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Antimicrobial resistance and molecular epidemiology of *Neisseria gonorrhoeae* in New Zealand, 2018-2019

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1. EXECUTIVE SUMMARY

The rise in antimicrobial resistance in *Neisseria gonorrhoeae* is of major public health importance. Of particular concern to New Zealand are international reports of isolates resistant to first line antibiotic treatment options, ceftriaxone and azithromycin, and the resultant change to treatment guidelines in both the UK (2018) and the US (2020) to single agent ceftriaxone. Such resistant strains may also impact New Zealand's ability to treat gonococcal infections in the future, and highlight the importance of continuing surveillance of this pathogen to help guide empiric treatment options, detect changes in antimicrobial resistance, identify dominant strains over time, and inform appropriate public health interventions. The current survey was undertaken between November 2018 to March 2019, and describes (1) the phenotypic antimicrobial susceptibility of *N. gonorrhoeae*, (2) the genotypic determinants associated with antimicrobial resistance, (3) the *N. gonorrhoeae* multi-antigen sequence type (NG-MAST) of all isolates, (4) the population structure of gonococci in New Zealand, and (5) changes in diversity since the previous *N. gonorrhoeae* survey that was carried out in 2014/2015.

A total of 344 non-duplicate *N. gonorrhoeae* isolates were received, with the majority submitted by laboratories in the Northern health region (170, 49.4%). Most isolates were obtained from males (74.1%) and from cases between 25 and 29 years of age (28.5%). Among males, 76.3% of isolates were cultured from urethral specimens, whereas in females most samples were from cervical or vaginal sites (89.8%).

Susceptibility testing to azithromycin, cefixime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, penicillin, spectinomycin and tetracycline was performed. Two (0.6%) isolates displayed decreased ceftriaxone susceptibility (minimum inhibitory concentration (MIC) ≥0.06 mg/L), three (0.9%) had decreased cefixime susceptibility (MIC ≥0.12 mg/L) and six isolates (1.7%) had an azithromycin MIC >1 mg/L. Two isolates had decreased susceptibility to both ceftriaxone and cefixime. There were no isolates with decreased susceptibility or resistance to both ceftriaxone and azithromycin. Rates of resistance to the other antibiotics tested were: ciprofloxacin, 23.8%; penicillin, 8.1%; spectinomycin, 0.0%; and tetracycline, 34.6%. There are no interpretive standards for ertapenem and gentamicin, but the MIC_{50} and MIC_{90} values for ertapenem were 0.016 mg/L and 0.03 mg/L, respectively, and for gentamicin both the MIC_{50} and MIC_{90} values were 8 mg/L.

A subset of 314/344 isolates were randomly selected to undergo Illumina-based sequencing to identify genotypic antimicrobial resistance determinants and to derive the *N. gonorrhoeae*

multi-antigen sequence type (NG-MAST). There was good correlation between genotypic and phenotypic susceptibility results and eighty-nine unique NG-MAST types were identified. The most common NG-MAST types comprised ST21017 (29.2%), ST13489 (26, 8.3%), ST21010 (21, 6.7%) and ST2400 (19, 6.1%), which differed from the 2014/2015 *N. gonorrhoeae* survey. The common NG-MAST types were found in all health regions except for ST2400 that was only found in the upper North island and ST12526 that was only found in the Southern health region.

Importantly, ST1407 and ST1903, associated with decreased ceftriaxone susceptibility and multidrug resistance, were not represented in the dataset. No novel resistance determinants were found, although one isolate with phenotypic resistance to azithromycin did not have a genotypic mechanism known to confer resistance identified.

Hierarchical cluster analysis identified eight subgroups in the 314 isolates sequenced. Each cluster consisted of isolates from both males and females, suggesting transmission between sexual risk groups. However, some clusters were dominated by isolates from male patients. Phylogenies of isolates provided evidence of a diverse population, with similar levels of diversity observed in both national surveys (2014/2015 and 2018/2019).

Overall, the rates of resistance found in this survey were similar to that observed in 2014/2015 and were generally lower than those reported by the Australian Gonococcal Surveillance Programme (AGSP). However, ongoing periodic surveillance is recommended given the rising rates of ceftriaxone and azithromycin resistance observed internationally.

2. INTRODUCTION

Antimicrobial resistance in *Neisseria gonorrhoeae* has been identified as a global public health threat by agencies such as the Centers for Disease Control and Prevention and the World Health Organization [1,2]. There are now a limited number of available treatment options for gonorrhoea, the sexually transmitted infection caused by this pathogen [3]. In many countries, including New Zealand, dual antibiotic therapy with ceftriaxone plus azithromycin is the recommended treatment [4], but there are increasing reports worldwide of isolates with resistance to ceftriaxone and azithromycin, along with treatment failures [5–7]. Concern about the impact of dual therapy on antimicrobial resistance in *N. gonorrhoeae* and co-occurring bacterial species has led to the recent change to single agent ceftriaxone as the recommended treatment for uncomplicated gonorrhea in both the UK and US [8,9].

In New Zealand, sexual health care is provided by specialist sexual health clinics and general practitioners across 20 District Health Boards (DHBs). Gonorrhoea is a notifiable infectious disease, and health practitioners are required to notify cases using an anonymised internet-based questionnaire in order to collect enhanced data, such as sexual behaviour, for notified cases. In addition, laboratory-based surveillance requires laboratories to submit *N. gonorrhoeae* testing data, including susceptibility data if available, to the Institute of Environment Science and Research Ltd (ESR) every month via a secure web-based portal. ESR publishes notification and surveillance data quarterly in a dashboard format. Dashboard data includes region, age, sex and ethnicity for all notifications, and sexual exposure data for approximately 35% of notifications. However, the widespread use of nucleic acid-based diagnosis of *N. gonorrhoeae* has limited the availability of cultured isolates for antimicrobial susceptibility testing and molecular characterisation [10]. Since the beginning of 2016, rates of gonorrhoea have increased from 70 per 100,000 population to 116 per 100,000 population in the year ending 31 March, 2019 [11].

To provide more detailed phenotypic and genotypic information for gonococcal strains causing infections in New Zealand, a survey of *N. gonorrhoeae* was undertaken by ESR for the first time in 2014/2015. Prior to this survey, ESR regularly collated national antimicrobial susceptibility data, but did not receive the isolates for additional susceptibility testing or sequencing. In the 2014/2015 ‘national’ survey, gonococci circulating in regions outside the greater Auckland area were under-represented [12]. The 2014/2015 survey found very few isolates with decreased susceptibility to ceftriaxone, of which none belonged to the globally disseminated ceftriaxone resistant NG-MAST clone ST1407 [13]. However, of concern was the

finding of isolates resistant (1.7%) or intermediate resistance (9.4%) to azithromycin, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints at that time. This is above the World Health Organisation (WHO) recommended limit of 5% resistance to recommended treatments [14]. EUCAST clinical breakpoints have since removed any resistant categorisation for *N. gonorrhoeae* and azithromycin, and instead include only the epidemiological cut-off (ECOFF) of 1 mg/L.

The aims of the current survey were to:

- provide information on phenotypic antimicrobial resistance among *N. gonorrhoeae*, including to second line antimicrobials, such as spectinomycin, gentamicin and ertapenem,
- *in silico* typing of *N. gonorrhoeae* based on whole genome sequencing of isolates to determine prevalent NG-MAST types,
- undertake a detailed analysis of the genetic determinants of antimicrobial resistance and correlate with phenotypic susceptibility data, and
- investigate the population structure of *N. gonorrhoeae* in New Zealand.

3. METHODS

3.1 ISOLATES AND PATIENT INFORMATION

All diagnostic laboratories were requested to refer all *N. gonorrhoeae* isolates cultured between 5 November 2018 and 10 March 2019 to ESR for the survey. Participating laboratories are listed in Table S1. Epidemiological data such as the national health index (NHI) number, sex, date of birth, and the site of the specimen were supplied with each isolate. The patient's domicile, and therefore DHB, was inferred from the location of the referring laboratory unless the referring laboratory specified otherwise. The three DHBs (Waitemata, Auckland and Counties Manukau) in the greater Auckland area were grouped together as the patient's specific DHB could not be inferred based on the referring laboratory. For similar reasons, the two DHBs (Capital & Coast and Hutt Valley) in the greater Wellington area were also grouped together. Enhanced data collected as part of the ESR gonorrhoea notification system was matched with the isolates ESR received as part of the laboratory-based survey using NHI and date of birth.

3.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility to azithromycin, cefixime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, penicillin, spectinomycin and tetracycline was determined by agar dilution according to the methods of the Clinical and Laboratory Standards Institute [15]. Ertapenem was purchased from TokuE (Bellingham, Washington, United States). All other antibiotic pure substances were purchased from Sigma-Aldrich (Saint Louis, Missouri, United States). β-lactamase production was determined using the chromogenic cephalosporin nitrocefin.

EUCAST clinical breakpoints [16] were used to interpret antimicrobial susceptibility data (Table 1). No interpretive standards were available for ertapenem or gentamicin. Decreased susceptibility to ceftriaxone was defined as MIC ≥0.06 mg/L and to cefixime was MIC ≥0.12 mg/L. These definitions are in line with the Australian Gonococcal Surveillance Programme (AGSP) but are lower than the EUCAST clinical breakpoint for resistance. The MIC_{50} and MIC_{90} values were defined as the MICs at which at least 50% and 90%, respectively, of isolates were inhibited.

Table 1: MIC breakpoints used to interpret antibiotic susceptibility results.

Antimicrobial	MIC breakpoint for susceptibility (mg/L)	MIC breakpoint for resistance (mg/L)
Azithromycin	≤1*	> 1*
Cefixime	≤0.12	>0.12
Ceftriaxone	≤0.12	>0.12
Ciprofloxacin	≤0.03	>0.06
Penicillin	≤0.06	>1
Spectinomycin	≤64	>64
Tetracycline	≤0.5	>1

* This is the ECOFF for detection of resistant mechanisms to azithromycin

3.3 EPIDEMIOLOGICAL DATA ANALYSIS

Based on prior research [12] we assumed the most prevalent resistance would be found in approximately 30% of isolates. In turn, a sample size greater than 303 was needed to detect resistance with 95% precision and characterise the population. A sample size of 320 isolates was targeted to allow for some inadequate samples during a four-month collection period. Statistical analyses were performed with SAS software v.9.3 (SAS Institute Inc, Cary, North Carolina, United States).

3.4 BIOINFORMATICS

A subset of 314 isolates was selected for whole genome sequencing (WGS) by randomly excluding isolates with no enhanced data available, based on region. Isolates were sequenced using the Illumina platform at ESR. Raw sequencing reads were put through the Nullarbor pipeline (Seemann et al., https://github.com/tseemann/nullarbor) for identification of potential contaminants, species confirmation and genome assembly.

3.1.1 Typing

Multi-antigen sequence typing (NG-MAST) that characterises variability in the *por* and *tbpB* genes was performed using ngmaster v0.5.5 (http://www.ng-mast.net) [17]. Where the *por* or *tbpB* allele was novel (i.e., not found in the NG-MAST database), or the combination of *por* and *tbpB* alleles was novel, temporary allele numbers and/or NG-MAST types were assigned. In this report, these temporary NG-MAST types commence with '21000' and have the number format '210nn'. Clonal complexes (CC) were defined by single locus variations in the allelic profile.

3.1.2 Characterisation of antimicrobial resistance determinants

All genomes were further screened for a variety of genetic determinants for antimicrobial resistance including acquired plasmids, the presence/absence of resistance genes and chromosomal point mutations (listed in Table S2).

Plasmids

A screen for the presence of *bla*$_{TEM}$ and *tetM* genes and plasmids was performed using abricate v.0.8.7 (Seemann T., https://github.com/tseemann/abricate) using the NCBI AMR database. For determination of plasmid types, an *in silico* PCR (Ipcress, exonerate v2.2.0), was run using the primers from Turner et al. (1999) to distinguish between the Dutch and American *tetM* plasmid types. Three types are known for the *bla*$_{TEM}$ plasmids (Asia, Africa, Toronoto/Rio type) and the primers from Dillon et al. (1999) were used to distinguish types based on amplicon size.

Mutations in 23S ribosomal RNA (23S rRNA) gene

The number of copies and presence of point mutations in the 23S rRNA gene was determined by mapping the raw reads to a reference with three masked copies of 23S rRNA, forcing mapping to a single copy of *N. gonorrhoeae* genome NCCP11945 using snippy v4.3.6 (Seemann T., https://github.com/tseemann/snippy). Copy numbers were determined using a custom python script (Kwong J., https://github.com/kwongj/ng23S-mutations).

Chromosomal mutations

Screening for chromosomal point mutations was performed using Ariba v. 2.14.4 [20] with a custom database created for *N. gonorrhoeae*. An *in silico* PCR was performed to test for the presence of *ermF, ermB, ermC, ereA, ereB*, and *mefAB* using existing primers [21,22].

3.1.3 Variant calling and phylogenetic tree inference

Raw sequencing reads were trimmed to remove adaptor sequences and low-quality bases using Trimmomatic v.0.39 [23]. Trimmed reads were aligned to the reference *N. gonorrhoeae* FA1090 (Genbank accession number: 21043) using bwa mem v0.7.17 [24], duplicate sequences were marked using Picard v2.23.6 [25]. Single Nucleotide Polymorphisms (SNPs) were called using Freebayes v1.3.2 [26]. Further SNP filtering was performed using vcftools v0.1.16 [27]. Repeat regions were identified using MUMmer v3.23 [28] and subsequently masked. Prophages present in the reference *N. gonorrhoeae* FA1090 were also masked in the alignment [29]. This filtering approach resulted in a total of 17,437 core SNPs present in 100% of isolates. Recombinant parts of the genome were identified and removed using gubbins v2.3.4 [30]. The final tree was built using IQ-TREE v1.6.9 [31].

A genomic dataset consisting of the 314 isolates from this study and the publicly available dataset of 401 isolates used in the New Zealand survey from 2014/2015 [32] was analysed using the same approach as described above, with a total of 19,506 core SNPs prior to removal of recombination.

3.1.4 Population structure

Genetic clusters were determined using the R package rhierbaps v1.1.3 implementing the hierBAPS algorithm [33] which performs a Bayesian Analysis of Population Structure (BAPS). Clustering was performed using two levels in the hierarchy and K = 65 as the upper bound for the number of clusters.

4. RESULTS

4.1 ISOLATES AND PATIENT DEMOGRAPHICS

A total of 344 non-duplicate N. gonorrhoeae isolates were received for the survey. Laboratories in the Northern health region referred the largest number of isolates (170, 49.4%), with most (164/170, 96.5%) from laboratories in Auckland. The Southern health region referred the next largest number of isolates (93, 27.0%), followed by the Midland health region (47, 13.7%) and the Central health region (34, 9.9%) (Table S1 and Table S3). Information about sexual behaviour was available for 59.0% (203/344) of these samples. There were differences in isolate referral rates from different regions; Canterbury DHB referred an isolate for 81.5% of laboratory notifications (84/103), compared to 11.5% (17/148) for Capital and Coast DHB (Wellington region) and four DHBs with no referrals (Tairawhiti, Taranaki, West Coast and Whanganui) (Table S4).

During the survey collection period, there were 1,636 laboratory notifications of gonorrhoea (Table S4) of which the majority were male (939/1,636, 57.4%). Enhanced data was available for 864 notified cases, of which the majority were male (574/864, 66.4%) and which comprised 61.1% (574/939) of all notified male cases. In contrast, 41.8% (290/693) of female notified cases had enhanced data available. Similarly, notified male cases were more likely to have a cultured isolate available (255/939, 27.2%) than female notified cases (88/693, 12.7%).

The age distribution of cases with referred isolates is shown in Table S5; the majority of all cases were between 25 and 29 years of age (28.5%) for both males and females. When compared to the total notifications, the highest percentage of isolates referred for the survey was in males between 35-39 years of age, but by contrast in females this age category had the lowest percentage of isolates referred for the survey (Figure S1). The majority of isolates were obtained from genital specimens, namely urethral specimens from males (76.3%) or cervical/vaginal specimens from females (89.8%) (Table S6).

Sexual behaviour data was available for 203 of the 344 study cases. Of these, 63 identified as men who have sex with men (MSM), 54 were heterosexual males and 86 were heterosexual females.

4.2 PHENOTYPIC ANTIMICROBIAL SUSCEPTIBILITY

The antimicrobial susceptibility results are shown in Table 2 and for the subset of the sequenced isolates in Table S7. The full MIC distribution data for each antimicrobial tested is presented in Table S8.

Table 2: Antimicrobial susceptibility among *Neisseria gonorrhoeae*, 2018-2019.

	Percent (number)				
Antimicrobial	**Susceptible**	**Intermediate/ decreased susceptibility[a]**	**Resistant**	**MIC_{50} (mg/L)**	**MIC_{90} (mg/L)**
Penicillin	1.45 (5)	90.4 (311)	8.1 (28)	0.25	1
Cefixime	99.1 (341)	0.9 (3)	0.0 (0)	0.008	0.03
Ceftriaxone	99.4 (342)	0.6 (2)	0.0 (0)	0.004	0.016
Ertapenem[b]	-	-	-	0.016	0.03
Ciprofloxacin	76.1 (262)	-	23.8 (82)	0.004	4
Tetracycline	38.1 (131)	27.3 (94)	34.6 (119)	0.5	32
Spectinomycin	100.0 (344)	0.0 (0)	0.0 (0)	32	32
Azithromycin	98.3 (338)	-	1.7 (6)	0.12	0.5
Gentamicin[2]	-	-	-	8	8

[a] The 'decreased susceptibility' category applies to cefixime and ceftriaxone.
[b] No interpretive standards available for ertapenem and gentamicin.

There were differences observed in the percentage of resistant isolates found in different health regions (Figure S2) and in different sites (Figure S3).

Two (0.6%) of the 344 isolates displayed decreased ceftriaxone susceptibility (MIC ≥0.06 mg/L), three isolates (0.9%) had decreased cefixime susceptibility (MIC ≥0.12 mg/L) and six isolates (1.7%) had an azithromycin MIC above the EUCAST ECOFF of 1 mg/L. Two isolates had decreased susceptibility to both ceftriaxone and cefixime but there were no isolates resistant to ceftriaxone or cefixime according to the EUCAST clinical breakpoints. There were no isolates with both decreased ceftriaxone susceptibility and an azithromycin MIC >1 mg/L. However, isolates with decreased ceftriaxone susceptibility were resistant to ciprofloxacin and tetracycline and were not susceptible to penicillin.

Both isolates with decreased ceftriaxone susceptibility and two of the three isolates with decreased cefixime susceptibility were from the Auckland region; the other isolate with decreased cefixime resistance was submitted from the Waikato. Isolates with an

azithromycin MIC >1 mg/L were submitted from the Auckland region (3 isolates), Waikato (1 isolate), Wellington (1 isolate) and Christchurch (1 isolate); none of these isolates displayed high-level azithromycin resistance (MIC >256 mg/L).

Sexual behaviour was available for a limited number of cefixime-, ceftriaxone- and azithromycin-nonsusceptible isolates; one isolate with decreased susceptibility to cefixime was from a heterosexual female, and one isolate with decreased susceptibility to ceftriaxone was from an MSM male. Five isolates with an azithromycin MIC >1mg/L had known sexual behaviour; two were from MSM, one was from a heterosexual male, and one was from a heterosexual female.

Among the 8.1% (28/344) of isolates that were penicillin resistant, 75.0% (21/28) produced β-lactamase. Among the 34.6% (119/344) of isolates that were tetracycline resistant, 73.1% (87/199) had MICs ≥16 mg/L indicating these isolates have high-level, plasmid-mediated tetracycline resistance due to the *tetM* gene, as discussed in section 4.3.2.

4.3 GENOTYPIC ANALYSIS

A subset of 314 isolates from the 344 isolates included in the survey were sequenced using an Illumina short-read approach. High quality sequencing data was available for all 314 isolates. Genome coverage to the *N. gonorrhoeae* FA1090 reference was 95.71% (± 0.23% SD) with median depth of 135 (interquartile range 77).

4.3.1 NG-MAST typing

A total of 89 unique NG-MAST types were found, of which 45 were present in the NG-MAST database and 44 (49.4%) were novel. The four most common sequence types were ST21017 (29, 9.2%), ST13489 (26, 8.3%), ST21010 (21, 6.7%) and ST2400 (19, 6.1%) (Table S9) This differs from the 2014/2015 survey in which 117 distinct NG-MAST types were identified, of which the four most common were ST4186 (11.3%), ST2400 (10.8%), ST 9368 (6.8%), ST10193 (3.8%). Importantly, ST10193 which appeared to be associated with clonal spread of decreased susceptibility to azithromycin in the 2014/2015 survey, was not found.

All other NG-MAST types were associated with less than 5% of isolates. A total of 55 NG-MAST types were found in only one isolate, and a further 11 types were found in two isolates each. Grouping isolates based on single-locus variation in allelic profiles resulted in 14 clonal complexes (291 isolates) and 15 singleton NG-MAST STs (23 isolates).

Among the isolates with decreased susceptibility to extended spectrum cephalosporins, three distinct NG-MAST sequence types were identified, which were assigned to distinct

clonal complexes. Decreased susceptibility to ceftriaxone was found in isolates with ST1513 and ST10386, whilst decreased susceptibility to cefixime was found in isolates with ST1513 and ST18982; hence ST1513 was found in a total of two isolates with either decreased susceptibility to both ceftriaxone and cefixime or cefixime alone. Five of the six isolates with an azithromycin MIC >1 mg/L were sequenced, which identified five NG-MAST types distinct from each other and distinct from isolates with decreased susceptibility to ceftriaxone and cefixime: ST7638, ST13892, ST14537, ST18146 and ST21033.

4.3.2 Genetic basis of antimicrobial resistance

The genetic basis of antimicrobial resistance identified in the 314 isolates that were sequenced, are summarised in Table 3 along with correlation to the resistance phenotype.

Azithromycin

Five isolates with an azithromycin MIC >1 mg/L underwent WGS. Four of these isolates had C2611T mutation in the 23S ribosomal RNA gene which results in a 50S target with reduced affinity for macrolides. This C2611T mutation was present in all four of the 23S rRNA gene alleles in three isolates and in three out of four alleles in one isolate. The remaining isolate did not carry any known mutation associated with azithromycin resistance. No acquired azithromycin resistance genes (*ermB, ermC, ermF, mefA/mefE*, *ereA, ereB*) or the 23S rRNA gene mutation associated with high-level resistance (A2059G) were found in any isolate.

Cefixime and ceftriaxone

Three isolates had decreased susceptibility to cefixime (MIC ≥0.12 mg/L) and two isolates had decreased susceptibility to ceftriaxone (MIC ≥0.06 mg/L). One isolate with decreased susceptibility to both cefixime and ceftriaxone had the same NG-MAST type as an isolate with reduced susceptibility to cefixime, ST1513. Both carried the mosaic XXXIV *penA* allele with the G545S, I312M and V316T mutations in penicillin-binding protein 2 (PBP2), as well as the A121N, G120K mutations in the gene encoding the outer membrane porin *porB1b*, and the L421P mutation in *ponA* (PBP1).

Of the remaining two isolates, one isolate with reduced susceptibility to ceftriaxone carried mutations in *porB1b* (G120K and A121D), *ponA* (L421P) and *penA* gene (A502V, P552S), whereas the remaining isolate with reduced susceptibility to cefixime had only the *mtrR* A39T mutation associated with β-lactam resistance via overexpression of the MtrCDE efflux pump. However, all other isolates (n=112) with the A39T *mtrR* mutation were susceptible to both cefixime and ceftriaxone.

Ciprofloxacin

Ciprofloxacin resistance was identified in 79 isolates (25.1%) that were characterised by WGS. All 79 isolates carried S91F mutations in *gyrA*, which reduce quinolone binding to DNA gyrase, with additional mutations in *gyrA* (D95G n=37, D95A n=41, or D95Y n=1). No mutations in *parE* were detected. Fourty-four isolates also carried mutations in *parC*, which reduces quinolone binding to topoisomerase IV subunit A, with mutations in D86N (n=19), E91K (n=3), S81I (n=1), S87R (n=16) and S87N (n=17). One isolate (MIC = 8 mg/L) had a mutation in *norM*, associated with overexpression of NorM efflux pump, alongside additional mutations in *parC* (S87N) and *gyrA* (S91F and D96G).

Penicillin

Isolates were screened for the presence of bla_{TEM} genes, as well as chromosomal point mutations known to confer resistance to penicillin. All isolates with a penicillin MIC ≥4 mg/L carried either the bla_{TEM-1} gene (n=15) or the $bla_{TEM-135}$ gene (n=1). All isolates, which tested positive for production of β-lactamase carried a bla_{TEM} gene.

The mutation L421P in *ponA* (penicillin binding protein 1a) was found for 99 isolates (33.5%). Forty-three percent of isolates possessed a mutation in *mtrR* (A39T, n=113 or G45D, n=25) resulting in overexpression of the MtrCDE efflux pump. A single base pair deletion in the *mtrR* promoter (-A) was present in 122 isolates. Three isolates had no reads mapped to the *mtrR* promoter. Forty-eight isolates had the G120K mutation in *porB1b*, an outer membrane porin, of which 44 isolates carried additionally either A121D (n=42) or A121N (n=2) and one isolate carried point mutations A121N and G120N. For 3.5% of isolates (n=11), no sequencing reads mapped to *porB1b* and for 5.7% (n=18) *porB1b* assembled with low coverage (<40x) with no variants identified. No isolates carried any mutations in the *pilQ* gene encoding the pore forming secretin PilQ of the type IV pili, presumably because type IV pili are also essential for *N. gonorrhoeae* pathogenesis.

Spectinomycin

All isolates were susceptible to spectinomycin and none of the isolates carried mutations associated with resistance to spectinomycin in the 16S rRNA or *rpsE* genes.

Tetracycline

The *tetM* gene, conferring high-level tetracycline resistance, was found in all isolates with a tetracycline MIC ≥8 mg/L (n=80). Of these 74 contained the “Dutch-type” plasmid and six contained the American-type plasmid.

A total of 193 isolates carried the mutation in *rpsJ* (61.6%) that reduces affinity of tetracycline to the 30S ribosome. No known mutations in *pilQ* were observed, but 44.0% of isolates carried mutations in *mtrR* (n=113 A39T mutation, n=25 G45D).

Others

The *folP* mutation (R228S) conferring resistance to sulphonamides was found in 272 isolates (86.6%). The mutation conferring resistance to rifampicin (H553N in *rpoB*) was detected in 21.0% of isolates (n=66) isolates.

The highly conserved *porA* pseudogene is a commonly used target for molecular detection of gonorrhoea, however there have been reports of test failures due to the presence of meningococcal *porA* in *N. gonorrhoeae* isolates. All isolates in this study contained the gonococcal *porA*.

Table 3: Number of isolates carrying plasmids or point mutations associated with antibiotic resistance and further correlation with resistant phenotypes.

Antimicrobial	Gene (protein)	Mutation	Number (%)	Phenotype Resistant Number (%)	Phenotype Intermediate resistance[1] / decreased susceptibility[2]
Penicillin	*bla*$_{TEM-1}$	-	18 (5.7%)	18 (100%)	-
	bla$_{TEM-135}$	-	1 (0.32%)	1 (100%)	-
Total	*mtrR* promoter	-	122 (39.9%)	4 (3.3%)	118 (96.7%)
N=314	*mtrR* (MtrR)	A39T	113 (36.0%)	19 (16.8%)	94 (83.2%)
		G45D	25 (8.0%)	-	25 (100 %)
	ponA (PBP1)	L421P	99 (31.5%)	10 (10.1%)	89 (89.9%)
	penA (PBP2)	A502V	4 (1.3%)	3 (75%)	1 (25%)
		G543S	8 (2.5%)	5 (62.5%)	3 (37.5%)
		G545S	2 (0.6%)	1 (50%)	1 (50%)
		I312M	3 (1.0%)	1 (66.6%)	2 (33.4%)
		P552S	2 (0.6%)	1 (50%)	1 (50%)
		V316T	3 (1.0%)	1 (66.6%)	2 (33.4%)
	penB (PorB1b)	A121D/N	45 (14.3%)	7 (15.6%)	38 (84.4%)
		G120K/N	49 (15.6%)	10 (20.4%)	39 (79.6%)
	pilQ (PilQ)	E666K	-	-	-
Ciprofloxacin	*gyrA* (GyrA)	D95G/A/Y	78 (25.2%)	78 (100%)	
		S91F	79 (25.2%)	79 (100%)	
Total	*parC* (ParC)	D86N	19 (6.1%)	19 (100%)	
N=314		E91K	3 (1.0%)	3 (100%)	NA
		S87I/N/R	25 (8.0%)	25 (100%)	
	parE (ParE)	-	-		
	norM promoter	-	1 (0.3%)	1 (100%)	
Tetracycline	*tetM*	-	80 (25.5%)	80 (100%)	-
	rpsJ	V57M	193 (61.5%)	110 (57%)	47 (24.4%)
Total	*mtrR* promoter	-	122 (39.9%)	65 (53.3%)	10 (8.2%)
N=314	*mtrR* (MtrR)	A39T	113 (36.0%)	43 (22.3%)	22 (11.4%)
		G45D	25 (8.0%)	1 (4 %)	-
	penB (PorB1b)	A121D/N	45 (14.3%)	31 (68.9%)	12 (26.7%)
		G120K/N	49 (15.6%)	35 (71.4%)	12 (24.5%)
	pilQ (PilQ)	E666K	-	-	-

Antimicrobial	Gene (protein)	Mutation	Number (%)	Phenotype Resistant Number (%)	Phenotype Intermediate resistance[1] / decreased susceptibility[2]
Azithromycin	23S	C2611T	4 (1.3%)	4 (100%)	
	mtrR promoter	-	122 (39.9%)	1 (0.8%)	NA
	mtrR (MtrR)	A39T	113 (36.0%)	0 (0%)	
Total	*mtrR* (MtR)	G45D	25 (8.0%)	1 (4 %)	
N=314	*ermB*	-	-	-	
	ermC	-	-	-	NA
	ermF	-	-	-	
	macAB promoter	-	-	-	
	mef	-	-	-	
Ceftriaxone/	*mtrR* (MtrR)	A39T	113 (36.0%)	-	1 (0.9%)
Cefixime		G45D	25 (8.0%)	-	1 (4%)
	ponA (PBP1)	L421P	99 (31.5%)	-	3 (3%)
Total	*penA* (PBP2)	A502V	4 (1.3%)	-	1 (25%)
N=314		G543S	8 (2.5%)	-	-
		G545S	2 (0.6%)	-	2 (100%)
		I312M	3 (1.0%)	-	2 (66.7%)
		P552S	2 (0.6%)	-	1 (50%)
		V316T	3 (1.0%)	-	2 (66.7%)
	penB (PorB1b)	A121D/N	45 (14.3%)	-	3 (6.7%)
		G120K/N	49 (15.6%)	-	3 (6.1%)
Spectinomycin	16S	C1192T	-	-	
Total	*rpsE*	-	-	-	NA
N=314					

[1] Intermediate resistance applies to penicillin and tetracycline
[2] Decreased susceptibility applies to ceftriaxone and cefixime

4.3.3 Phylogeny and population structure

A Maximum Likelihood (ML) phylogeny (Figure 1) was inferred to assess the phylogenetic relationship of the 314 isolates and the reference genome *N. gonorrhoeae* FA1090. The phylogeny was based on the core genome alignment after removal of recombinant regions consisting of 5,059 sites (including 3,871 informative sites). For identification of population structure, a hierarchical clustering analysis was performed using Bayesian Analysis of Population Structure (BAPS) software to identify subpopulations in the sequence collection. Fifteen BAPS derived clusters (subpopulations) were identified. The diversity within clusters was low and all isolates belonging to the same cluster grouped together with the exception of isolates from cluster 11 and 8, which were split up in different portions of the tree. Clusters contained isolated from both males and females. The phylogenetic tree in Figure 2 highlights the phenotypic susceptibility testing results for each isolate, showing multi-resistant isolates clustering together.

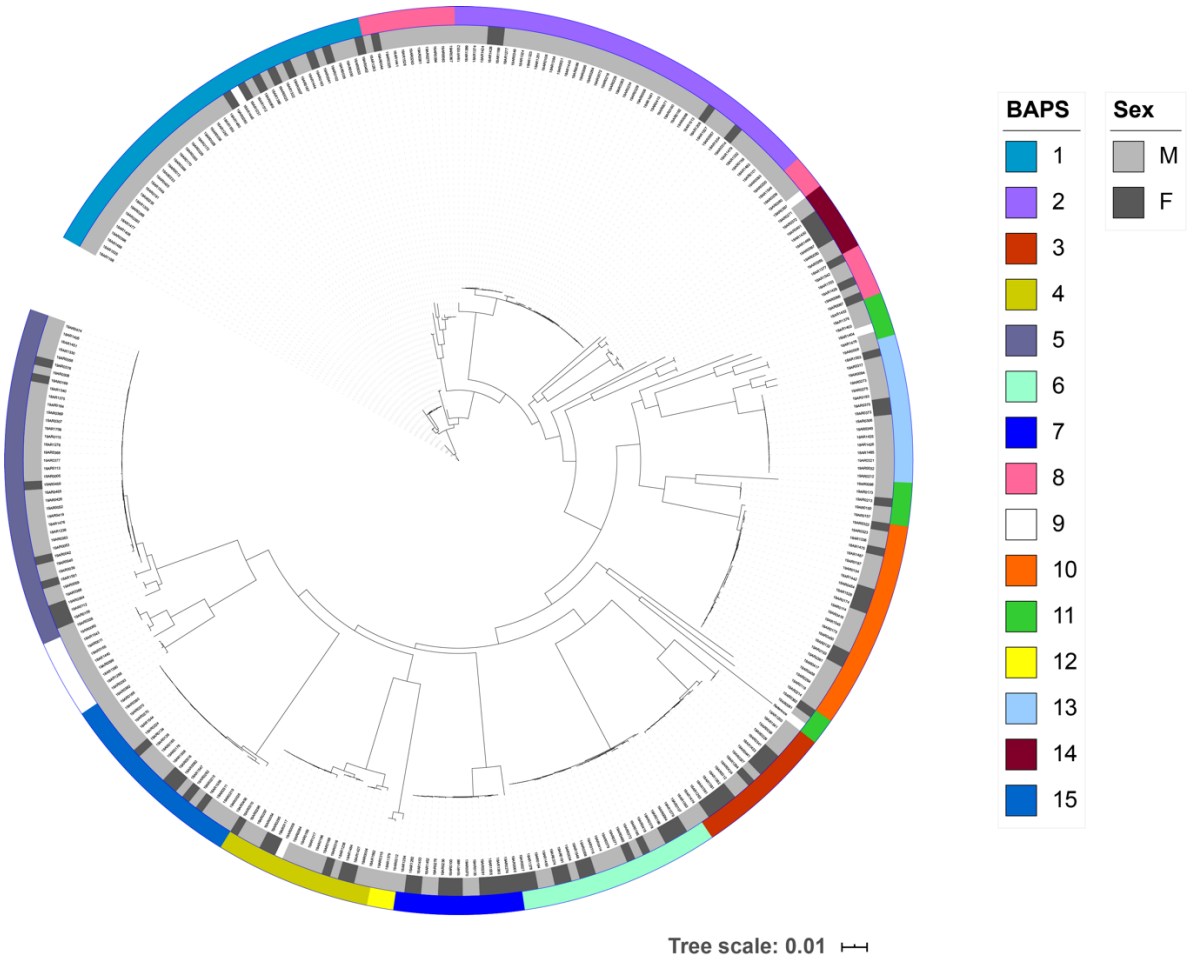

Figure 1: Population structure of *Neisseria gonorrhoeae* circulating in New Zealand in 2018-2019. The Maximum Likelihood phylogeny is based on 314 *N. gonorrhoeae* isolates and the reference strain FA1090. The ML tree was built using IQ-TREE and is based on a recombinant-free core genome alignment of 5,059 sites. The inner ring illustrates patient sex and the outer ring illustrates the BAPS clusters.

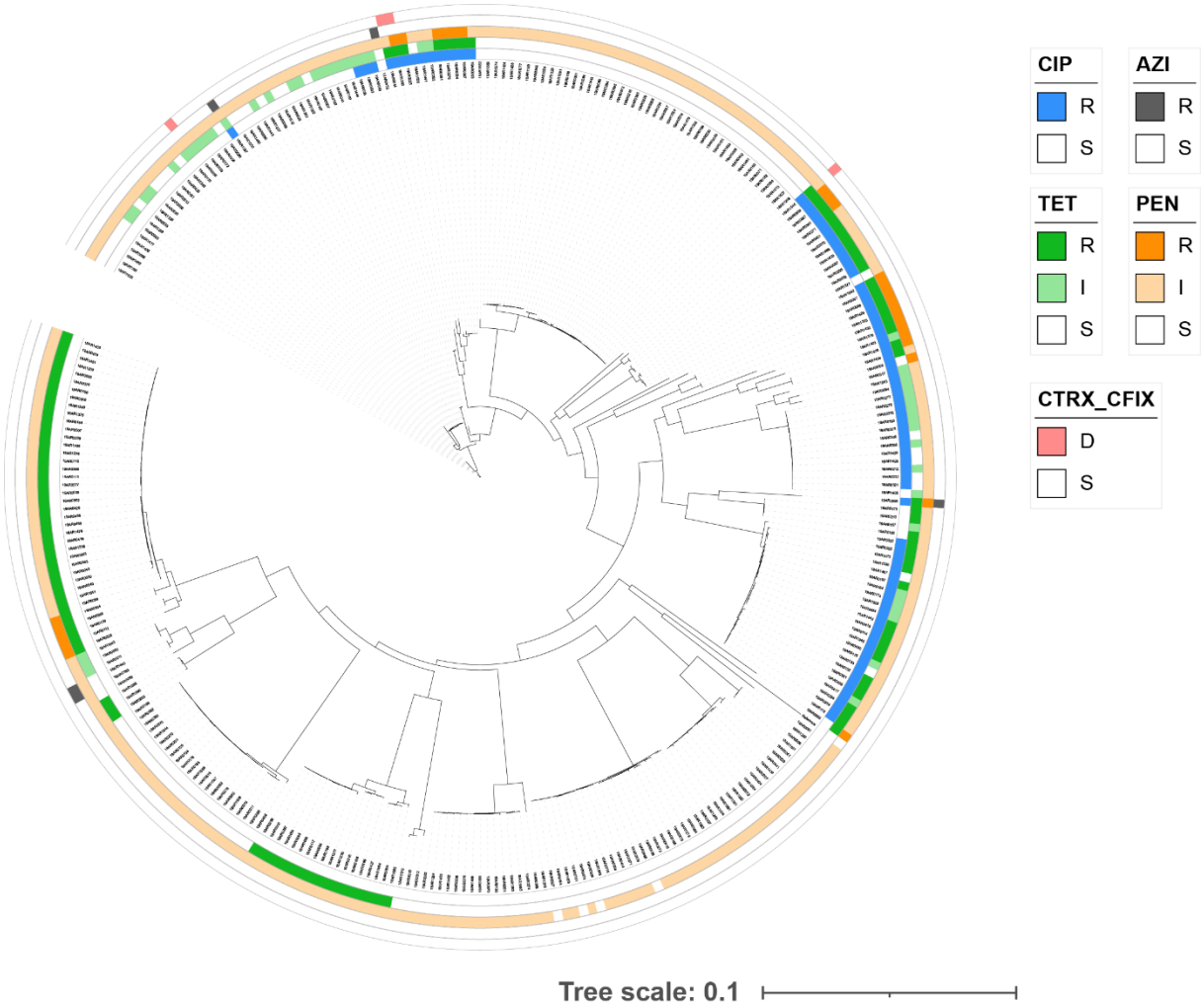

Figure 2: Maximum Likelihood phylogeny of 314 *Neisseria gonorrhoeae* isolates and susceptibility/resistance based on MICs for the different antibiotics tested. The inner ring reflects results for ciprofloxacin (CIP), followed by results for tetracycline (TET), Penicillin (PEN), azithromycin (AZI) and ceftriaxone/cefixime (CTRX_CFIX).

Isolates from the 2018/2019 survey were compared to the 401 isolates from New Zealand's 2014/2015 national *N. gonorrhoeae* survey [12,32] to illustrate diversity of isolates from both national surveys. The datasets were combined to a total of 716 samples (401 isolates from 2014/2015, 314 isolates from 2018/2019, *N. gonorrhoeae* FA10190 reference) and a ML phylogeny was built on a core genome alignment of 4,813 sites with 3,269 informative sites. The tree (Figure 3) illustrates the two *N. gonorrhoeae* datasets have similar levels of diversity.

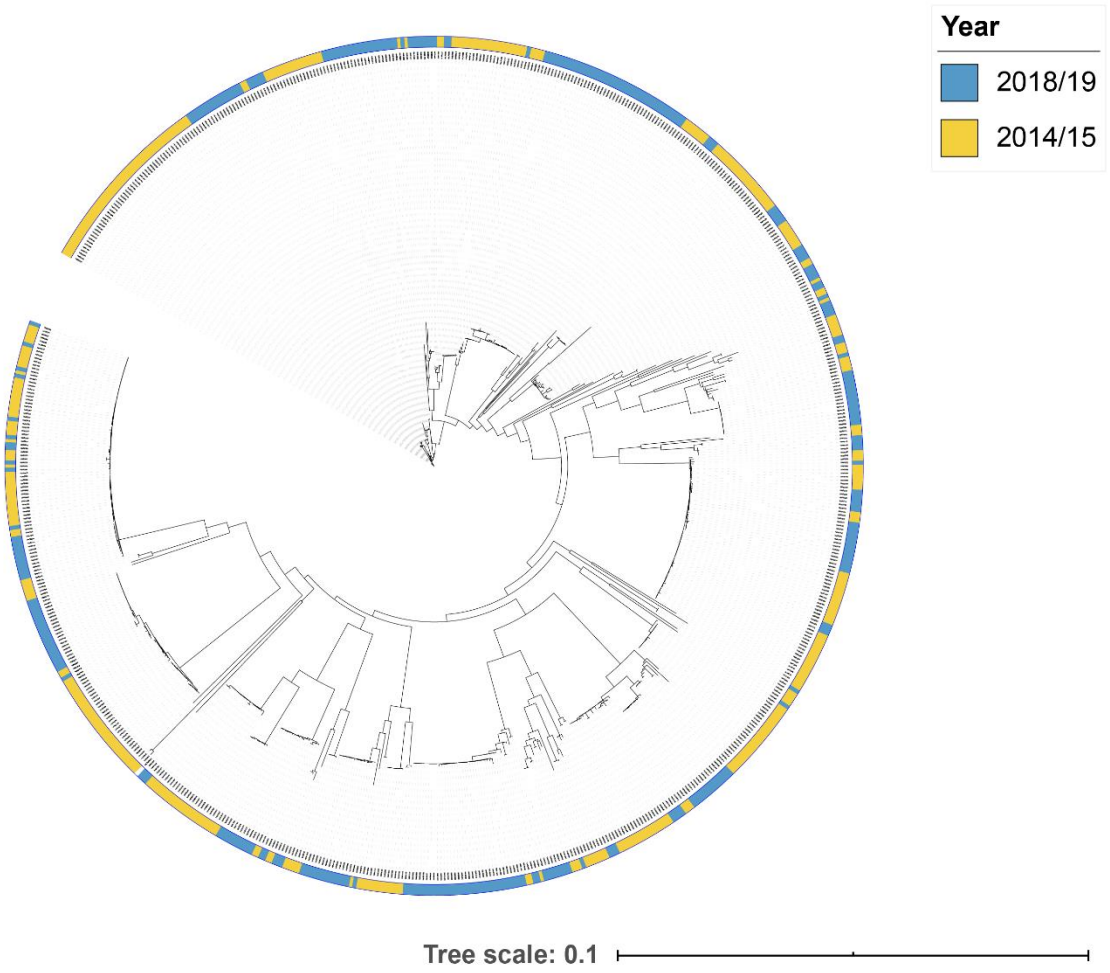

Figure 3: Maximum Likelihood phylogeny of 716 isolates from the two national *Neisseria gonorrhoeae* surveys. All samples from the 2014/2015 survey are highlighted in yellow and the isolates from 2018/2019 are illustrated in blue.

5. DISCUSSION

5.1 IMPORTANCE

The increasing resistance of *N. gonorrhoeae* to the extended-spectrum cephalosporins and azithromycin observed globally, combined with reports of treatment failures highlights the need for ongoing epidemiological surveillance of *N. gonorrhoeae* [1,5,34]. This report contributes to our understanding of both the antibiotic resistance and molecular epidemiology of this pathogen in New Zealand.

5.2 LIMITATIONS

The move to nucleic acid-based diagnosis of gonorrhoea has limited the availability of isolates for susceptibility testing and sequencing. During the study period, 79.0% (1,292/1,636) of notified gonorrhoea cases did not have a cultured isolate referred to ESR. With such a low overall sampling fraction, it is difficult to make any firm conclusions about *N. gonorrhoeae* circulating in New Zealand. Laboratories are encouraged to review their culture procedures to optimise the recovery of isolates. The difference in recovery rates between regions likely reflects differences in laboratory and sampling practices. Development of culture-independent methods which include detection of resistance mechanisms may help better inform the true prevalence of antimicrobial resistance in the future. However, this technology is not yet widely available, and the wide array of potential genotypic resistance determinants will prove challenging to interpret without correlation to the phenotype.

There is an over-representation of males in this study population compared to total laboratory notifications and more males had enhanced data available, as 27.2% of notified cases in males had a culture isolate available, compared to only 12.7% for females. This survey is therefore less representative of gonorrhoea infections occurring in women. One reason to explain the gender discrepancy may be that males are more likely to attend a sexual health clinic for testing, and therefore be more likely to have enhanced surveillance data and a dedicated sample for culture collected. Information about sexual behaviour was not available for 41.0% (141/344) of isolates and analysis of travel history data was not undertaken.

5.3 ANTIMICROBIAL RESISTANCE

This is the second New Zealand *N. gonorrhoeae* survey comparing phenotypic antimicrobial susceptibility methods with genotypic mutations likely to confer resistance. The survey

demonstrates that, with the exception of azithromycin and tetracycline, rates of resistance have remained stable or decreased. Rates of resistance are lower than Australia[12,32,35] (Table 4), but the sampled populations may not be directly comparable due to differing interpretative standards.

Table 4: Comparison of antimicrobial resistance among *Neisseria gonorrhoeae* in New Zealand and Australia.

Antimicrobial	Antimicrobial resistance[a]		
	NZ 2014-2015[b] *n = 425*	**NZ 2018-2019[c]** *n = 344*	**AGSP 2019[d]** *n = 7835*
	% (number)	% (number)	% (number)
Penicillin	12.0 (51)	8.1 (28)	22.1 (2,136)
Azithromycin	1.7[e] (7)	1.7 (6)	4.6 (488)
Ciprofloxacin	32.2 (137)	23.8 (82)	28.4 (2,743)
Ceftriaxone decreased susceptibility[f]	2.6 (11)	0.6 (2)	1.3 (126)
High-level tetracycline	15.8 (67)	25.3 (87)	27.0 (994)
Spectinomycin	0.0 (0)	0.0 (0)	0.0 (0)

a Different versions of interpretative standards have been used for the three studies, so breakpoints may be different.
b Data from New Zealand's 2014/2015 survey [12,32].
c Data from this survey.
d Data from the Australian Gonococcal Surveillance Programme 2019 [35].
e Two (0.5%) of these isolates have azithromycin MIC >1 mg/L, which is the cut-off used in the 2018/2019 survey.
f Defined as MIC ≥0.06 mg/L.

Reassuringly, decreased susceptibility to ceftriaxone was detected in very few isolates (2/344). Similarly, there were only six isolates with an azithromycin MIC above the current EUCAST ECOFF of 1 mg/L. However, the proportion of isolates with an azithromycin MIC above the current ECOFF has increased in the last four years from 0.5% (2/425) in 2014/2015 to 1.7% (6/344) in 2018/2019. It should be noted that the azithromycin resistance rate reported in 2014/2015 of 1.7% (7/425) is based on an azithromycin breakpoint of >0.5 mg/L in place at that time. No isolates with acquired high-level azithromycin resistance (MIC >256 mg/L) were identified and none of the isolates belonged to the globally disseminated NG-MAST ST1407 associated with decreased ceftriaxone susceptibility, or the multidrug resistant ST1903 [36–38].

N. gonorrhoeae uses multiple mechanisms to facilitate resistance to antibiotics, which may be the result of mutations in chromosomal genes or through acquisition of extra-chromosomal elements such as plasmids. Our understanding of the genes and mutations that confer antibiotic resistance has increased in recent years. In this study there was a strong correlation between the antibiotic resistant phenotype and genotype, with known genetic determinants for the antibiotic resistance being found in all isolates, except for one isolate which was phenotypically resistant to azithromycin, but did not carry any known determinants involved with azithromycin resistance in the genotype to explain the resistance.

Penicillinase producing *N. gonorrhoeae* have high-level resistance to penicillin, conferred by a TEM β-lactamase gene, with eight different types described currently [39]. All isolates with high-level resistance (MIC >4 mg/L) in New Zealand were beta-lactamase positive and carried the β-lactamase TEM-1 gene, although one isolate contained the TEM-135 gene, which differs from TEM-1 by a single nucleotide substitution at position 135 (M182T). The $bla_{\text{TEM-135}}$ plasmid is common in Australia but has not previously been observed in New Zealand (including in the 2014/2015 survey), suggesting acquisition outside New Zealand, or introduction from overseas. Importantly, the $bla_{\text{TEM-135}}$ gene requires only one additional SNP to produce an extended spectrum beta-lactamase [39].

There are an increasing number of molecular assays available for characterisation of *N. gonorrhoeae,* but these are not yet widely available in New Zealand. All sequenced isolates carried gonococcal *porA,* the target of most diagnostic tests used in New Zealand. Genomic data from this survey is useful to inform suitability of new molecular assays for the New Zealand context. The large number of unique NG-MAST types identified in this survey highlights the importance of this work to continue to better characterise and catalogue gonococcal isolates in New Zealand.

5.4 POPULATION STRUCTURE AND TYPING ANALYSIS

Phylogenies of gonorrhoea isolates provide evidence of a diverse population, with similar levels of diversity observed in both national surveys (2014/2015 and 2018/2019). The hierarchical cluster analysis for the 2018/2019 survey isolates, identified 15 subgroups in the sampled population. Each cluster consisted of isolates from both males and females, suggesting transmission between sexual risk groups is occurring. However, some clusters were dominated by isolates from male patients.

Comparing the most common NG-MAST types circulating in NZ with the six most common types in Australia in 2019 [40] found no overlap, as expected, since variations in prevalence of NG-MAST types between countries are common. Prevalence in NG-MAST types also varies over time, as has been shown in other countries [37] and the same has been observed here. In 2014/2015 the most prevalent sequence types were ST4186 (11.3%) and ST2400 (10.8%), whereas in 2018/2019 the most common types were ST21017 (9.2%), ST13489 (8.3%), followed by ST21010 (6.7%) and ST2400 (6.1%). Particularly in Europe, the spread of ST1407, a multi-drug resistant *N. gonorrhoeae* clone has caused concern [37], but no isolates with this ST were detected in this survey (Table S9).

While NG-MAST types associated with age, gender, sexual behaviour and region could provide some important information on transmission networks, the large number of distinct NG-MAST types and small number of isolates associated with each NG-MAST type means that a larger dataset is needed to draw statistically significant conclusions. It also highlights the need for better cataloguing of gonococcal isolates in New Zealand.

Two isolates with decreased susceptibility to the extended spectrum cephalosporins belonged to the same clonal complex (ST1513) and had the same genotypic resistance determinants, including the XXXIV *penA* allele. Phenotypic susceptibility results were similar, with all MICs being either the same or differing by one doubling dilution. It is not clear if there was an epidemiological link between the two isolates, although both patients resided in the upper North Island.

In conclusion, this survey draws emphasis to the increasing burden of gonorrhoea infection in New Zealand and that clusters involve both male and female patients. A rise in antimicrobial resistance has not been demonstrated, but the threat remains very high. Current AMR surveillance activity for *N. gonorrhoeae* is inadequate to detect early introduction of azithromycin and ceftriaxone resistance or sequence types of concern due to the sporadic nature of national surveys and the limited numbers of culture isolates available; diagnostic laboratories are encouraged to maintain culture capability and ensure sufficient

samples are received for surveillance of antimicrobial resistance, particularly for women. There was good correlation between genotypic and phenotypic resistance patterns. There is a high degree of diversity within *N. gonorrhoeae* isolates, with replacement of common sequence types since the previous 2014/2015 survey. Surveillance activities should be strengthened for this important drug resistant pathogen to inform public health programmes within New Zealand and globally. In particular, all isolates with high level resistance to azithromycin and/or resistance to extended spectrum cephalosporins should continue to be routinely referred to ESR for phenotypic confirmation and genotypic characterisation.

6. APPENDIX

Table S1: Number of isolates referred by each laboratory

Laboratory	Isolates referred for survey		Number of isolates characterised by WGS
	Number of isolates	Percentage of total	
Whangarei Hospital*	6	1.7	6
LabPlus*	56	16.3	50
Middlemore Hospital	10	2.9	9
North Shore Hospital	8	2.3	7
Labtests*	90	26.2	84
Waikato Hospital*	23	6.7	22
Pathlab Bay of Plenty*	25	7.3	22
Southern Community Labs, Hastings*	14	4.1	13
Medlab Central*	2	0.6	2
Wellington Southern Community Labs	17	4.9	17
Canterbury Health Laboratories*	46	13.4	40
Canterbury SCL	40	11.6	35
Southern Community Labs, Dunedin*	7	2.0	7
Total	**344**	**100**	**314**

* Denotes laboratories that were requested to refer gonococcal isolates for the survey. No isolates were received from four laboratories that were requested to refer isolates.

Table S2: Antimicrobial resistance determinants screened for in this survey. Target gene, characteristics and description for antimicrobial resistance mechanism. PEN = penicillin, CIP = ciprofloxacin, TET = tetracycline, CFX = cefixime, CTRX = ceftriaxone, SPEC = spectinomycin, AZI = azithromycin.

Target	Characteristic	PEN	CIP	TET	CFIX/ CTRX	SPEC	AZI	Reference
tetM 30S ribosome	plasmid			x				41,42
rpsJ 30S ribosome	V57M			x				43
pilQ Type IV pili	E666K	x		x				44,45
penB (porB1b) PorinB1b	G120K(D/N/R) G120K & A121D(G/N/S)	x		x	x			46,47
mtrR promoter MtrCDE efflux pump	Single nucleotide deletion (A)	x		x	x		x	48–54
mtrR MtrCDE efflux pump	A39T A40D G45D	x		x	x		x	55,56
23S rRNA	C2611T A2059G						x	57,58
ermF *ermB* *ermC*	presence						x	21,59
macAB MacAB efflux pump	promoter mutation						x	60
mefA, mefE efflux pump	presence						x	59
ereA, ereB	presence						x	22
rplD	G70D						x	61,62
rplV	3’ tandem duplications						x	63
ponA PBP1	L421P	x			x			64
bla$_{TEM-1}$ βlactamase	Plasmid	x						65
bla$_{TEM-135}$ βlactamase	Plasmid	x						65
gyrA DNA gyrase	S91F D95N/G/Y/A		X					47,66
parC topoisomerase IV	D86N S87R/I/W/N S88P E91K		X					47,52,66
parE topoisomerase IV	G410V		x					67
norM NorM efflux pump	promoter mutation		x					68

Table S2. cont

Target	Characteristic	PEN	CIP	TET	CFIX/ CTRX	SPEC	AZI	Reference
16S rRNA	C1192T					x		69
rpsE 30S ribosome	K28E T24P Codon 27 deletion					x		70,71
penA (PBP2)	A311V, I312M, G545S V316T(P) T483S, A501V(P/T) G542S P551S	x			x			51,72
porA	*N. meningitis porA* gene sequences							52,73
folP sulphonamide R	R228S							52,74
rpoB rifampicin R	H553N							52,75

Table S3: Distribution of isolates referred for the survey by district health board and region.

Region/District health board	Number of isolates	Percentage of total	Number of isolates characterised by WGS
Northern	**170**	**49.4**	**156**
Northland	6	1.7	6
Waitemata/Auckland/Counties Manukau	164	47.7	150
Midland	**47**	**13.7**	**43**
Waikato	31	9.0	29
Lakes	4	1.2	2
Bay of Plenty	12	3.5	12
Tairawhiti			
Taranaki			
Central	**34**	**9.9**	**33**
Hawke's Bay	15	4.4	14
Whanganui			
MidCentral	2	0.6	2
Capital & Coast/Hutt Valley	17	4.9	17
Wairarapa			
Southern	**93**	**27.0**	**82**
Nelson Marlborough	2	0.6	2
West Coast			
Canterbury/South Canterbury	85	24.7	74
Southern	6	1.7	6
Total	**344**	**100.0**	**314**

Table S4. Notifications of *Neisseria gonorrhoeae* cases during the New Zealand 2018/2019 survey period.

	Laboratory notifications		Enhanced data		Laboratory-based survey		Percentage of notifications with a culture
	n	%	n	%	n	%	
Sex							
Male	939	57.4	574	66.4	255	74.1	27.2
Female	693	42.4	290	34.0	88	25.6	12.7
Unknown	4	0.2	0	0.0	1	0.3	20
TOTAL	1636		864		344		
Age							
0-9	1	0.1	6	0.7	1	0.3	33.3
10-19	237	14.5	96	11.1	33	9.6	13.7
20-29	842	51.5	422	48.8	171	49.7	21.9
30-39	411	25.1	185	21.4	95	27.6	28.0
40-49	145	8.9	61	7.1	43	12.5	23.1
Unknown	0	0.0	94	10.9	1	0.3	25.0
TOTAL	1636		864		344		
DHB							
Auckland Region	903	55.2	366	42.4	164	47.7	20.2
Wellington Region	148	9.0	87	10.1	17	4.9	9.6
Waikato	113	6.9	76	8.8	31	9.0	22.1
Canterbury °	103	6.3	118	13.7	84	24.4	84.8
Northland	63	3.9	24	2.8	6	1.7	8.6
Bay of Plenty	50	3.1	26	3.0	12	3.5	20.3
Hawke's Bay	77	4.7	40	4.6	15	4.4	27.8
Southern	55	3.4	37	4.3	6	1.7	13.6
Lakes	31	1.9	23	2.7	4	1.2	12.5
MidCentral	22	1.3	16	1.9	2	0.6	8.7
Nelson/Mar	14	0.9	12	1.4	2	0.6	11.1
Tairawhiti	23	1.4	11	1.3	0	0.0	0
Whanganui	8	0.5	5	0.6	0	0.0	0
West Coast	4	0.2	1	0.1	0	0.0	0
South Canterbury	9	0.6	4	0.5	1	0.3	33.3
Taranaki °	13	0.8	18	2.1	0	0.0	0
TOTAL	1636		864		344		

° Laboratory data and enhanced data mutually exclusive. Enhanced data manually entered by clinicians, therefore higher number of enhanced data may represent reporting date differences.

Table S5: Age and sex of patients from whom *Neisseria gonorrhoeae* were referred.

Age group (years)	Laboratory notifications Number (column percent)						Isolates referred for survey Number (column percent)[1]:						Isolates characterised using WGS Number (column percent)[1]:					
	Female		Male		Total[2]		Female		Male		Total		Female		Male		Total	
0-4	0	(0.0)	1	(0.1)	1	(0.1)	0	(0.0)	1	(0.4)	1	(0.3)	0	(0.0)	1	(0.3)	1	(0.3)
5-9	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
10-14	7	(1.0)	2	(0.2)	9	(0.6)	2	(2.3)	0	(0.0)	2	(0.6)	2	(2.4)	0	(0.0)	2	(0.6)
15-19	154	(22.2)	73	(7.8)	227	(13.9)	16	(18.2)	15	(5.9)	31	(9.0)	16	(19.5)	15	(6.5)	31	(9.9)
20-24	187	(27.0)	227	(24.2)	414	(25.4)	20	(22.7)	53	(20.8)	73	(21.2)	18	(22.0)	46	(19.9)	64	(20.4)
25-29	153	(22.1)	275	(29.3)	428	(26.2)	25	(28.4)	73	(28.6)	98	(28.5)	22	(26.8)	68	(29.4)	90	(28.7)
30-34	88	(12.7)	168	(17.9)	256	(15.7)	12	(13.6)	48	(18.8)	60	(17.4)	11	(13.4)	45	(19.5)	56	(17.8)
35-39	65	(9.4)	87	(9.3)	152	(9.3)	4	(4.5)	31	(12.2)	35	(10.2)	4	(4.9)	28	(12.1)	32	(10.2)
≥40	39	(5.6)	106	(11.3)	145	(8.9)	9	(10.2)	33	(12.9)	43	(12.5)	9	(11.0)	27	(11.7)	37	(11.8)
Total[1]	**693**		**939**		**1632**		**88**		**255**		**344**		**82**		**231**		**314**	

[1] Age not reported for one male patient and gender not reported for one patient. These patients are included in the totals for each category.

[2] Unknown sex for four patients

Table S6. Body site of isolation of *Neisseria gonorrhoeae.*

	Number (column percent)[1]:					
Site	**Female**		**Male**		**Total**	
Urethral[1]	2	(2.3)	171	(76.3)	174	(50.6)
Cervix/vagina	79	(89.8)	0	(-)	79	(23.0)
Penile	0	(-)	32	(14.3)	32	(9.3)
Throat/pharynx	1	(2.3)	10	(4.5)	11	(3.2)
Urogenital (not otherwise specified)	4	(4.6)	4	(1.8)	8	(2.3)
Urine	0	(-)	3	(1.3)	3	(0.9)
Anorectal	0	(-)	33	(12.9)	33	(9.6)
Other[2]	2	(2.3)	2	(0.8)	4	(1.1)
Total	88	(100)	224	(100)	344	(100)

1 Gender not reported for one patient. This patient is included in the totals for each category, but not in the denominator used to calculate the percentages for each age or gender group
2 Includes two isolates from eyes, one from a knee aspirate and one from a wrist aspirate

Table S7: Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates characterised using WGS.

Antimicrobial	Percent (number):			MIC_{50} (mg/L)	MIC_{90} (mg/L)
	Susceptible	Intermediate/ decreased susceptibility[1]	Resistant		
Penicillin	1.3 (4)	90.5 (284)	8.3 (26)	0.25	1
Cefixime	99.0 (311)	1.0 (3)	0.0. (0)	0.008	0.03
Ceftriaxone	99.4 (312)	0.6 (2)	0.0. (0)	0.004	0.016
Ertapenem[2]	-	-	-	0.016	0.03
Ciprofloxacin	74.8 (235)	-	25.2 (79)	0.004	4
Tetracycline	38.2 (120)	26.8 (84)	110 (35.0)	0.5	32
Spectinomycin	100.0 (314)	0.0 (0)	0.0 (0)	32	32
Azithromycin	98.4 (309)	-	1.6 (5)	0.12	0.5
Gentamicin[2]	-	-	-	8	8

[1] The 'decreased susceptibility' category applies to cefixime and ceftriaxone.
[2] No interpretive standards available for ertapenem and gentamicin.

Table S8: Distribution of minimum inhibitory concentrations (MICs) of the 344 *Neisseria gonorrhoeae* isolates included in the survey.

Antimicrobial	Percent of isolates with an MIC (mg/L) of:																
	0.002	**0.004**	**0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
penicillin – all						1.5	25.9	49.7	8.4	6.4	2.9	0.6	0.3	4.4			
β-lactamase +ve											14.3	9.5	4.8	71.4			
β-lactamase -ve						1.5	27.6	52.9	9.0	6.8	2.2						
cefixime		8.7	57.6	20.1	9.6	3.2	0.9										
ceftriaxone	11.3	53.5	19.8	7.6	7.3	0.6											
ertapenem		0.3	36.1	46.8	14.8	1.5	0.6										
ciprofloxacin	14.0	57.6	4.4	0.6	0.0	0.0	0.0	0.0	0.0	0.9	5.2	10.2	7.3				
tetracycline						0.3	12.5	25.3	12.2	15.1	8.4	0.3	0.6	10.5	14.8		
spectinomycin													0.3	31.7	68.0		
azithromycin					11.1	13.1	30.2	32.0	10.8	1.2	0.6	0.9	0.3				
gentamicin											0.3	9.0	84.0	6.7			

The white fields represent the range of antibiotic concentrations tested. MIC values less than or equal to the lowest concentration tested are presented as this lowest concentration. MIC values greater than the highest concentration tested are presented as the next highest concentration after the highest concentration tested. The vertical lines indicate the breakpoints between the susceptibility categories. For antibiotics where there are two vertical lines (ie, penicillin, tetracycline and spectinomycin), the first line represents the breakpoint between susceptible and intermediate, and the second line represents the breakpoint between intermediate and resistant. For cefixime and ceftriaxone the vertical line on the left represents the decreased susceptibility breakpoint. For ertapenem and gentamicin there are no vertical lines as there are no interpretive standards for these antibiotics. However, the MIC50 and MIC90 are represented by light grey shading. For gentamicin the MIC50 = MIC90

Table S9: NG-MAST types identified among the 314 *Neisseria gonorrhoeae* isolates sequenced.

2018/2019 NG-MAST (ST)	Number (%)	2014/2015 NG-MAST (ST)	Number (%)
21017	29 (9.2)	4186	45 (11.3)
13489	26 (8.3)	2400	43 (10.8)
21010	21 (6.7)	9368	27 (6.8)
2400	19 (6.1)	10193	15 (3.8)
1498, 4186	15 (4.8)	90018	14 (3.5)
5049, 12526	14 (4.5)	90006	13 (3.3)
21020	12 (3.8)	90019	12 (3.0)
356, 5441, 21003, 21016	8 (2.6)	7753, 90010	10 (2.5)
18980	7 (2.2)	7803	9 (2.3)
18982, 21009, 21023	5 (1.6)	7175	8 (2.0)
8433, 9650, 11461, 16487, 18791, 21008	3 (1.0)	10998	7 (1.8)
1513, 9918, 15589, 17538, 21001, 21013, 21019, 21021, 21024, 21026, 21034	2 (0.6)	299, 90020	6 (1.5)
		356, 4244, 9166, 90011, 90023,	5 (1.3)
5, 8, 338, 4487, 5061, 5624, 6360, 7268, 7638, 7758, 8115, 9208, 9510, 9925, 10121, 10386, 13892, 14537, 14994, 15344, 15922, 17370, 17448, 18146, 19413, 20163, 21000, 21002, 21004, 21005, 21006, 21007, 21011, 21012, 21014, 21015, 21018, 21022, 21025, 21027, 21028, 21029, 21030, 21031, 21032, 21033, 21035, 21036, 21037, 21038, 21039, 21040, 21041, 21042, 21043	1 (0.3)	90021, 90022, 90024, 90042	3 (0.8)
		5624, 6950, 7577, 9650, 11520, 90033, 90046, 90052	2 (0.5)
		217, 402, 1407, 2501, 7268, 8522, 8570, 8709, 8883, 9909, 11760, 90003, 90005, 90009, 90012, 90013, 90026, 90037, 90045, 90048, 90050, 90055, 90065 Other (62 different STs)	1 (0.3)

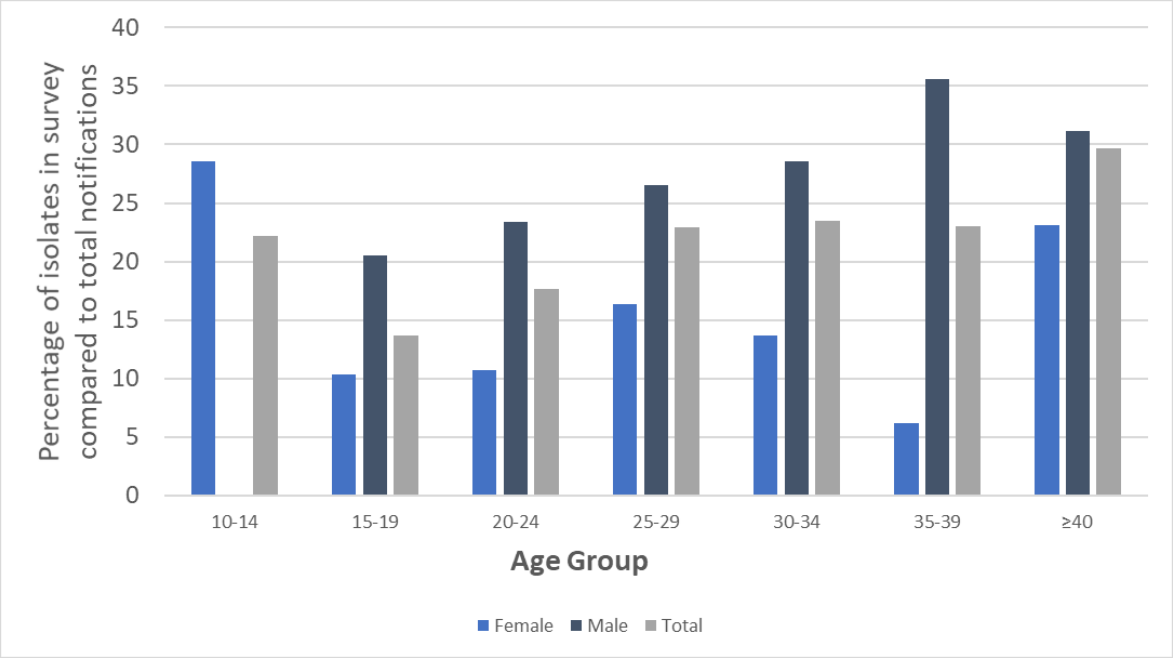

Figure S1: *Neisseria gonorrhoeae* cultures by gender as a percentage of total notifications, 2018-2019.

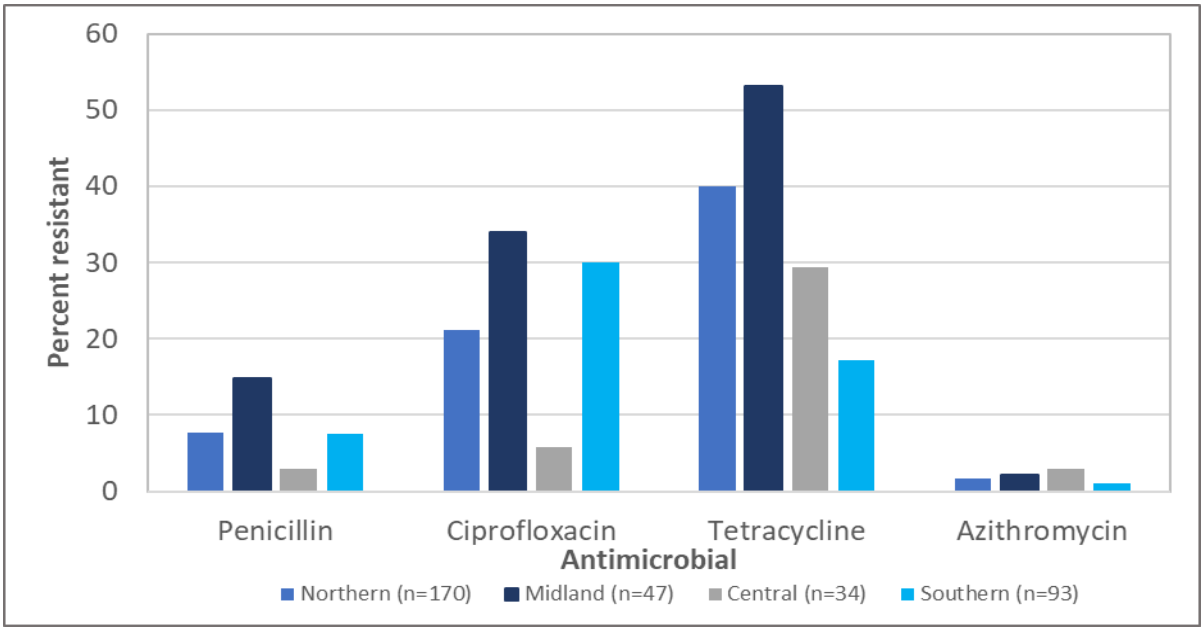

Figure S2: Antimicrobial resistance by health region among *Neisseria gonorrhoeae*, 2018-2019.

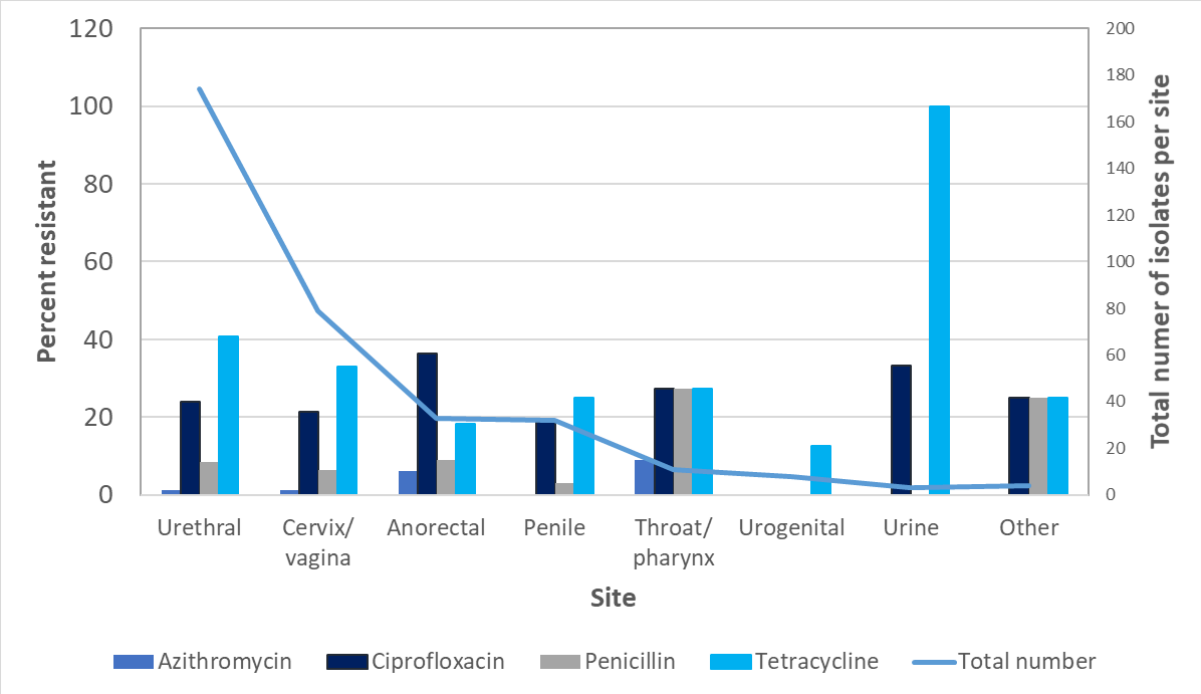

Figure S3: Antimicrobial resistance by site among *Neisseria gonorrhoeae*, 2018-2019.

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