Whole genome sequencing reveals transmission of gonococcal antibiotic resistance among men who have sex with men: an observational study

SUPPLEMENTARY APPENDIX

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed using methods of the Australian Gonococcal Surveillance Programme (AGSP) and in accordance with the National Neisseria Network of Australia.[1 2] Interpretative criteria are listed in Table S1.

Table S1: AGSP Interpretative Criteria for *N. gonorrhoeae* MIC values

Antibiotic MIC (mg/L)	Sensitive	Less susceptible / Decreased susceptibility	Resistant
Penicillin	<0.06	0.06–0.5	>0.5 [#]
Ceftriaxone	<0.06	0.06–0.25	Not defined
Azithromycin	<1	Not defined	≥1
Ciprofloxacin	<0.06	0.06–0.5	>0.5
Spectinomycin	≤64	Not defined	>64

[#] All penicillinase-producing N. gonorrhoeae (PPNG) isolates are considered resistant to penicillin, irrespective of the MIC value

BIOINFORMATICS METHODS

Raw sequencing reads were processed using a customised pipeline to streamline and automate initial handling of sequence data (https://github.com/tseemann/nullarbor). Within this pipeline, Illumina Nextera adapters and low-quality sequence (Phred score <10) were trimmed from raw sequencing reads, with the trimmed sequences subsequently undergoing *de novo* assembly (*SPAdes* v3.7.1),[3] annotation (*Prokka* v1.12-beta),[4] MLST (https://github.com/tseemann/mlst), resistance gene detection (https://github.com/tseemann/abricate), and pan-genome analysis (*Roary* v3.6.6).[5] To generate an alignment of core genome single nucleotide polymorphisms (SNPs), sequence reads for each isolate were aligned to the NCCP11945 reference genome (GenBank accession NC_011035.1)[6] within the *Nullarbor* pipeline using *Snippy* v3.1 (https://github.com/tseemann/snippy). Sites that were covered by less than 10 reads were considered absent for that isolate. The remaining non-absent sites were considered to be a SNP if at least 90% of the reads covering that site were different to the reference. The 90% read concordance threshold was set to minimise sequencing error. Any site for which at least one of the isolates had a SNP and none of the isolates were absent, were considered as "core SNP" sites. These were used as input to determine the phylogenomic relatedness of the isolates. SNPs identified between isolates from within an individual or partnership were manually inspected and verified.

The Nullarbor makefile was generated with the following command:

```
$ nullarbor.pl --name partners.20160926 --mlst neisseria --ref
NCCP11945.gbk --input samples.tab --outdir partners.20160926 --accurate
```

Within Nullarbor, the following commands were run for each isolate:

```
# Trim reads with Trimmomatic
$ trimmomatic PE -threads $(CPUS) -phred33
/mnt/seq/MDU/READS/sample1/sample1_R1.fastq.gz
/mnt/seq/MDU/READS/sample1/sample1_R2.fastq.gz sample1/clipped_R1.fq.gz
/dev/null sample1/clipped_R2.fq.gz /dev/null
ILLUMINACLIP:conf/trimmomatic.fa:1:30:11 LEADING:10 TRAILING:10
MINLEN:30

# Basic QC estimation of sequencing yield and depth
$ fq --quiet --ref ref.fa sample1/clipped_R1.fq.gz
sample1/clipped_R2.fq.gz > sample1/yield.clean.tab

# QC check species and contamination with Kraken
$ kraken --threads $(CPUS) --preload --paired sample1/clipped_R1.fq.gz
sample1/clipped_R2.fq.gz | kraken-report > sample1/kraken.tab
```

```
# Read alignment with Snippy
$ snippy --cpus $(CPUS) --force --outdir sample1/sample1 --ref
NCCP11945.gbk --R1 sample1/R1.fq.gz --R2 sample1/R2.fq.gz

# De novo draft genome assembly with SPAdes
$ spades.py -o sample1/spades --careful --tmp-dir /tmp --only-assembler
-t $(CPUS) -k 21,33,55,77,97,107,117,127 -1 sample1/clipped_R1.fq.gz -2
sample1/clipped_R2.fq.gz

# Basic assembly QC
$ fa —e —t sample1/contigs.fa

# In silico detection of acquired resistance mechanisms
$ abricate sample1/contigs.fa > abricate.tab

# Draft genome auto-annotation with Prokka
$ prokka --centre X --compliant --force --fast --locustag sample1
--prefix sample1 --outdir sample1/prokka --cpus $(CPUS) --proteins
NCCP11945.gbk sample1/contigs.fa
```

In addition, the following were run on the subsequent output:

```
# Generate core genome SNP alignment and pseudo-whole-genome alignment
$ snippy-core sample1/sample1 sample2/sample2 ... sampleN/sampleN

# Generate table of pairwise SNP distances
$ afa-pairwise.pl core.aln > distances.tab

# Pan-genome analysis with Roary
$ roary -f sample1/roary -v -p $(CPUS) */prokka/*.gff

# Approximate maximum likelihood tree estimation with FastTree
$ FastTree —nt —gtr < core.aln > tree.newick
```

A summary report was generated for each set of isolates run through Nullarbor including quality control metrics, MLST, resistome and pan-genome comparison. NG-MAST and MLST results were inferred *in silico* with *NGMASTER*,[7] and *mlst* (https://github.com/tseemann/mlst) respectively:

```
# In silico MLST
$ mlst --scheme neisseria sample1/contigs.fa > mlst.tab

# In silico NG-MAST
$ ngmaster sample1/contigs.fa > ngmast.tab
```

The pan-genome output from *Roary* was formatted (https://github.com/kwongj/roary2fripan) for visualisation and inspected in *FriPan* (http://drpowell.github.io/FriPan/).

Resistance-determining regions of individual genomes were identified in *de novo* draft assemblies using *BLAST*.[8] Resistance gene sequences were extracted, aligned with *MUSCLE*,[9] and manually inspected in *Geneious* v10.0.5 (https://www.geneious.com/) for comparison between strains. Additionally, to verify the mutations detected in draft assemblies, sequence reads were aligned to the FA1090 reference genome (GenBank accession AE004969.1) using Snippy v3.2-dev. The resulting output (snps.tab file) was searched for resistance determinants:

```
# Run snippy to align reads to FA1090 reference
$ snippy --outdir sample1 --ref FA1090.gbk --pe1
sample1/clipped_R1.fq.gz --pe2 sample1/clipped_R2.fq.gz

# Search snps.tab file for resistance determinants
$ for f in */snps.tab ; do if grep NGO1812 $f | grep missense | grep
120/348 ; then echo $f ; fi ; done
```

To detect 23S allele mutations, sequencing reads were aligned to a mutated NCCP11945 reference genome with three copies of the 23S region masked to force alignment to a single locus. An in-house script (https://github.com/kwongj/ng23S-mutations) was used to examine the relative proportion of reads with the mutation and determine how many 23S copies had the mutation.

```
# Run snippy to align reads to masked NCCP11945 reference
$ snippy --outdir sample1-23S --ref NG_NCCP11945_23S-masked.gbk --pe1
sample1/clipped_R1.fq.gz --pe2 sample1/clipped_R2.fq.gz

# Run ng23S-mutations script to print results to stdout
$ ng23S-mutations.py sample1-23S sample2-23S ... sampleN-23S
```

For phylogenetic analysis, recombination was identified with *ClonalFrameML*,[10] and filtered using a custom python script (https://github.com/kwongj/cfml-maskrc) using the following commands:

```
# Resolve polytomies in the tree from Nullarbor
$ nw_multifurcation.py --nosupport tree.newick > core.tree

# Trim the pseudo-whole-genome alignment from Nullarbor
$ trim-aln.py --end 2232025 core.full.aln > core.chr.full.aln

# Run ClonalFrameML
$ ClonalFrameML core.tree core.chr.full.aln cfml –
ignore_incomplete_sites true

# Filter predicted regions of recombination from alignment
$ cfml-maskrc.py --aln core.chr.full.aln --out maskrc.full.aln cfml
```

A maximum likelihood phylogeny was inferred from the filtered alignment using *RAxML* v8.2.8[11] to compare isolates:

```
# Exclude invariant and non-core sites using snp-sites
$ snp-sites —c —o maskrc.core.aln maskrc.full.aln

# Run RAxML
$ raxmlHPC-PTHREADS -s maskrc.core.aln -n raxml.maskrc.tree -m GTRGAMMA
-f a -p 13579 -x 13579 -# 1000 -T 64
```

SUPPLEMENTARY RESULTS

A summary table of isolate and genome characteristics is provided in Supplementary Table S2. For each isolate, over 89% of the reference genome was covered by aligned reads for identifying SNPs. A total of 15,981 core SNP sites were identified, with 1619 genes identified as core genes shared by >99% of isolates from a total of 5229 putative genes.

SUPPLEMENTARY TABLES AND FIGURES

Table S2: List of 94 isolates included in the study

ID	Date collected	Couple	Partner	Source	Sex	Age yrs	NG-MAST	POR	TBPB	MLST	abcZ	adk	aroE	fumC	gdh	pdhC	pgm	Penicillin - MIC	Spectinomycin - MIC	Ceftriaxone - MIC	Ciprofloxacin - MIC	Tetracycline - MIC	Azithromycin - MIC	Beta-lactamase	penA	penA_allele	penB/porB1b	pilQ	ponA	mtr_promoter	mtrR	blaTEM	tetM	gyrA	parC	parE	16S rRNA	rpsE	23S rRNA	macA	macB	macAB promoter	norM	norM promoter	GGI
AUSMDU00005522	28/03/2006	05	A	Pharyngeal	M	24	40	30	35	-	126	39	67	78	150	153	65	0.06	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II*	penA_294					G45D									NEIS0488_655	NEIS0489_834		NEIS0763_93		yes
AUSMDU00005523	28/03/2006	05	B	Urethra	M	22	40	30	35	-	126	39	67	78	150	153	65	0.06	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II*	penA_294					G45D									NEIS0488_655	NEIS0489_834		NEIS0763_93		yes
AUSMDU00005524	27/04/2006	04	A	Rectal	M	26	-	new	1812	8123	59	39	170	78	148	153	65	0.25	<=64.0	<=0.03	4	<=8.0	0.25	Not detected	NonMosaic_II	penA_23	G120K, A121N			-A	G45D			S91F, D95G	D86N					NEIS0488_294	NEIS0489_338		NEIS0763_8		no
AUSMDU00005525	27/04/2006	04	B	Urethra	M	21	1419	912	33	1579	109	39	67	111	148	153	65	2	<=64.0	<=0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005526	22/05/2006	10	A	Urethra	M	28	210	59	4	8122	59	142	67	156	188	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_XXII	penA_20	A121S			+T										NEIS0488_460	NEIS0489_593		NEIS0763_338		yes
AUSMDU00005527	22/05/2006	10	A	Pharyngeal	M	28	210	59	4	8122	59	142	67	156	188	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	<=0.06	Not detected	NonMosaic_XXII	penA_20	A121S			+T										NEIS0488_460	NEIS0489_593		NEIS0763_338		yes
AUSMDU00005528	22/05/2006	10	A	Rectal	M	28	210	59	4	8122	59	142	67	156	188	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_XXII	penA_20	A121S			+T										NEIS0488_460	NEIS0489_593		NEIS0763_338		yes
AUSMDU00005529	22/05/2006	10	B	Urethra	M	21	210	59	4	8122	59	142	67	156	188	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_XXII	penA_20	A121S			+T										NEIS0488_460	NEIS0489_593		NEIS0763_338		yes
AUSMDU00005530	22/05/2006	10	B	Rectal	M	21	210	59	4	8122	59	142	67	156	188	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_XXII	penA_20	A121S			+T										NEIS0488_460	NEIS0489_593		NEIS0763_338		yes
AUSMDU00005531	27/06/2006	11	A	Urethra	M	21	1	1	24	1584	59	39	67	156	150	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II*	penA_294														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005532	27/06/2006	11	B	Pharyngeal	M	41	1	1	24	1584	59	39	67	156	150	153	65	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II*	penA_294														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005533	19/07/2006	01	A	Urethra	M	34	1419	912	33	1579	109	39	67	111	148	153	65	2	<=64.0	<=0.03	<=0.03	<=8.0	0.5	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005534	19/07/2006	01	A	Rectal	M	34	1419	912	33	1579	109	39	67	111	148	153	65	1	<=64.0	<=0.03	<=0.03	<=8.0	0.5	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005535	19/07/2006	01	B	Pharyngeal	M	24	1419	912	33	1579	109	39	67	111	148	153	65	2	<=64.0	<=0.03	<=0.03	<=8.0	0.5	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005536	19/07/2006	01	B	Rectal	M	24	1419	912	33	1579	109	39	67	111	148	153	65	2	<=64.0	<=0.03	<=0.03	<=8.0	0.5	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005537	14/11/2006	02	B	Urethra	M	34	147	53	21	1582	59	39	170	78	148	154	65	0.25	<=64.0	<=0.03	8	<=8.0	0.5	Not detected	NonMosaic_II	penA_23	G120K, A121D			-A	G45D			S91F, D95G	D86N					NEIS0488_294	NEIS0489_338		NEIS0763_8		no
AUSMDU00005538	14/11/2006	02	B	Pharyngeal	M	34	147	53	21	1582	59	39	170	78	148	154	65	0.25	<=64.0	<=0.03	8	<=8.0	0.5	Not detected	NonMosaic_II	penA_23	G120K, A121D			-A	G45D			S91F, D95G	D86N					NEIS0488_294	NEIS0489_338		NEIS0763_8		no
AUSMDU00005539	14/11/2006	02	A	Pharyngeal	M	36	147	53	21	1582	59	39	170	78	148	154	65	0.25	<=64.0	<=0.03	8	<=8.0	0.25	Not detected	NonMosaic_II	penA_23	G120K, A121D			-A	G45D			S91F, D95G	D86N					NEIS0488_294	NEIS0489_338		NEIS0763_8		no
AUSMDU00005540	14/11/2006	02	A	Rectal	M	36	147	53	21	1582	59	39	170	78	148	154	65	0.25	<=64.0	<=0.03	8	<=8.0	0.25	Not detected	NonMosaic_II	penA_23	G120K, A121D			-A	G45D			S91F, D95G	D86N					NEIS0488_294	NEIS0489_338		NEIS0763_8		no
AUSMDU00005541	11/02/2008	07	A	Pharyngeal	M	29	225	4	4	1901	109	39	170	111	148	153	65	2	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_283	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005542	11/02/2008	07	B	Urethra	M	30	225	4	4	1901	109	39	170	111	148	153	65	2	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_283	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005543	22/12/2008	14	A	Urethra	M	34	225	4	4	1901	109	39	170	111	148	153	65	>2.0	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_283	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005544	22/12/2008	14	A	Pharyngeal	M	34	225	4	4	1901	109	39	170	111	148	153	65	>2.0	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_283	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005545	22/12/2008	14	B	Rectal	M	33	225	4	4	1901	109	39	170	111	148	153	65	>2.0	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_283	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005546	15/01/2009	15	B	Rectal	M	17	-	new	29	1580	126	39	67	111	148	153	133	0.25	<=64.0	<=0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_II	penA_166	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		yes
AUSMDU00005547	15/01/2009	15	B	Pharyngeal	M	17	-	new	29	1580	126	39	67	111	148	153	133	0.25	<=64.0	<=0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_II	penA_166	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		yes
AUSMDU00005548	15/01/2009	15	A	Urethra	M	26	-	new	29	1580	126	39	67	111	148	153	133	0.25	<=64.0	<=0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_II	penA_166	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		yes
AUSMDU00005549	15/01/2009	15	A	Pharyngeal	M	26	-	new	29	1580	126	39	67	111	148	153	133	0.25	<=64.0	<=0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_II	penA_166	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		yes
AUSMDU00005550	30/06/2009	17	B	Pharyngeal	M	47	225	4	4	1901	109	39	170	111	148	153	65	2	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005551	30/06/2009	17	A	Rectal	M	UNK	225	4	4	1901	109	39	170	111	148	153	65	2	<=64.0	<=0.03	16	<=8.0	0.25	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005552	21/07/2009	18	B	Urethra	M	21	-	new	new	-	126	39	67	78	150	153	133	0.25	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II	penA_23														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005553	21/07/2009	18	B	Pharyngeal	M	21	-	new	new	-	126	39	67	78	150	153	133	0.25	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II	penA_23														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005554	21/07/2009	18	A	Pharyngeal	M	21	-	new	new	-	126	39	67	78	150	153	133	0.125	<=64.0	<=0.03	<=0.03	<=8.0	0.125	Not detected	NonMosaic_II	penA_23														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005555	20/04/2010	16	B	Rectal	M	35	-	new	33	1579	109	39	67	111	148	153	65	2	<=64.0	0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005556	20/04/2010	16	A	Urethra	M	26	-	new	33	1579	109	39	67	111	148	153	65	2	<=64.0	0.03	<=0.03	<=8.0	0.25	Not detected	NonMosaic_IX	penA_285	G120K, A121D		L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005557	10/05/2010	21	A	Urethra	M	25	2992	1808	29	9362	126	39	67	238	147	153	133	0.25	<=64.0	<=0.008	<=0.03	<=8.0	0.5	Not detected	NonMosaic_II	penA_166														NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005558	10/05/2010	21	B	Rectal	M	21	2992	1808	29	9362	126	39	67	238	147	153	133	0.25	<=64.0	<=0.008	<=0.03	<=8.0	0.5	Not detected	NonMosaic_II	penA_166														NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005559	16/08/2010	09	B	Urethra	M	25	5268	19	25	10317	126	142	67	156	230	153	65	1	<=64.0	<=0.008	<=0.03	<=8.0	0.25	Detected	NonMosaic_II*	penA_294						blaTEM-1B								NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005560	16/08/2010	09	A	Pharyngeal	M	27	5268	19	25	10317	126	142	67	156	230	153	65	1	<=64.0	<=0.008	<=0.03	<=8.0	0.25	Detected	NonMosaic_II*	penA_294						blaTEM-1B								NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005561	21/10/2010	34	A	Rectal	M	22	2992	1808	29	9363	126	39	67	238	148	153	133	0.25	<=64.0	<=0.008	<=0.03	<=8.0	0.5	Not detected	NonMosaic_II	penA_166				A>C															no
AUSMDU00005562	21/10/2010	34	B	Rectal	M	20	2992	1808	29	9363	126	39	67	238	148	153	133	0.25	<=64.0	<=0.008	<=0.03	<=8.0	0.5	Not detected	NonMosaic_II	penA_166				A>C															no
AUSMDU00005563	15/02/2011	19	B	Urethra	M	27	2992	1808	29	9363	126	39	67	238	148	153	133	0.125	<=64	<=0.008	<=0.03	<=4.0	1	Not detected	NonMosaic_II	penA_166				A>C										NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005564	15/02/2011	19	A	Rectal	M	52	2992	1808	29	9363	126	39	67	238	148	153	133	0.125	<=64	<=0.008	<=0.03	<=4.0	0.5	Not detected	NonMosaic_II	penA_166				A>C										NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005565	20/07/2011	22	B	Urethra	M	21	-	new	267	1584	59	39	67	156	150	153	65	0.125	<=64	<=0.008	<=0.03	<=4.0	0.125	Not detected	NonMosaic_II*	penA_294														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005566	20/07/2011	22	B	Rectal	M	21	-	new	267	1584	59	39	67	156	150	153	65	0.125	<=64	<=0.008	<=0.03	<=4.0	0.25	Not detected	NonMosaic_II*	penA_294														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005567	20/07/2011	22	A	Pharyngeal	M	21	-	new	267	1584	59	39	67	156	150	153	65	0.125	<=64	<=0.008	<=0.03	<=4.0	0.25	Not detected	NonMosaic_II*	penA_294														NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005568	24/11/2011	08	A	Urethra	M	45	6940	4143	29	9363	126	39	67	238	148	153	133	0.25	<=64	<=0.008	<=0.03	<=4.0	0.5	Not detected	NonMosaic_II	penA_166														NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005569	24/11/2011	08	B	Rectal	M	30	6940	4143	29	9363	126	39	67	238	148	153	133	0.25	<=64	<=0.008	<=0.03	<=4.0	0.5	Not detected	NonMosaic_II	penA_166														NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005570	7/02/2012	24	A	Urethra	M	21	1407	908	110	1901	109	39	170	111	148	153	65	2	<=64	0.06	16	<=4.0	0.25	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005571	7/02/2012	24	B	Rectal	M	32	1407	908	110	1901	109	39	170	111	148	153	65	2	<=64	0.06	16	<=4.0	0.25	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005572	5/03/2012	25	B	Urethra	M	33	-	new	703	9364	435	39	67	158	148	71	65	0.5	<=64	0.016	16	>8.0	0.125	Not detected	NonMosaic_V**	penA_228	G120K, A121G		L421P				tet(M)-5	S91F, D95G	S87N, E91K					NEIS0488_298	NEIS0489_339		NEIS0763_50		no
AUSMDU00005573	5/03/2012	25	A	Rectal	M	23	-	new	703	9364	435	39	67	158	148	71	65	1	<=64	0.016	16	>8.0	0.125	Not detected	NonMosaic_V**	penA_228	G120K, A121G		L421P				tet(M)-5	S91F, D95G	S87N, E91K					NEIS0488_298	NEIS0489_339		NEIS0763_50		no
AUSMDU00005574	10/04/2012	23	B	Urethra	M	22	359	301	29	8126	126	39	67	78	148	153	133	1	<=64	0.016	<=0.03	<=4.0	0.125	Not detected	NovelMosaic_XLI	penA_357	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005575	10/04/2012	23	B	Rectal	M	22	359	301	29	8126	126	39	67	78	148	153	133	1	<=64	0.016	<=0.03	<=4.0	0.125	Not detected	NovelMosaic_XLI	penA_357	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005576	10/04/2012	23	A	Pharyngeal	M	30	359	301	29	8126	126	39	67	78	148	153	133	1	<=64	0.016	<=0.03	<=4.0	0.125	Not detected	NovelMosaic_XLI	penA_357	A121S				G45D									NEIS0488_12	NEIS0489_287		NEIS0763_8		no
AUSMDU00005577	3/08/2012	13	B	Rectal	M	27	8120	4834	33	1579	109	39	67	111	148	153	65	1	<=64	0.016	<=0.03	<=4.0	0.25	Not detected	NonMosaic_IX	penA_285			L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005578	3/08/2012	13	A	Urethra	M	27	8120	4834	33	1579	109	39	67	111	148	153	65	1	<=64	0.016	<=0.03	<=4.0	0.25	Not detected	NonMosaic_IX	penA_285			L421P	-A										NEIS0488_296	NEIS0489_340		NEIS0763_8		yes
AUSMDU00005579	18/02/2013	28	B	Urethra	M	29	3550	4	49	1901	109	39	170	111	148	153	65	1	<=64	0.016	16	<=4.0	0.125	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005580	18/02/2013	28	B	Pharyngeal	M	29	3550	4	49	1901	109	39	170	111	148	153	65	1	<=64	0.016	16	<=4.0	0.125	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005581	18/02/2013	28	A	Pharyngeal	M	23	3550	4	49	1901	109	39	170	111	148	153	65	2	<=64	0.03	16	<=4.0	0.25	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005582	18/02/2013	28	A	Rectal	M	23	3550	4	49	1901	109	39	170	111	148	153	65	2	<=64	0.016	16	<=4.0	0.25	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005583	18/02/2013	28	A	Rectal	M	23	3550	4	49	1901	109	39	170	111	148	153	65	1	<=64	0.03	16	<=4.0	0.25	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005584	18/02/2013	28	B	Rectal	M	29	3550	4	49	1901	109	39	170	111	148	153	65	1	<=64	0.03	16	<=4.0	0.25	Not detected	NonMosaic_XII	penA_21	G120K, A121D		L421P	-A				S91F, D95G	S87R					NEIS0488_292	NEIS0489_336		NEIS0763_207		yes
AUSMDU00005585	8/04/2013	27	B	Rectal	M	25	5268	19	25	10317	126	142	67	156	230	153	65	1	<=64	<=0.008	<=0.03	<=4	0.125	Detected	NonMosaic_II*	penA_294						blaTEM-1B								NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005586	8/04/2013	27	A	Urethra	M	21	5268	19	25	10317	126	142	67	156	230	153	65	2	<=64	<=0.008	<=0.03	<=4	0.125	Detected	NonMosaic_II*	penA_294						blaTEM-1B								NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005587	8/04/2013	27	A	Pharyngeal	M	21	5268	19	25	10317	126	142	67	156	230	153	65	2	<=64	<=0.008	<=0.03	<=4	0.125	Detected	NonMosaic_II*	penA_294						blaTEM-1B								NEIS0488_301	NEIS0489_345		NEIS0763_11		yes
AUSMDU00005588	22/04/2013	03	A	Urethra	M	30	4822	1903	29	1901	109	39	170	111	148	153	65	0.5	<=64	0.03	4	<=4	<=0.06	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005589	22/04/2013	03	B	Rectal	M	33	4822	1903	29	1901	109	39	170	111	148	153	65	0.5	<=64	0.03	4	<=4	<=0.06	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005590	22/04/2013	03	B	Pharyngeal	M	33	4822	1903	29	1901	109	39	170	111	148	153	65	1	<=64	0.03	4	<=4	<=0.06	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005591	29/04/2013	06	A	Urethra	M	55	4822	1903	29	1901	109	39	170	111	148	153	65	2	<=64	0.06	16	<=4	0.25	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005592	29/04/2013	06	B	Pharyngeal	M	46	4822	1903	29	1901	109	39	170	111	148	153	65	2	<=64	0.06	8	<=4	0.125	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005593	29/04/2013	06	B	Rectal	M	46	4822	1903	29	1901	109	39	170	111	148	153	65	2	<=64	0.06	8	<=4	0.125	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N		L421P	-A				S91F, D95G	S87R					NEIS0488_12	NEIS0489_12		NEIS0763_11		yes
AUSMDU00005594	3/06/2013	30	A	Urethra	M	22	8602	5093	5	-	59	39	67	78	682	153	65	2	<=64	<=0.008	2	>8	<=0.06	Detected	NonMosaic_II*	penA_294			L421P			blaTEM-1B	tet(M)-12	S91F, D95G	D86N						NEIS0489_81		NEIS0763_327		no
AUSMDU00005595	3/06/2013	30	B	Rectal	M	33	8602	5093	5	-	59	39	67	78	682	153	65	1	<=64	<=0.008	2	>8	<=0.06	Detected	NonMosaic_II*	penA_294			L421P			blaTEM-1B	tet(M)-12	S91F, D95G	D86N						NEIS0489_81		NEIS0763_327		no

AUSMDU00005596	3/06/2013	30	B	Pharyngeal	M	33	8602	5093	5	-	59	39	67	78	682	153	65	1	<=64	<=0.008	2	>8	<=0.06	Detected	NonMosaic_II*	penA_294		L421P		blaTEM-1B	tet(M)-12	S91F, D95G	D86N			NEIS0489_81	NEIS0763_327	no
AUSMDU00005597	3/10/2013	29	B	Pharyngeal	M	31	-	new	563	7363	59	39	67	78	148	153	65	0.5	<=64	0.03	8	<=4	0.125	Not detected	NonMosaic_IX*	penA_645	G120K, A121D	L421P	-A			S91F, D95G	E91G	NEIS0488_635	NEIS0489_808	NEIS0763_332	yes
AUSMDU00005598	3/10/2013	29	B	Rectal	M	31	-	new	563	7363	59	39	67	78	148	153	65	0.5	<=64	0.03	8	<=4	0.125	Not detected	NonMosaic_IX*	penA_645	G120K, A121D	L421P	-A			S91F, D95G	E91G	NEIS0488_635	NEIS0489_808	NEIS0763_332	yes
AUSMDU00005599	3/10/2013	29	A	Rectal	M	33	-	new	563	7363	59	39	67	78	148	153	65	0.5	<=64	0.03	8	<=4	0.125	Not detected	NonMosaic_IX*	penA_645	G120K, A121D	L421P	-A			S91F, D95G	E91G	NEIS0488_635	NEIS0489_808	NEIS0763_332	yes
AUSMDU00005600	30/12/2013	26	A	Urethra	M	20	8030	4786	563	7363	59	39	67	78	148	153	65	0.5	<=64	0.03	8	<=4	0.125	Not detected	NonMosaic_IX*	penA_645	G120K, A121D	L421P	-A			S91F, D95G	E91G	NEIS0488_635	NEIS0489_808	NEIS0763_332	yes
AUSMDU00005601	31/12/2013	31	A	Urethra	M	27	10162	5957	188	8123	59	39	170	78	148	153	65	0.125	<=64	<=0.008	<=0.03	>8	<=0.06	Not detected	NonMosaic_II	penA_166					tet(M)-5			NEIS0488_514	NEIS0489_804	NEIS0763_11	no
AUSMDU00005602	31/12/2013	31	B	Rectal	M	33	10162	5957	188	8123	59	39	170	78	148	153	65	0.125	<=64	<=0.008	<=0.03	>8	<=0.06	Not detected	NonMosaic_II	penA_166					tet(M)-5			NEIS0488_514	NEIS0489_804	NEIS0763_11	no
AUSMDU00005603	30/12/2013	26	B	Rectal	M	24	8030	4786	563	7363	59	39	67	78	148	153	65	0.5	<=64	0.03	4	<=4	0.125	Not detected	NonMosaic_IX*	penA_645	G120K, A121D	L421P	-A			S91F, D95G	E91G	NEIS0488_12	NEIS0489_808	NEIS0763_332	yes
AUSMDU00005604	3/07/2014	32	A	Pharyngeal	M	19	1614	1053	33	7828	126	39	67	158	148	153	133	0.25	<=64	<=0.008	1	<=4	<=0.06	Not detected	NonMosaic_IX	penA_285	G120K, A121D	L421P	-A			S91F, D95G		NEIS0488_70	NEIS0489_842	NEIS0763_332	no
AUSMDU00005605	3/07/2014	32	A	Rectal	M	19	1614	1053	33	7828	126	39	67	158	148	153	133	0.25	<=64	<=0.008	1	<=4	<=0.06	Not detected	NonMosaic_IX	penA_285	G120K, A121D	L421P	-A			S91F, D95G		NEIS0488_70	NEIS0489_842	NEIS0763_332	no
AUSMDU00005606	3/07/2014	32	B	Urethra	M	43	1614	1053	33	7828	126	39	67	158	148	153	133	0.25	<=64	0.016	1	<=4	<=0.06	Not detected	NonMosaic_IX	penA_285	G120K, A121D	L421P	-A			S91F, D95G		NEIS0488_70	NEIS0489_842	NEIS0763_332	no
AUSMDU00005607	14/08/2014	20	A	Rectal	M	50	8709	2656	1579	-	126	39	-	238	148	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.25	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005608	14/08/2014	20	A	Pharyngeal	M	50	8709	2656	1579	-	126	39	-	238	148	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.25	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005609	14/08/2014	20	B	Urethra	M	42	8709	2656	1579	-	126	39	-	238	148	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.25	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005610	8/09/2014	12	A	Urethra	M	26	9654	1808	1744	-	126	39	170	238	-	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.125	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005611	8/09/2014	12	A	Rectal	M	26	9654	1808	1744	-	126	39	170	238	-	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.25	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005612	8/09/2014	12	B	Pharyngeal	M	22	9654	1808	1744	-	126	39	170	238	-	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.25	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005613	8/09/2014	12	B	Rectal	M	22	9654	1808	1744	-	126	39	170	238	-	153	133	0.25	<=64	<=0.008	<=0.03	<=4	0.25	Not detected	NonMosaic_II	penA_166								NEIS0488_12	NEIS0489_287	NEIS0763_8	no
AUSMDU00005614	22/09/2014	33	A	Urethra	M	35	1407	908	110	7360	109	39	170	78	148	153	65	1	<=64	0.03	8	<=4	0.25	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N	L421P	-A			S91F, D95G	S87R	NEIS0488_12	NEIS0489_12	NEIS0763_327	yes
AUSMDU00005615	22/09/2014	33	B	Rectal	M	24	1407	908	110	7360	109	39	170	78	148	153	65	1	<=64	0.06	8	<=4	0.25	Not detected	Mosaic_XXXIV	penA_266	G120K, A121N	L421P	-A			S91F, D95G	S87R	NEIS0488_12	NEIS0489_12	NEIS0763_327	yes

Table S3: Comparison between distinguishable partner isolates

Partner	**4A**	**4B**	**25A**	**25B**	**26A**	**26B**
Site	Urethra	Rectum	Urethra	Rectum	Urethra	Rectum
Susceptibility						
Penicillin MIC (mg/L)	2·0	0·25	0·5	1·0	0·5	0·5
PPNG	ND	ND	ND	ND	ND	ND
Ceftriaxone MIC (mg/L)	0·03	0·03	0·016	0·016	0·03	0·03
Azithromycin MIC (mg/L)			0·125	0·125	0·125	0·125
Ciprofloxacin MIC (mg/L)	≤ 0·03	4	16	16	8	4
Spectinomycin MIC (mg/L)	≤ 64	≤ 64	≤ 64	≤ 64	≤ 64	≤ 64
Typing						
NG-MAST	1419	New	New	New	8030	8030
MLST	1579	8123	9364	9364	7363	7363
WGS comparison						
Number of predicted CDS	2055	2088	2050	2035	2084	2107
SNPs unfiltered	4063	4063	163	163	73	73
SNPs filtered	1498	1498	1	1	25	25

MIC = minimum inhibitory concentration

PPNG = penicillinase-producing *N. gonorrhoeae*

ND = not detected

NG-MAST = N. gonorrhoeae multi-antigen sequence typing

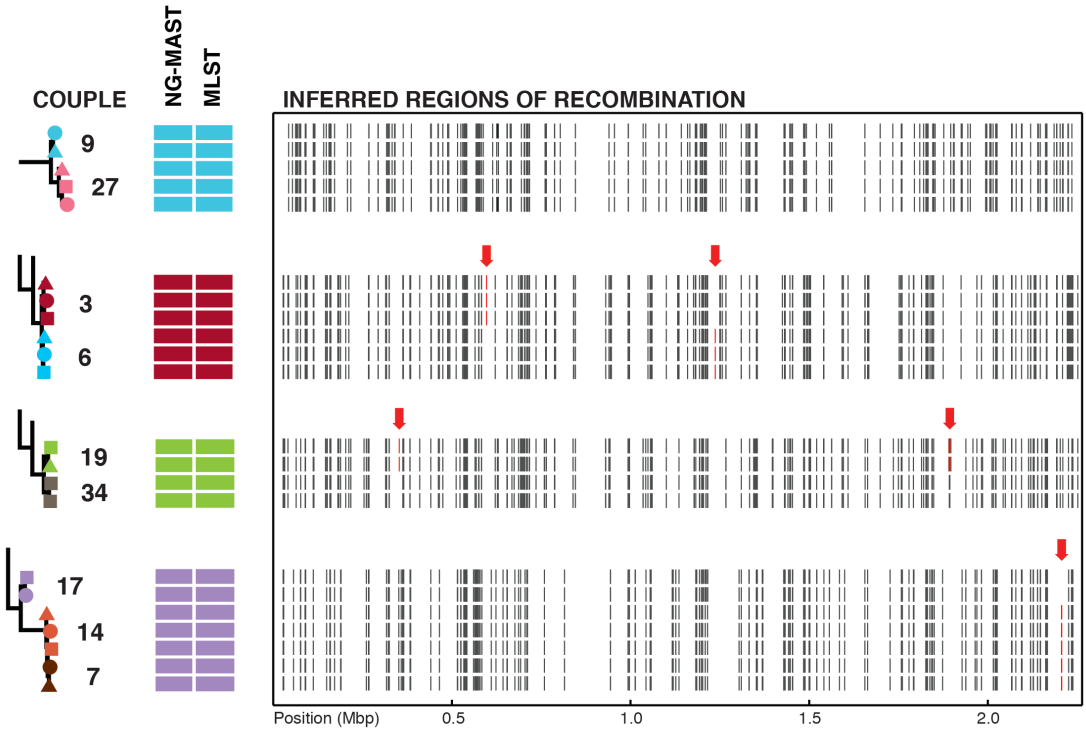
MLST = multi-locus sequence typing

WGS = whole-genome sequencing

CDS = coding DNA sequences

SNP = single nucleotide polymorphism

Figure S1: Comparison of four instances where couples had isolates with the same NG-MAST and MLST as another couple. The portions of the tree on the left illustrate the phylogenetic relationships between the isolates. The panel on the right is a heat map of recombinant regions represented by grey vertical lines at the corresponding position across the *N. gonorrhoeae* genome. Red arrows and lines indicate regions where recombination patterns were different between the couples in each instance.



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