Supplementary Materials:

Table S1: Antibiotic Disk Diffusion Assay (DDA) results for 4 commensal *Neisseria* species and 4 *N. gonorrhoeae* strains. Average (± SEM) diameter (mm) of the zones of inhibition from 3 biological replicates, of 3 independent experiments, were measured using ImageJ [2].

	Neisseria species							
ZoI (mm)	*Nla*	*Nci*	*Nmu*	*Nel*	*Ngo* FA19[a]	*Ngo* MS11[b]	*Ngo F89*[c]	*Ngo H041*[d]
Penicillin -10 UI	32.5 (±0.3)	29.8 (±0.6)	34.0 (±0.3)	29.7 (±0.3)	46.7 (±1.0)	26.9 (±0.8)	31.6 (±0.3)	24.4 (±0.3)
Ampicillin -10ug	31.6 (±1.0)	28.3 (±0.3)	29.2 (±1.0)	29.5 (±0.4)	42.5 (±0.4)	30.5 (±0.1)	34.2 (±0.5)	24.8 (±0.3)
Ceftriaxone -30ug	41.2 (±0.8)	30.1 (±1.1)	37.4 (±0.2)	32.4 (±0.5)	50.2 (±0.6)	37.2 (±0.7)	31.5 (±0.4)	30.8 (±0.3)
Azithromycin -15ug	24.6 (±0.7)	15.5 (±0.3)	21.4 (±0.3)	20.9 (±0.2)	35.9 (±0.8)	31.5 (±0.4)	33.0 (±1.1)	34.2 (±0.3)
Erythromycin -15ug	21.4 (±0.4)	14.9 (±0.2)	15.9 (±0.4)	18.0 (±1.0)	33.1 (±0.7)	26.0 (±0.5)	26.2 (±1.0)	28.4 (±0.4)
Chloramphenicol -30ug	35.1 (±1.2)	25.6 (±1.2)	27.3 (±0.4)	26.3 (±0.4)	42.2 (±0.5)	24.8 (±0.5)	29.7 (±0.6)	26.3 (±0.2)
Kanamycin -30ug	30.7 (±0.6)	21.5 (±0.1)	31.4 (±0.2)	23.1 (±0.3)	33.8 (±0.5)	27.8 (±0.6)	31.9 (±0.6)	32.2 (±0.3)
Streptomycin -10ug	18.7 (±0.3)	14.0 (±0.2)	19.7 (±0.4)	13.8 (±0.4)	16.9 (±0.5)	6.0 (±0.1)	15.2 (±0.4)	16.1 (±0.3)
Tetracycline -30ug	32.8 (±0.5)	25.6 (±0.4)	24.9 (±0.4)	25.3 (±0.2)	37.8 (±0.6)	28.0 (±0.4)	30.8 (±0.4)	29.7 (±0.3)
Gentamicin -10ug	19.9 (±0.3)	17.8 (±0.2)	21.8 (±0.3)	17.9 (±0.2)	20.2 (±0.2)	17.5 (±0.3)	18.2 (±0.4)	19.0 (±0.1)

Zones of inhibition (ZoI) of and similar (<20% diameter reduction) to *N. gonorrhoeae* FA19 are highlighted in grey. ZoI highlighted in pink are > 33% smaller than those of *N. gonorrhoeae* FA19, and denote high resistance levels. Intermediate species display ZoI smaller than those of *N. gonorrhoeae* FA19 by 20% to 33% and are highlighted in yellow. The antimicrobial disk measures 6 mm in diameter, which is the minimum ZoI measurable. Species: Nla, *N. lactamica*; Nci, *N. cinerea*; Nmu, *N. mucosa*; Nel, *N. elongata*; Ngo, *N. gonorrhoeae*

[a] *N. gonorrhoeae* FA19 is considered susceptible to most antibiotics presented, used as reference [3].

[b] *N. gonorrhoeae* MS11 is considered highly resistant to streptomycin [4].

[c] *N. gonorrhoeae* F89 is considered highly resistant to cefixime and ceftriaxone [5].

[d] *N. gonorrhoeae* H041 is considered highly resistant to ceftriaxone [6].

Table S2: Commensal *Neisseria* minimal inhibitory concentrations (MIC, µg/mL) to antibiotics (penicillin, azithromycin, ceftriaxone, erythromycin, chloramphenicol, gentamicin).

MIC (µg/mL)	Penicillin	Ceftriaxone	Azithromycin	Erythromycin	Chloramphenicol	Gentamicin
N. lactamica	0.25	0.008	0.25	2	0.5	2
N. cinerea	0.125-0.25	0.128	0.25-0.5	2	0.5	2
N. mucosa	2	0.064	0.5	8	4	2
N. elongata	0.5-1	0.064	0.5	4	1	8
N. gonorrhoeae FA19			0.063[a]	0.25[a]		4
N. gonorrhoeae Su[b]	≤ 0.06	≤ 0.015	≤ 0.5	NA	NA	4
N. gonorrhoeae Re[b]	≥ 2	≥ 0.25[c]	≥ 2[c]	NA	NA	8

MIC values 16-fold or higher compared to susceptible *N. gonorrhoeae*'s values are highlighted in pink, while values 4-8 fold higher are highlighted in yellow.

[a] N. gonorrhoeae FA19 values according to Zarantonelli *et al.* [7].

[b] *N. gonorrhoeae*'s MIC breakpoint (µg/mL) values according to CLSI [8].

[c] *N. gonorrhoeae*'s MIC values of wild-type distribution according to Kirkcaldy *et al.* [9].

Table S3: Known mutations confirmed or likely involved in antimicrobial resistance, with observed cognate mutations in commensal *Neisseria*

Antibiotic class	Antibiotic	gene	Known Mutation[a]	Observed Cognate Mutation in commensal *Neisseria*	Proportion present	Reference
Macrolide	Azithromycin, Erythromycin	rplV	I4L		0	[10]
Macrolide	Azithromycin, Erythromycin	rplV	L6Q	L6Q	9/11	[10]
Macrolide	Azithromycin, Erythromycin	rplV	K6Q		0	[10]
Macrolide	Azithromycin, Erythromycin	rplV	T72A	T72V	11/11	[10]
Macrolide	Azithromycin, Erythromycin	rplV	S101T	S101C	11/11	[10]
Macrolide	Azithromycin, Erythromycin	rplV	I103L		0	[10]
Macrolide	Azithromycin, Erythromycin	rplV	K83N		0	[10]
Macrolide	Azithromycin, Erythromycin	rplV	D94H	D94N	11/11	[10]
Macrolide	Azithromycin, Erythromycin	rplV	K98N	R99Q	11/11	[10]
Macrolide	Azithromycin, Erythromycin	rplV	V17I	V17A	11/11	[10]
Macrolide	Azithromycin, Erythromycin	rplV	L46Q	V45Q	11/11	[10]
Macrolide	Azithromycin, Erythromycin	rplD	V52I		0	[10]
Macrolide	Azithromycin, Erythromycin	rplD	V52L		0	[10]
Macrolide	Azithromycin, Erythromycin	rplD	A37S		0	[10]
Macrolide	Azithromycin, Erythromycin	rplD	K74T	K74S	26/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	D91E	D91N	26/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	V120I	V120A	9/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	V120I	V121I	10/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	K123S	K123A	14/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	K123S	K123S	1/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	K123S	K123D	1/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	K123S	K123E	14/26	[10]

Macrolide	Azithromycin, Erythromycin	rplD	D154E		0	[10]
Macrolide	Azithromycin, Erythromycin	rplD	T173N	T173Q	16/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	T173N	T173H	9/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	A190V	A190K	13/26	[10]
Macrolide	Azithromycin, Erythromycin	rplD	A190V	A190R	8/27	[10]
Macrolide	Azithromycin, Erythromycin	mtrR	A39T			[11]
Macrolide	Azithromycin, Erythromycin	mtrR	G45D			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	penA	F504L			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	penA	A510V			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	penA	A516G			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	penA	P551L			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	penA	I312M			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	penA	V316T			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	ponA	L421P			[11]
Beta-lactams/ Cephalosporin	Penicillin, Ampicillin, Ceftriaxone	porB	G120K		0	[11]
Tetracycline	Tetracycline	rpsJ	V57M		0	
Tetracycline	Tetracycline	tetM	plasmid mediated	Not observed	0	
Chloramphenicol	Chloramphenicol	CAT	plasmid mediated	Not observed	0	
Aminoglycoside	Streptomycin, Spectinomycin, Kanamycin	str		Not observed	0	
Aminoglycoside	Gentamicin	N/A		Not observed	0	

[a] Known mutations may have been identified in *N. gonorrhoeae* or in other model organisms such as *Escherichia coli*.

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