1		96	US_NmUC
ATL#2		128	US_NmUC
FA19		48	<i>N. gonorrhoeae</i>
FA1090		96	<i>N. gonorrhoeae</i>
H041		192	<i>N. gonorrhoeae</i>
MS11		96	<i>N. gonorrhoeae</i>
M7		64	<i>N. meningitidis</i>
FAM18		384	<i>N. meningitidis</i>
F-M7		192	<i>N. meningitidis</i>

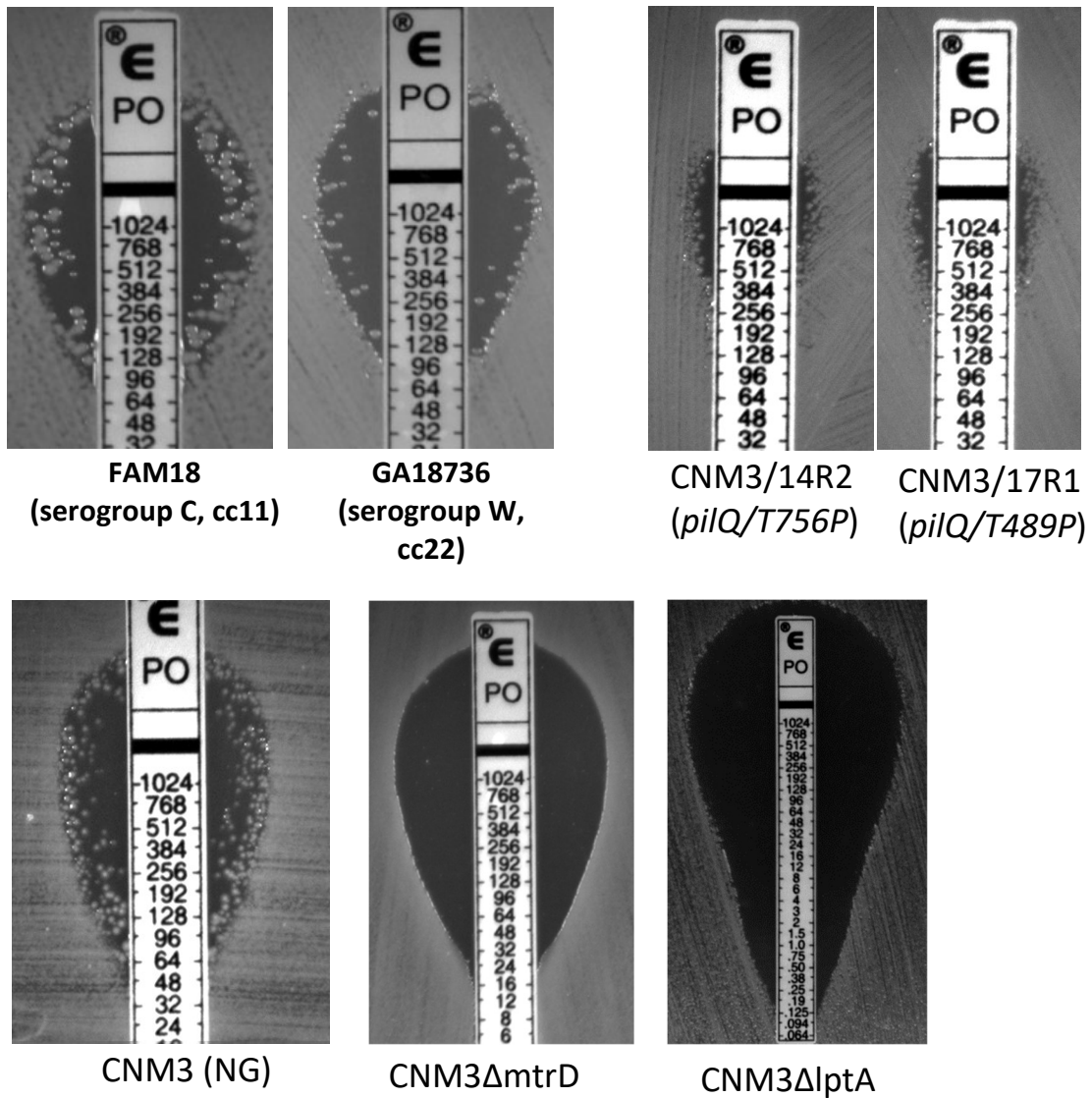
**Supplemental Table S2.** Comparison of antibiotic resistance levels of the PmB heteroresistant mutant by disc diffusion

Antibiotics	CNM3	3R4	MS11	CNG20	FA19
<b>Disc diffusion (mm)</b>					
Penicillin G	<b>32.7 ± 1.3</b>	<b>26.9 ± 0.9</b>	30	40	45
Cefuroxime	<b>31.7 ± 0.6</b>	<b>26.3 ± 0.6</b>	32	42	50
Ceftriaxone	41.6 ± 3.4	39.6 ± 2.7	47	50	58
Streptomycin	<b>11.6 ± 1.3</b>	<b>9.6 ± 0.5</b>	6	19	16
Kanamycin	<b>15.5 ± 1.0</b>	<b>12.4 ± 0.8</b>	16	15	18
Chloramphenicol	<b>35.5 ± 1.2</b>	<b>30.7 ± 1.1</b>	28	41	45
Tetracycline	<b>34.0 ± 0.0</b>	<b>30.7 ± 1.2</b>	31	30	38
Ciprofloxacin	43.0 ± 1.0	43.0 ± 1.0	46	48	54

#. Data are presented as the mean ± standard deviation (N=3-8). The values in bold are statistically different between CNM3 and 3R4 by student's t test. A single data point was shown for each gonococcal strain for comparison.

**Supplemental Table S3.** Sequences of primers used in this study

<b>Primer name</b>	<b>Sequence (5'-3')</b>
mtrC-F1	TTATGAAGCAGGTCTGGAAAGCG
mtrDF2-ER	cggaattcCGAATTGGGCGGCGGTTACA
mtrE3R1-ER	cggaattcGTTTTCGGCGCGGTCAGTT
lgtG1	GCAACCAACAACCTCAAACACG
lgtG2	CGTTCATGACCTCTGTACATGC
pilQ-F1	CGATATGCGCCAACAAGGGAACA
pilQ-5RA3	ttcctcctagttagtcaccccAAACATATGCCCTTATTTGACGC
pilQ-3FA3	cctggaggaataatgaccCATATGCCCTTCTTTATGCTTTTT
pilQ-3R2	GGGTTCGGGCTTGTCGGGTTAC
YT113	TCCCATCCGCCGAATAAATAGTC
pilM-5Ra	ttcctcctagttagtcaccccCGGGACGGACGCGATGATGT
pilM-3Fa	cctggaggaataatgaccGAACTTGATGCGCCGACACTG
pilM-R2	CTGCCGCTGAGCCGATAAAGAGT
pilM-3R	GCCGCCTTACGATGGGTTGT
aphA3-SmF	GGGTGACTAACTAGGAGGAA
aphA3-SmR	GGGTCATTATTCCTCCAGG
mutL.1a	TATGCCACGCATTGCCGCCCTGCC
mutL.1b	CGGTTTATTGTCCGCGCAAGAACAG



**Supplemental Figure 1.** Polymyxin B E-test results of *N. meningitidis* isolates and mutants