

**Supplemental information for:**

**Prevalence and detailed mapping of the gonococcal genetic island in *Neisseria meningitidis***

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Table S1. Results of GGI screen in *N. meningitidis*

Strain	Serogroup	GGI +/-	GGI type	Source
ATCC 13077	A	Neg	—	ATCC
M06993	A	Neg	—	CDC
M07221	A	Neg	—	CDC
M14761	A	Neg	—	CDC
M00970	A	Neg	—	CDC
M0009C	A	Neg	—	CDC
M020N1	A	Neg	—	CDC
M016N3	A	Neg	—	CDC
M13943	A	Neg	—	CDC
M10050	A	Neg	—	CDC
M09285	A	Neg	—	CDC
M07126	A	Neg	—	CDC
M07339	A	Neg	—	CDC
M07795	A	Neg	—	CDC
M07797	A	Neg	—	CDC
M07799	A	Neg	—	CDC
M07801	A	Neg	—	CDC
M03389	A	Neg	—	CDC
M04656	A	Neg	—	CDC
M04239	A	Neg	—	CDC
M01421	A	Neg	—	CDC
WSLH B-1	B	Neg	—	WSLH
WSLH B-2	B	Neg	—	WSLH
WSLH B-3	B	Neg	—	WSLH
WSLH B-4	B	Neg	—	WSLH
WSLH B-5	B	Neg	—	WSLH
WSLH B-6	B	Neg	—	WSLH
WSLH B-7	B	Neg	—	WSLH
WSLH B-8	B	Neg	—	WSLH
WSLH B-9	B	Neg	—	WSLH
WSLH B-10	B	Neg	—	WSLH
ATCC 23254	B	Neg	—	ATCC
ATCC 13090	B	Neg	—	ATCC
NM00151	B	Neg	—	L.H. Harrison
NM00207	B	Neg	—	L.H. Harrison
NM00254	B	Neg	—	L.H. Harrison
NM00268	B	Neg	—	L.H. Harrison
NM00275	B	Neg	—	L.H. Harrison
NM00290	B	Neg	—	L.H. Harrison
NM00292	B	Neg	—	L.H. Harrison

NM00329	B	Neg	—	L.H. Harrison
NM01036	B	Neg	—	L.H. Harrison
NM01039	B	Neg	—	L.H. Harrison
NM02758	B	Neg	—	L.H. Harrison
WSLH C-1	C	Neg	—	WSLH
WSLH C-2	C	Neg	—	WSLH
WSLH C-3	C	Pos	5	WSLH
WSLH C-4	C	Pos	5	WSLH
WSLH C-5	C	Pos	5	WSLH
WSLH C-6	C	Pos	5	WSLH
WSLH C-7	C	Pos	5	WSLH
WSLH C-8	C	Neg	—	WSLH
WSLH C-9	C	Neg	—	WSLH
WSLH C-10	C	Neg	—	WSLH
NM00002	C	Neg	—	L.H. Harrison
NM00041	C	Neg	—	L.H. Harrison
NM00141	C	Neg	—	L.H. Harrison
NM00178	C	Neg	—	L.H. Harrison
NM00199	C	Neg	—	L.H. Harrison
NM00200	C	Neg	—	L.H. Harrison
NM00272	C	Neg	—	L.H. Harrison
NM00273	C	Neg	—	L.H. Harrison
NM00306	C	Neg	—	L.H. Harrison
NM00344	C	Neg	—	L.H. Harrison
NM01028	C	Neg	—	L.H. Harrison
ATCC 13102	C	Pos	2	ATCC
ATCC 13113	D	Neg	—	ATCC
98/250521	H	Pos	3	PHLS
97/252675	H	Pos	5	PHLS
WSLH W135-1	W135	Pos	3	WSLH
WSLH W135-2	W135	Neg	—	WSLH
WSLH W135-3	W135	Pos	3	WSLH
WSLH W135-4	W135	Pos	3	WSLH
43744	W135	Neg	—	L.H. Harrison
35559	W135	Neg	—	L.H. Harrison
NM00076	W135	Neg	—	L.H. Harrison
NM00079	W135	Neg	—	L.H. Harrison
NM00110	W135	Neg	—	L.H. Harrison
NM00112	W135	Neg	—	L.H. Harrison
NM00134	W135	Neg	—	L.H. Harrison
NM00174	W135	Neg	—	L.H. Harrison
NM02978	W135	Pos	3	L.H. Harrison
A22	W135	Pos	3	D. A. Caugant
$\alpha$ 275*	W135	Pos	4	—

WSLH X-2	X	Neg	—	WSLH
WSLH X-3	X	Neg	—	WSLH
NM00255	X	Neg	—	L.H. Harrison
NM00358	X	Neg	—	L.H. Harrison
M08210	X	Neg	—	CDC
M07186	X	Neg	—	CDC
M09664	X	Pos	5	CDC
M04222	X	Neg	—	CDC
M04370	X	Neg	—	CDC
M00328	X	Neg	—	CDC
M15113	X	Pos	1	CDC
WSLH Y-1	Y	Neg	—	WSLH
WSLH Y-2	Y	Neg	—	WSLH
WSLH Y-3	Y	Neg	—	WSLH
WSLH Y-4	Y	Neg	—	WSLH
WSLH Y-5	Y	Neg	—	WSLH
WSLH Y-6	Y	Neg	—	WSLH
WSLH Y-7	Y	Neg	—	WSLH
WSLH Y-8	Y	Neg	—	WSLH
WSLH Y-9	Y	Neg	—	WSLH
WSLH Y-10	Y	Neg	—	WSLH
NM00080	Y	Neg	—	L.H. Harrison
NM00083	Y	Neg	—	L.H. Harrison
NM00127	Y	Neg	—	L.H. Harrison
NM00130	Y	Neg	—	L.H. Harrison
NM00176	Y	Neg	—	L.H. Harrison
NM00190	Y	Neg	—	L.H. Harrison
NM00191	Y	Neg	—	L.H. Harrison
NM00211	Y	Neg	—	L.H. Harrison
NM00220	Y	Neg	—	L.H. Harrison
NM00233	Y	Neg	—	L.H. Harrison
NM00286	Y	Neg	—	L.H. Harrison
NM00293	Y	Neg	—	L.H. Harrison
NM02767	Y	Neg	—	L.H. Harrison
WSLH Z-1	Z	Neg	—	WSLH
WSLH Z-2	Z	Pos	5	WSLH
WSLH Z-3	Z	Neg	—	WSLH
NM00140	Z	Pos	5	L.H. Harrison
NM00183	Z	Pos	5	L.H. Harrison
00/240868	Z	Pos	5	PHLS
01/241422	Z	Pos	5	PHLS
01/241471	Z	Pos	1	PHLS

\* Only the draft genome sequence of  $\alpha$ 275 was used

Table S2. Primers used for cloning and Southern blotting

Name	Sequence (5'→3')	Target	Plasmid
SacI-195F	GCC CGA GCT CAT TCT GAC GCT CCT GCA TCT	<i>yea</i>	pSB2
SacII-64R	GAA TCC GCG GCC GAA CGT GAC CAA CTT CAG	<i>yecA</i>	
BamHI-kanF	CAG GAT CCA AAG CCA GTC CGC AGA AAC G	KanR and promoter	pKH99
BamHI-kanR	CTG GAT CCT GGG CGA AGA ACT CCA GCA T	KanR	
RpsL F	CAG CTC GAG TGA TTG TGA GGG ATG TCG G	<i>rpsL</i>	pNMD42
RpsL R	TTA GGT ACC GGC AGT ACG TCG CGC TTG G	<i>rpsL</i>	
SpeI-77F	CAT ACT AGT TAA CAG CAG ACG CTC CAT TC	upstream of GGI	pNMD37
dif-Sac 4R	ACT GAG CTC AGA TTG CAT AAT ATA CAT TAT GCG AAC T	<i>difB</i>	
PspOMI-183F	GAC TTC GGG CCC TTC AGC CTA TTG CGA GCT GT	<i>yfd</i>	pKL22
KpnI-144R	GCG GTA CCA TGT GCG TCA GTT TGC TCA G	<i>yfeA</i>	
pMOB F	GTA AAA CGA CGG CCA GTG AGC GCG	polylinker of pIDN	pKL19
SacI-pKL18 R	GTT CGA GCT CCG GAA TCG CCA CGA ACA AAC CC	<i>exbB</i>	
XmaI-pGCU R	CAT GCC CGG GAA CGT TGT TGC CAT TGC TGC	DUS sequence	pKL18
XmaI-pKL16 F	GAA CAA CCC GGG ATT TAG GCG GTT GTC GCG GCA GTT TGC	pKL16	pKL17
XmaI-pKL16 R	CAG GCA CCC GGG ATC CAT AAT TCG GGC ATA ATA ATA GC	pKL16 for tonB	
BamHI-tonB F	GAA GCG GAT CCT GAG CCA GCG GCA TCT GAT ACG	<i>NGO1380</i>	pKL16
PstI-tonB R	GAA GTC CTG CAG CGG AAT CGC CAC GAA CAA ACC C	<i>exbB</i>	
pKL13 F2	CTG GTA GCA GCA ATT TGG ATG	pKL13	pKL15
HindIII-pGCU R2	CCG CAT AAG CCA AGC TTA ACG TTG TTG CCA TTG CTG C	DUS sequence	
SacI-83R	GCG AGC TCA TCT CTA GGC TTC CAG TAG A	<i>yaa</i>	pKL13
53F	TAG ATG CGA TCA CCC TTA CG	<i>tral</i>	
235R	TTG TTC TGG ATA CAG CGT GCT AGC GG	<i>tral</i>	
247F	ATG ACA CTA AGA AGC TTG GG	<i>yaf</i>	

254R	CAT GCG GCG TTA TAA GCA CC
193F	CCG GTC AGT TGA AGT AAT CG
218R	AAA GTT TGG CGA GTC TCA GC
197F	TTC AGC CAG ATA CTG CTC AC
72R	TAG CGG TTT CTA ACC CTG AC

*oriT*

*ydhA*

*ydhB*

*yea*

*yeb*

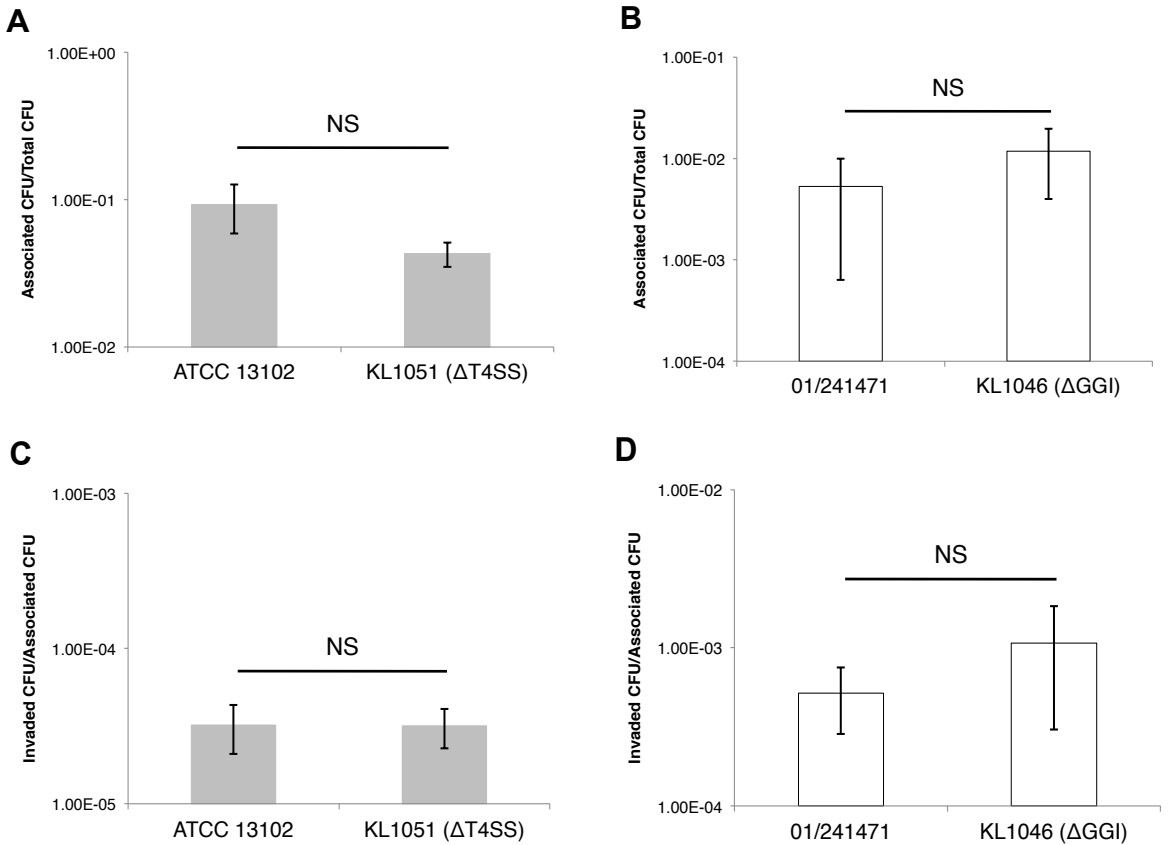
Table S3. Primers used in PCR walk of meningococcal GGIs

Primer pair	Sequence (5'-->3')	Target
77F-76R	TAA CAG CAG ACG CTC CAT TC	<i>difB</i> flanking DNA
	ATG GCG TTT CGT CAA CGT TT	<i>traD</i>
135F-118R	CCG GCC TTA TTC AAT AAC GG	<i>traD</i>
	AAC CGG TGA CAG CAG AAC AA	<i>traI</i>
255F-254R	TCA GAA ACT ATG GCT TCG AG	<i>traI</i>
	CAT GCG GCG TTA TAA GCA CC	<i>ltgX</i>
51F-254R	TAT CAA TTG TGC CGT AAC CC	<i>traI</i>
	CAT GCG GCG TTA TAA GCA CC	<i>ltgX</i>
53F-48R	ACG GCG GTA AGG TTG CGT CT	<i>traI</i>
	CCA AGC CAA TAA ATC GTC TG	<i>traL</i>
111F-46R	CCG CTG ACA AGC ATT AGT G	<i>traI</i>
	CTC AAG ATC ACC CTC CAT AA	<i>traK</i>
45F-124R	CCG CGA GCT ACT TAT TTC TT	<i>traE</i>
	TGC GGT ATA GTC TGT CAT GC	<i>dsbC</i>
55F-60R	ACG GCG GTA AGG TTG CGT CT	<i>traB</i>
	CCA AGC CAA TAA ATC GTC TG	<i>traC</i>
59F-58R	GAT TAG CGG TAT GGG TAA GG	<i>traB</i>
	TCA ACC TCT TTG TCG ATA GC	<i>traC</i>
119F-132R	GAC CAT TTG AGC GGA CTA TC	<i>traC</i>
	CAT ACG CCT AAT GCC AAT GC	<i>ybe</i>
89F-94R	GCC GTG TCC GTA AGT ACA AA	<i>ybe</i>
	AAT TTA GCG GCC TGC ACT TC	<i>traU</i>
35F-34R	GCA CAA AAA TAC CGC ACT GCC TCA C	<i>trbC</i>
	AAC GGT GCA GCA ATA ATC GGC AG	<i>traN</i>
221F-28R	AAG GTG GTA TGC GAG AAA TCA GG	<i>ybi</i>
	TCT AAC TGT TCC AAT TCC TGT GCG	<i>traN</i>
225F-TRAH4R	TCA GAT TGG TGC GTT GGG TTT CTC	<i>traN</i>
	CTG TGT ATT TGT CCT TAT CCC	<i>traH</i>
27F-18R	CGG TTG ACA ACG CGG ATA TTT CAG G	<i>traH</i> promoter
	CTC AGC AGC AGA ACG AAT CAA GG	<i>traG</i>
traG1F-PID2R	GCA ACT GAC TCA AGA CTG GGC	<i>traG</i>
	CTT TGG GCG ATA GTT ACG AG	<i>eppA</i>
PID3F-1105-8R	AAT CCG GTG CGA CTG TTA GT	<i>traG</i>
	GGA TTA CAA GTG ACA GCA TCC AAG	<i>yda</i>
CSHR-1105-6R	GGC TAA GAT GTA GAA AAA GCC CAG	<i>cspA</i>
	CCG AAA AAG CAT GGG ATT CTG	<i>ydbA</i>
1105-7F-1105-10R	GCC AAG TCC CGC CAA TTT ACT GTC	<i>yda</i>

	TTG ACC AGG AGG AAG GCG ACC	<i>ydbA</i>
248F-150R	ACT GAC TGG CAG CGA TGA CA	<i>ydcB-ydcA</i> intergenic region
	ATT GGT GCT CCT GTC GTT TG	<i>ydeA</i>
63F-196R	ATA AGG AAA CCC GCA GGT TG	<i>ydeA-ydd</i> intergenic region
	CTT CGG GAT GTA CGG CAT TT	<i>ydg-ydf</i> intergenic region
257F-256R	AAA TGC CGT ACA TCC CTA AG	<i>ydg-ydf</i> intergenic region
	TTT CTT CGC ACG TAC CTT CG	<i>ydhA</i>
105F-190R	CGA AGG TAC GTG CGA AGA AA	<i>ydhA</i>
	ACA GGG CAT CGG TAT ATC TC	<i>yea-ydi</i> intergenic region
81F-88R	TCC TTG GGT TCC TGT TTG AC	<i>ydi-ydhB</i> intergenic region
	GGC GCA TTC AGT AGC TCA AA	<i>yea</i>
147F-72R	CCA GCA TGT CAG CTT CTT TC	<i>yea</i>
	TAG CGG TTT CTA ACC CTG AC	<i>yeb-yea</i> intergenic region
149F-64R	GAA CAG GTC TTC CGC ATA AC	<i>yea</i>
	CCG AAC GTG ACC AAC TTC AG	<i>yecA</i>
155F-192R	CAG TCC AAA CCC GCC ATA TT	<i>yeb</i>
	GGA GGC ATA TTC CCA AAC TT	<i>yedA-yecB</i> intergenic region
155F-220R	CAG TCC AAA CCC GCC ATA TT	<i>yeb</i>
	GCG CCA CCT CTT ACT TAG AT	<i>yedA-yecB</i> intergenic region
219F-202R	TCA GGC GCG GAC AGC TTA AA	<i>yedA</i>
	TTA AAC CGG CCA CGT GAA GT	<i>yeg-yee</i> intergenic region
203F-142R	ACC TCC GAC GAT ACG AAG AT	<i>yeg-yee</i> intergenic region
	CCG ATA ACG ACA GCC AAA TG	<i>topB</i>
215F-160R	TTC GGG AAA CCT ACT ACA CC	<i>topB</i>
	TGT TAC CTG GGC ATT TGG TC	<i>topB</i>
99F-166R	GTA CCT GCA TCT GCC AAA CT	<i>topB</i>
	GTA TAA TGG CGG TCG GAT GA	<i>yfb</i>
117F-168R	GGC AGA ATC TCT TGC TCA AC	<i>yfb</i>
	ACA GCT CGC AAT AGG CTG AA	<i>yfd</i>
179F-144R	CAT TGC AGC AGT CTT CAG AG	<i>yfd</i>
	ATG TGC GTC AGT TTG CTC AG	<i>yfeB</i>
183F-120R	TTC AGC CTA TTG CGA GCT GT	<i>yfd</i>
	AAA CGG GAG CTA AGA GTG AG	<i>parB</i>
177F-106R	GCT TTG CGG TAA TAC CTG CT	<i>yfeA</i>
	CGC AGC ATT TGC TGC TAC AT	<i>parA</i>
259F-260R	CTC ACT CTT AGC TCC CGT TT	<i>parB</i>
	TAC GAG CCA TCA TCT ACG CAC AAG	<i>parA</i>
165F-hlhggiR	TGC GTA GAT GAT GGC TCG TA	<i>parA</i>



	CAG GCA AAC AGC TAT TTG AG	<i>ung</i>
83F-82R	TCT ACT GGA AGC CTA GAG AT	downstream of <i>yaa</i>
	ATC GGT GAC GAA TTC ATT CC	<i>traD</i>
137F-118R	CCA GGG TGG TGT TTA TCT AA	<i>traD</i>
	AAC CGG TGA CAG CAG AAC AA	<i>traI</i>
45F-46R	CCG CGA GCT ACT TAT TTC TT	<i>traE</i>
	CTC AAG ATC ACC CTC CAT AA	<i>traK</i>
113F-1105-6R	CTG CCT CAA GAC GCA ACT TA	<i>yag</i>
	CCG AAA AAG CAT GGG ATT CTG	<i>ydbA</i>
155F-106R	CAG TCC AAA CCC GCC ATA TT	<i>yeb</i>
	CGC AGC ATT TGC TGC TAC AT	<i>parA</i>
63F-148R	ATA AGG AAA CCC GCA GGT TG	<i>ydeA-ydd</i> intergenic region
	ACG TCT GAC CTT GCC AAA CAG AGA	<i>ydeB</i>



**Figure S1.** *N. meningitidis* do not exhibit T4SS-dependent differences in association to and invasion of ME180 epithelial cells. ME180 cells were infected with meningococci for 3.5 hrs at MOI=100. Supernatants and saponin lysates were collected and plated onto GCB agar to determine total CFU counts. Cells were extensively washed, lysed, and plated onto GCB agar to enumerate associated CFU. Additional wells were treated with 25  $\mu$ g/ml Gm for one hour, washed, lysed, and plated to determine the number of intracellular bacteria. (A) and (C) The association index is a ratio of associated CFU:total CFU. (B) and (D) The invasion index is a ratio of intracellular CFU:associated CFU. Columns represent the geometric mean of three independent experiments, each performed in triplicate. Error bars represent the standard error of the mean.  $p > 0.05$  by ANOVA and Tukey-Kramer method. NS-no significance.