Genomic Surveillance of *Neisseria gonorrhoeae* in the Philippines from 2013-2014

Alfred S. Villamin[a†], Silvia Argimón[b†], Manuel C. Jamoralin, Jr.[a], Karis D. Boehme[a], Melissa L. Masim[a], June M. Gayeta[a], Marietta L. Lagrada[a], Agnettah M. Olorosa[a], Victoria Cohen[b], Lara T. Hernandez[a], Benjamin Jeffrey[b], Khalil Abudahab[b], Charmian M. Hufano[a], Sonia B. Sia[a], John Stelling[c], Matthew T.G. Holden[d], David M. Aanensen[b,e*] and Celia C. Carlos[a*] on behalf of the Philippines Antimicrobial Resistance Surveillance Program

[a] Antimicrobial Resistance Surveillance Reference Laboratory, Research Institute for Tropical Medicine, Muntinlupa, Philippines.
[b] Centre for Genomic Pathogen Surveillance, Wellcome Genome Campus, Hinxton, UK.
[c] Brigham and Women's Hospital, Boston, MA, USA.
[d] University of St Andrews School of Medicine, St Andrews, Scotland.
[e] Big Data Institute, University of Oxford, Oxford, UK.

[†] These authors contributed equally to this work.
* These authors contributed equally to this work.
email: david.aanensen@sanger.ac.uk, ccarlosphl@yahoo.com

Abstract

Antimicrobial-resistant *Neisseria gonorrhoeae* is a major threat to public health, and of particular concern in the Western Pacific Region, where the incidence of gonorrhoea is high. The Antimicrobial Resistance Surveillance Program (ARSP) has been capturing information on resistant gonorrhoea since 1996, but studies of the genomic epidemiology of gonorrhoea in the Philippines are lacking.

We sequenced the whole genomes of 21 *N. gonorrhoeae* isolates collected in 2013-2014 by the ARSP. The multi-locus sequence type, multi-antigen sequence type, presence of antimicrobial resistance (AMR) determinants, and relatedness between the isolates were all derived from the sequence data. The concordance between phenotypic and genotypic resistance was also determined.

Ten out of 21 isolates were resistant to penicillin, ciprofloxacin and tetracycline, mostly linked to the presence of bla_{TEM} gene, the S91F mutation in the *gyrA* gene, and the *tetM* gene, respectively. None of the isolates were resistant to azithromycin, ceftriaxone or cefixime, although we identified the A24- deletion in the *mtrR* promoter in one isolate. The concordance between phenotypic and genotypic resistance was 92.38% overall for 5 antibiotics in 4 classes. Despite the small number of isolates studied, they were genetically diverse, as shown by the sequence types, the NG-MAST types and the tree. Comparison with global genomes placed the Philippine genomes within global Lineage A and led to the identification of an international transmission route.

This first genomic survey of *N. gonorrhoeae* isolates collected by ARSP will be used to contextualize ongoing prospective surveillance, and it highlights the importance of genomic surveillance in the Western Pacific and other endemic regions to understand the spread of drug-resistant gonorrhoea worldwide.

Introduction

Neisseria gonorrhoeae is a leading cause of sexually-transmitted disease (STD), with an estimated 78 million cases of gonorrhoea each year worldwide, including 35.2 million in the WHO Western Pacific Region. [1] In the Philippines, the prevalence of gonorrhoea was reported to be <2% for both men and women in 2002, [2] with higher rates of 7.7% and 10.8% among men having sex with men (MSM) for two different sites in 2005. [3]

N. gonorrhoeae has developed resistance to first-line antibiotics, such as sulfonamides, penicillins, tetracyclines, macrolides, fluoroquinolones and early cephalosporins. Currently recommended monotherapy for gonorrhoea is limited to one last effective class of antimicrobials, the extended-spectrum cephalosporins (ESCs, e.g. cefixime and ceftriaxone), but due to the recent emergence of resistance to these drugs, dual therapy with the injectable ceftriaxone plus oral azithromycin is the recommended treatment in many countries. [4] While resistance to azithromycin has also increased globally, [1] resistance to the dual therapy remains low. [5]

The increase in *N. gonorrhoeae* infections resistant to front-line antibiotics triggered a global action plan from the World Health Organization (WHO) to control the spread and impact of gonococcal resistance and a call for international collaborative action, especially in the Western Pacific Region. [1] The WHO Gonococcal Antimicrobial Surveillance Programme (GASP) has operated in the Western Pacific and South East Asian regions since 1992, but surveillance of gonococcal antimicrobial resistance (AMR) remains limited in Asia Pacific. [6] A recent report showed that 18 of 21 countries in the region reported isolates with decreased susceptibility to ceftriaxone and/or isolates resistant to azithromycin between 2011 and 2016. [6] The Philippines Department of Health - Antimicrobial Resistance Surveillance Program (DOH-ARSP) has been contributing AMR surveillance data to the Western Pacific GASP since 1996 and has not confirmed isolates with decreased susceptibility or resistance to these antibiotics during 2011-2016, [6] while high gonococcal resistance rates against other first-line antibiotics has been long reported (Figure 1). Ongoing surveillance is thus key to detect potential events of emergence or introduction of resistance to current treatment options.

Molecular methods for epidemiology of gonococci include both *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST) [7] and multi-locus sequence typing (MLST), [8] though NG-MAST is more widely used to investigate specific gonococcal antimicrobial resistance (AMR) phenotypes.[8] Whole genome sequencing (WGS) was recently shown to provide better resolution and accuracy than NG-MAST and MLST. [9] A good understanding of the population structure and the mechanisms of resistance of *N. gonorrhoeae* in the Philippines can enable the detection of high-risk clones associated with high-risk groups and contribute to the clinical management of gonococcal-related diseases and the creation of policies to prevent the spread of drug-resistance. Here we describe the results of the first genomic survey of gonococcal isolates from the Philippines.

Methods

Bacterial Isolates

A total of 51 *N. gonorrhoeae* isolates were collected by ARSP sentinel sites in 2013 and 2014 (Table 1). Out of the 36 isolates referred to the ARSP reference laboratory (ARSRL) for confirmation, 22 isolates from 7 sentinel sites were resuscitated and submitted for WGS.

Antimicrobial Susceptibility Testing (AST)

All *N. gonorrhoeae* isolates from this study were tested at ARSRL for antimicrobial susceptibility to 5 antimicrobials representing 4 different classes, namely penicillin (PEN), ciprofloxacin (CIP), tetracycline (TCY), ceftriaxone (CRO), and cefixime (CFX), using disk diffusion and gradient diffusion method (Etest, BioMerieux). The minimum inhibitory concentrations (MIC) were interpreted as resistant, intermediate and susceptible based on the interpretative criteria from the 26th edition of the Clinical and Laboratory Standards Institute (CLSI). [10] All isolates were screened for beta-lactamase production using cefinase paper discs (BD BBL).

DNA Extraction and Whole-Genome Sequencing

DNA was extracted from a single colony of each isolate with the Wizard® Genomic DNA Purification Kit (Promega). The DNA quantity and quality were determined using the Quantus Fluorometer (Promega) with picogreen and a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). The DNA extracts were then shipped to Wellcome Trust Sanger Institute for sequencing on the Illumina HiSeq platform (Illumina, CA, USA) with 100-bp paired-end reads. Raw sequence data were deposited in the European Nucleotide Archive (ENA) under the study accession PRJEB17615. Run accessions are provided on the Microreact projects.

Bioinformatics analysis

Genome quality was evaluated based on metrics generated from assemblies, annotation files, and the alignment of the isolates to the reference genome of *N. gonorrhoeae* strain TCDC-NG08107 (accession CP002441.1), as previously described. [11] Annotated assemblies were produced using the pipeline previously described. [12] Twenty-one high-quality *N. gonorrhoeae* genomes were included in this study.

The MLST sequence types (STs) and NG-MAST types, as well as the presence of AMR determinants (known genes or mutations) and clustering of the isolates based on genetic similarity (tree), were predicted *in silico* using Pathogenwatch, (https://pathogen.watch). To confirm the clustering results obtained with Pathogenwatch, the evolutionary relationships between the isolates were inferred from single nucleotide polymorphisms (SNPs) by mapping the paired-end reads to the reference genomes of *N. gonorrhoeae* strain FA 1090 (accession AE004969.1) as described in detail previously. [11] Mobile genetic elements (MGEs) and recombination regions detected with Gubbins were masked from the alignment of pseudogenomes. SNPs were extracted with snp_sites [13] and maximum likelihood phylogenetic trees were generated with RAxML [14] based on the generalised time reversible (GTR) model with GAMMA method of correction for among-site rate variation.

To contextualize the genomes from this study with public global genomes we combined them with two surveys available on Pathogenwatch, a European survey of 1054 genomes [15] and a global survey of 419 genomes. [16]

The outputs from Pathogenwatch (tree, genotyping and AMR predictions) together with the metadata collected from the sentinel sites were visualised with Microreact. [11]

The genomic predictions of AMR obtained with Pathogenwatch (test) were compared to the phenotypic results (reference) and the concordance between the two methods was computed for each of 5 antibiotics (105 total comparisons). Isolates found to be either resistant or intermediately resistant were considered resistant for comparison purposes.

Results

Demographic and Clinical Characteristics of the *N. gonorrhoeae* Isolates

The 21 genomes included in this study represented 7 sentinel sites, with VSM contributing the most isolates (n=11). The highest incidence was from the age group of 15-24 years old (47.6 %), followed by the age group 5-14 years old (28.6 %), age 25-34 years old (14.3%) and age 1-4 years old (9.5%, Table 2). The majority of the isolates were from females (N=11 vs N=10 from males). The specimen source was most frequently the vagina (N=8) for female patients and penile discharge (N=5) for males. All the patients were outpatients.

Concordance Between Phenotypic and Genotypic AMR

Isolates were tested for susceptibility to 5 antibiotics representing 4 different classes. In line with the resistance trends shown in Figure 1, the most prevalent resistance profile was PEN CIP TCY, identified in 10 isolates from 5 different sentinel sites, and mostly linked to the presence of bla_{TEM} gene, the S91F mutation in GyrA, and the *tetM* gene, respectively (Table 3). One penicillin-resistant isolate (13ARS_DMC0024) harboured three mutations, the deletion of A24 nucleotide in the *mtrR* promoter, and the non-synonymous substitutions in *ponA* (L421P) and *porB* (A121D) that that may contribute to high-level penicillin resistance (Mortimer and Grad, 2019). The partially-assembled bla_{TEM} gene was also detected in this genome but not considered for the concordance analysis. We also identified the non-synonymous mutation in the *folP* gene (R228S) in 20 out of 21 genomes, which confers resistance to sulfonamides. However, the isolates are not routinely tested for resistance to this antibiotic class.

The concordance between the AST results and genotypic predictions (Table 3) was larger than 95% for all antibiotics except for tetracycline (66.67%), bringing the overall concordance to 92.38% (Table 3). The discrepancies were attributed to 7 false positive results (isolates with a susceptible phenotype but AMR determinants in their genomes), all of which contained the mutation V57M in the *rpsJ* gene, with 2 isolates also carrying the *tetM* gene (Eyre 2017).

Genotypic findings

In silico genotyping

A total of 15 different STs were identified, with only 4 (9364, 10316, 1582 and 8133) represented by more than 1 isolate. Nine genomes were assigned to 8 known NG-MAST types, and 12 genomes to 9 novel types. Only 3 sentinel sites, namely MMH, VSM and ZPH submitted more than 1 isolate, and they were all represented by almost as many STs as isolates submitted. Details of the number and most common STs and NG-MAST types found in each of the sentinel sites are shown on Table 4.

Population structure of N. gonorrhoeae *in the Philippines*

The diverse gonococcal population was represented by a tree with three deep-branching clades and no clear geographical signal (Figure 2). Clades II and III were characterized by a different repertoire of AMR genes and mutations. Clade II contained mostly isolates susceptible or with reduced susceptibility to tetracycline with the V57M mutation in the *rpsJ* gene alone, and clade III was composed of isolates resistant to tetracycline also containing the *tetM* gene. Similarly, while both clades contained isolates resistant to ciprofloxacin, clade II was characterized by the presence of 2 mutations on the *gyrA* gene (S91Fand D95G), alone or in combination with the D86N mutation in *parC*, while clade III was characterized by the presence of only *gyrA*_S91F. Clades II and III showed different geographic distribution, although both were present in the VSM and ZPH sentinel sites that submitted the largest number of isolates. The genome from sentinel site DMC with a deletion in the *mtrR* promoter (A24-) was found on a separate branch (I) in the tree and also carried a different complement of AMR determinants, indicating that it is genetically distinct from the rest (Figure 2).

N. gonorrhoeae *from the Philippines in Global context*

The Philippine genomes were contextualized with two recently published collections [15, 16] available in Pathogenwatch. A recent global collection of 419 genomes from 58 countries (including the Philippines) defined two major lineages with different evolutionary strategies. The genomes from this study were mostly found within a sub-clade of Lineage A, a multidrug-resistant lineage associated with infection in high-risk sexual networks (Figure 3A), and mixed with genomes from Europe, Pakistan, and SE Asia, including previously isolated genomes from the Philippines (1998 and 2008, [16]). The genome from DMC was found within a separate subclade with genomes from India, Pakistan, and Europe, which also carried the *mtrR*_A24- promoter deletion.

We also contextualized our isolates with 1054 genomes from 20 countries collected in 2013 by a European survey. [15] Notably, the genome of strain 14ARS_VSM0347 isolated from a female on 25/04/2014 in the Philippines was highly similar to that of ECDC_GC_088 isolated from a male on 31/12/2013 in Norway (Figure 3B), with no SNP differences found in the Pathogenwatch core genome (1542 genes). One SNP was identified in the reference-based alignment of the two pseudogenomes, confirming that these two genomes are highly similar. The two strains also shared the same complement of AMR genes and SNPs (Figure 3B). The genetic distance between the two genomes is consistent with isolates from the same location, [15] suggesting a route of international transmission. The gonococcal reference laboratory at the Norwegian Institute of Public Health confirmed that the isolate was also resistant to penicillin and ciprofloxacin, and susceptible to ESCs (tetracycline not tested), and that the Norwegian male had visited the Philippines and claimed to have contracted gonorrhoea during his stay (personal communication).

Discussion

Whole-genome sequencing showed that the *N. gonorrhoeae* genomes from the Philippines are genetically diverse and carry a variety of AMR determinants, such as chromosomal mutations and acquired genes (Table 3 and Figure 2). The concordance between phenotypic and genotypic resistance was high (>95%) for most antibiotics, but only 66% for tetracycline (Table 3). Susceptible isolates carrying the *rpsJ*_V57M

mutation alone, which confers low-level resistance, have been shown before. [17] The two susceptible isolates also carrying the *tetM* gene were re-tested by disk diffusion and their susceptibility confirmed. Although *N. gonorrhoeae* remains largely susceptible to ESCs and azithromycin in the Philippines, we identified one isolate (13ARS_DMC0024) with a mutation in the *mtrR* promoter. While this isolate was susceptible to azithromycin and cefixime, the A24- deletion in the *mtrR* promoter results in the overexpression of the MtrCDE efflux pump and, in combination with other mutations, can lead to increased MIC values for these antibiotics (as well as penicillin and tetracycline). [4, 18] Prospective surveillance with WGS can detect in real-time the acquisition of additional mutations that could result in decreased susceptibility.

There was no evidence of geographical clustering of the isolates, in line with previous global datasets. [16, 19] A recent report suggested rapid recent intercontinental transmission of gonorrhoea with common introductions from Asia to the rest of the world. [16] In agreement with this, the Philippine genomes were interspersed with those from other countries (Figure 3A). In addition, we found evidence of an introduction event from the Philippines to Norway, which was associated with travel of a Norwegian male to the Philippines (Figure 3B).

The Philippine genomes belonged to lineage A, which is associated with infection in high-risk sexual networks. Combining WGS with epidemiological information can reveal transmission routes and risk factors, which can be used to design better control measures. [20] The size of our retrospective dataset (N=21) and the linked epidemiological data did not permit any inferences about sexual networks or risk factors, but the number of reported isolates by ARSP has since increased to more than 100 per year, and data on risk factors is now being collected, which will enable a more comprehensive analysis in future reports.

Our results represent the first genomic survey of *N. gonorrhoeae* isolates collected by ARSP, and will constitute the background to contextualize ongoing prospective surveillance. In addition, it highlights the importance of genomic surveillance in the Western Pacific and other endemic regions to understand the spread of drug-resistant gonorrhoea worldwide.

Acknowledgements

Funding provided by Newton Fund, Medical Research Council (UK), Philippine Council for Health Research and Development. We are grateful to Dr. Leonor Sánchez-Busó and Dr. Magnus Unemo for the critical reading of the manuscript.

Tables

	Number of Isolates		
	2013	**2014**	**Total**
Total ARSP	24	27	51
Referred to ARSRL	16	20	36
Submitted for WGS	8	14	22
High-quality genomes	8	13	21
By sentinel site			
CVM		1	1
DMC	1		1
EVR	1		1
MMH	1	1	2
NMC		1	1
VSM	5	6	11
ZPH		4	4
By AMR profile			
PEN CIP TCY	2	8	10
PEN CIP	4	4	8
PEN	2		2
CIP TCY		1	1

Table 1. Number of *N. gonorrhoeae* isolates analysed by the ARSP and referred to the ARSRL during 2013 and 2014, isolates submitted for whole-genome sequencing, and high-quality *N. gonorrhoeae* genomes obtained, discriminated by sentinel site and AMR profile.

Characteristic	No. Isolates
Sex	
Male	10
Female	11
Age (in years)	
1-4	2
5-14	6
15-24	10
25-34	3
Patient Type	
In-Patient	0
Out-Patient	21
Specimen Origin	
Community	21
Hospital	0
Specimen Type	
Vagina	8
Penile Discharge	5
Cervix	3
Genital discharge, male	2
Decubitus ulcer	1
Urine	1
Other	1

Table 2. Demographic and clinical characteristics of 21 *N. gonorrhoeae* isolates.

Antibiotic Class	Antibiotic	Resistant Isolates	False Positive	False Negative	Concordance (%)	Resistance Genes/SNPs
Cephalosporin	Cefixime	0	0	0	100	*mtrR*_promoter_A24-
Cephalosporin	Ceftriaxone	0	0	0	100	*mtrR*_promoter_A24-
Penicillin	Penicillin	18	0	1	95.24	bla_{TEM}, *mtrR*_promoter_A24-, *ponA1*_L421P, *porB1b*_A121D, *porB1b*_G120K
Tetracycline	Tetracycline	14	7	0	66.67	*tetM*, *rpsJ*-V57M, *mtrR*_promoter_A24-
Fluoroquinolone	Ciprofloxacin	19	0	0	100	*gyrA*_D95G, *gyrA*_S91F, *parC*_D86N

Table 3. Comparison of genomic predictions of antibiotic resistance with laboratory susceptibility testing at the ARSRL.

Laboratory	No. of Isolates	STs (no. of isolates)	NG-MAST types (no. of isolates)	Resistance Profiles (no. of isolates)	Resistance Genes and SNPs
CVM	1	8780	novel	PEN CIP TCY	*bla*$_{TEM}$, *ponA1*_L421P, *gyrA*_S91F, *tetM*, *rpsJ*-V57M
DMC	1	1903	novel	PEN CIP TCY	*ponA1*_L421P, *porB1b*_A121D, *mtrR*_promoter_A24-, *gyrA*_D95G, *gyrA*_S91F, *tetM*, *rpsJ*-V57M
EVR	1	1582	13796	PEN CIP	*bla*$_{TEM}$, *gyrA*_D95G, *gyrA*_S91F, *rpsJ*-V57M
MMH	2	11956, 11208	1631, novel	PEN CIP	*bla*$_{TEM}$, *porB1b*_A121D, *porB1b*_G120K, *gyrA*_D95G, *gyrA*_S91F, *parC*_D86N
NMC	1	1588	novel	PEN CIP TCY	*bla*$_{TEM}$, *ponA1*_L421P, *gyrA*_S91F, *tetM*, *rpsJ*-V57M
VSM	11	8133 (2), 9364 (2), 10316 (1), 1587 (1), 11431 (1), 11963 (1), 1582 (1), 11956 (1), 4313 (1)	2187(2), 1691 (1), 1498 (1),1797 (1), novel (6)	PEN CIP (4) PEN CIP TCY (4) CIP TCY (1) PEN (2)	*bla*$_{TEM}$, *ponA1*_L421P, *porB1b*_A121D, *porB1b*_G120K, *tetM*, *rpsJ*-V57M, *gyrA*_D95G, *gyrA*_S91F, *parC*_D86N
ZPH	4	10316 (2), 9364 (1), 8130 (1)	2020, 11821, novel	PEN CIP TCY (3) PEN CIP (1)	*bla*$_{TEM}$, *porB1b*_A121D, *gyrA*_D95G, *gyrA*_S91F, *parC*_D86N, *tetM*, *rpsJ*-V57M

Table 4. Distribution of isolates, STs, NG-MAST types, resistance profiles and resistance genes and mutations across the 7 sentinel sites. The genetic resistance mechanisms are listed for all the isolates from each site.

Figure Legends

Figure 1. Yearly resistance rates of *N. gonorrhoeae* between 2000 and 2014 for penicillin (PEN), ciprofloxacin (CIP) and tetracycline (TCY).

Figure 2. Genomic surveillance of *N. gonorrhoeae* from the Philippines 2013-2014. Phylogenetic tree of 21 isolates from the Philippines, inferred with Pathogenwatch from 6563 variant sites. The tree leaves are coloured by sentinel site (map). The MLST sequence type (ST), the resistance phenotype for 5 antibiotics and the distribution of AMR determinants (red: present, grey: not found) are shown as blocks next to the tree. The data are available at https://microreact.org/project/ARSP_NGO_2013-14 and https://pathogen.watch/collection/hrgcb1kog51f-arspngo2013-2014

Figure 3. Global context of *N. gonorrhoeae* from the Philippines. A) Tree of 440 genomes from this study and a recent global collection, inferred with Pathogenwatch from 23444 variant sites in 1543 core genes. Purple nodes indicate genomes from the Philippines from this study (circles) or the global collection (squares). The data are available at https://pathogen.watch/collection/t5ja1h5pd4be-see-and-sequence-ngo-global-context. **B)** Detail of the subtree of closely related genomes from the Philippines (orange nodes) and Norway (blue node), inferred with Pathogenwatch. The numbers on the nodes indicate the pairwise SNP differences between isolates. The ST, NG-MAST type and presence of AMR determinants are indicated as blocks next to the tree. The full collection is available at https://pathogen.watch/collection/73stpaz0nqc7-see-and-sequence-ngo-european-context.

Bibliography

1. Wi T, Lahra MM, Ndowa F, Bala M, Dillon JR, Ramon-Pardo P, et al. Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. PLoS Med. 2017;14(7):e1002344.
2. Wi TEC, Saniel OP, Ramos ER, MacNeil J, Neilsen G. RTI/STI Prevalence in Selected Sites in the Philippines. 2002.
3. WHO Western Pacific Region. External Review of the National Health Sector response to HIV and Sexually Transmitted Infections 2013: Republic of the Philippines. 2015.
4. Unemo M, Shafer WM. Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. Clin Microbiol Rev. 2014;27(3):587-613.
5. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, et al. Failure of Dual Antimicrobial Therapy in Treatment of Gonorrhea. N Engl J Med. 2016;374(25):2504-6.
6. George CRR, Enriquez RP, Gatus BJ, Whiley DM, Lo YR, Ishikawa N, et al. Systematic review and survey of Neisseria gonorrhoeae ceftriaxone and azithromycin susceptibility data in the Asia Pacific, 2011 to 2016. PLoS One. 2019;14(4):e0213312.
7. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis. 2004;189(8):1497-505.
8. Jolley KA, Maiden MC. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics. 2010;11:595.
9. Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, Sednaoui P. High-level cefixime- and ceftriaxone-resistant Neisseria gonorrhoeae in France: novel penA mosaic allele in a successful international clone causes treatment failure. Antimicrob Agents Chemother. 2012;56(3):1273-80.
10. Clinical and Laboratory Standards Institute. M100-S26 Performance Standards for Antimicrobial Susceptibility Testing. 26th Edition. 2016.
11. Argimon S, Masim MA, Gayeta JM, Lagrada ML, Macaranas PK, Cohen V, et al. See and Sequence: Integrating Whole-Genome Sequencing Within the National Antimicrobial Resistance Surveillance Program in the Philippines. BioRxiv. 2019.
12. Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, Harris SR, et al. Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data. Microb Genom. 2016;2(8):e000083.
13. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb Genom. 2016;2(4):e000056.
14. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30(9):1312-3.
15. Harris SR, Cole MJ, Spiteri G, Sanchez-Buso L, Golparian D, Jacobsson S, et al. Public health surveillance of multidrug-resistant clones of Neisseria gonorrhoeae in Europe: a genomic survey. Lancet Infect Dis. 2018;18(7):758-68.
16. Sanchez-Buso L, Golparian D, Corander J, Grad YH, Ohnishi M, Flemming R, et al. The impact of antimicrobials on gonococcal evolution. Nat Microbiol. 2019;4(11):1941-50.
17. Bailey AL, Potter RF, Wallace MA, Johnson C, Dantas G, Burnham CA. Genotypic and Phenotypic Characterization of Antimicrobial Resistance in Neisseria gonorrhoeae: a Cross-Sectional Study of Isolates Recovered from Routine Urine Cultures in a High-Incidence Setting. mSphere. 2019;4(4).
18. Mortimer TD, Grad YH. Applications of genomics to slow the spread of multidrug-resistant Neisseria gonorrhoeae. Ann N Y Acad Sci. 2019;1435(1):93-109.
19. Bennett JS, Jolley KA, Sparling PF, Saunders NJ, Hart CA, Feavers IM, et al. Species status of Neisseria gonorrhoeae: evolutionary and epidemiological inferences from multilocus sequence typing. BMC Biol. 2007;5:35.

20. Didelot X, Dordel J, Whittles LK, Collins C, Bilek N, Bishop CJ, et al. Genomic Analysis and Comparison of Two Gonorrhea Outbreaks. MBio. 2016;7(3).

Figures

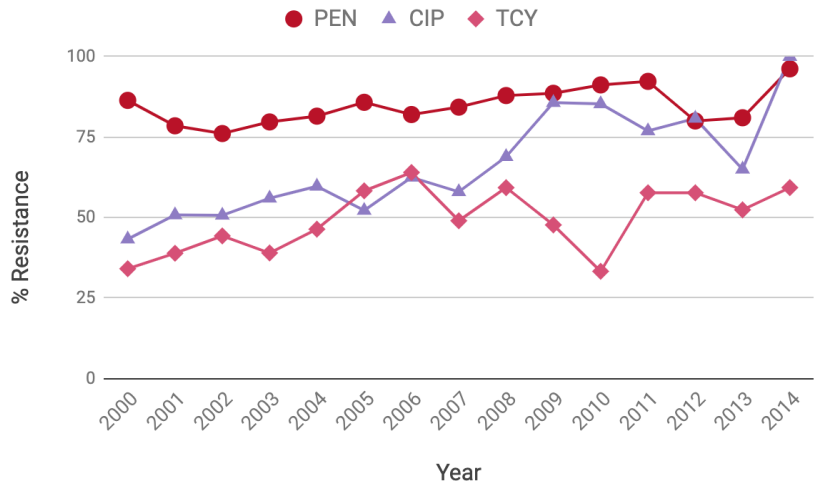

Figure 1. Yearly resistance rates of *N. gonorrhoeae* between 2000 and 2014 for penicillin (PEN), ciprofloxacin (CIP) and tetracycline (TCY).

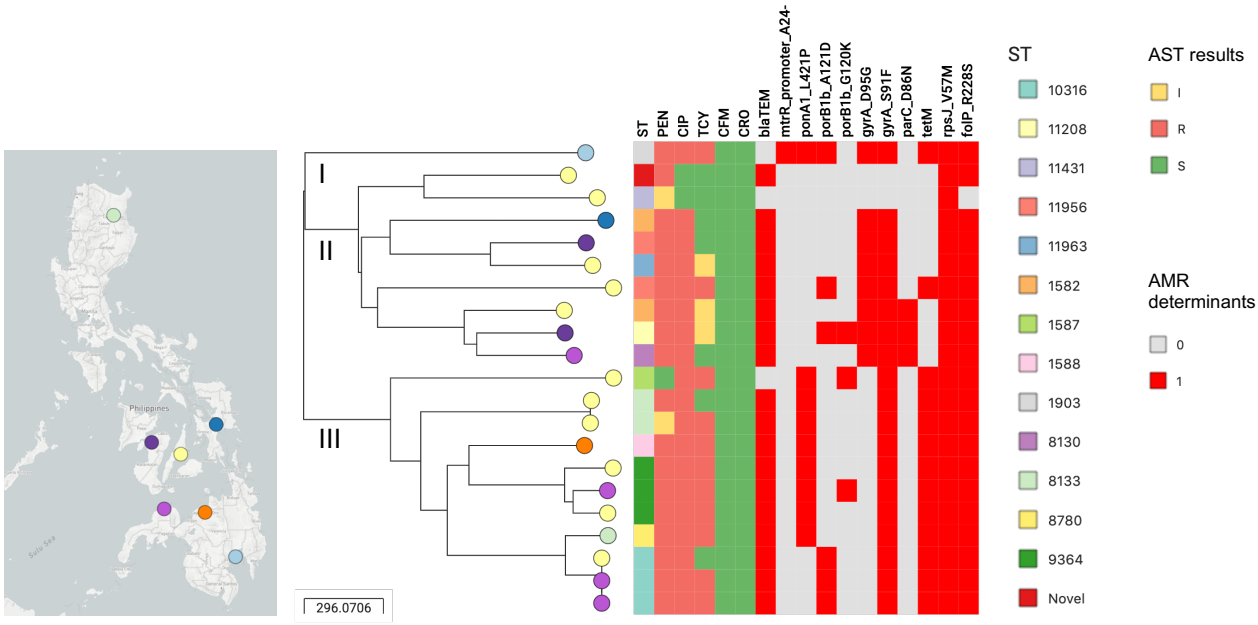

Figure 2. Genomic surveillance of *N. gonorrhoeae* from the Philippines 2013-2014. Phylogenetic tree of 21 isolates from the Philippines, inferred with Pathogenwatch from 6563 variant sites. The tree leaves are coloured by sentinel site (map). The MLST sequence type (ST), the resistance phenotype for 5 antibiotics and the distribution of AMR determinants (red: present, grey: not found) are shown as blocks next to the tree. The data are available at https://microreact.org/project/ARSP_NGO_2013-14 and https://pathogen.watch/collection/hrgcb1kog51f-arspngo2013-2014

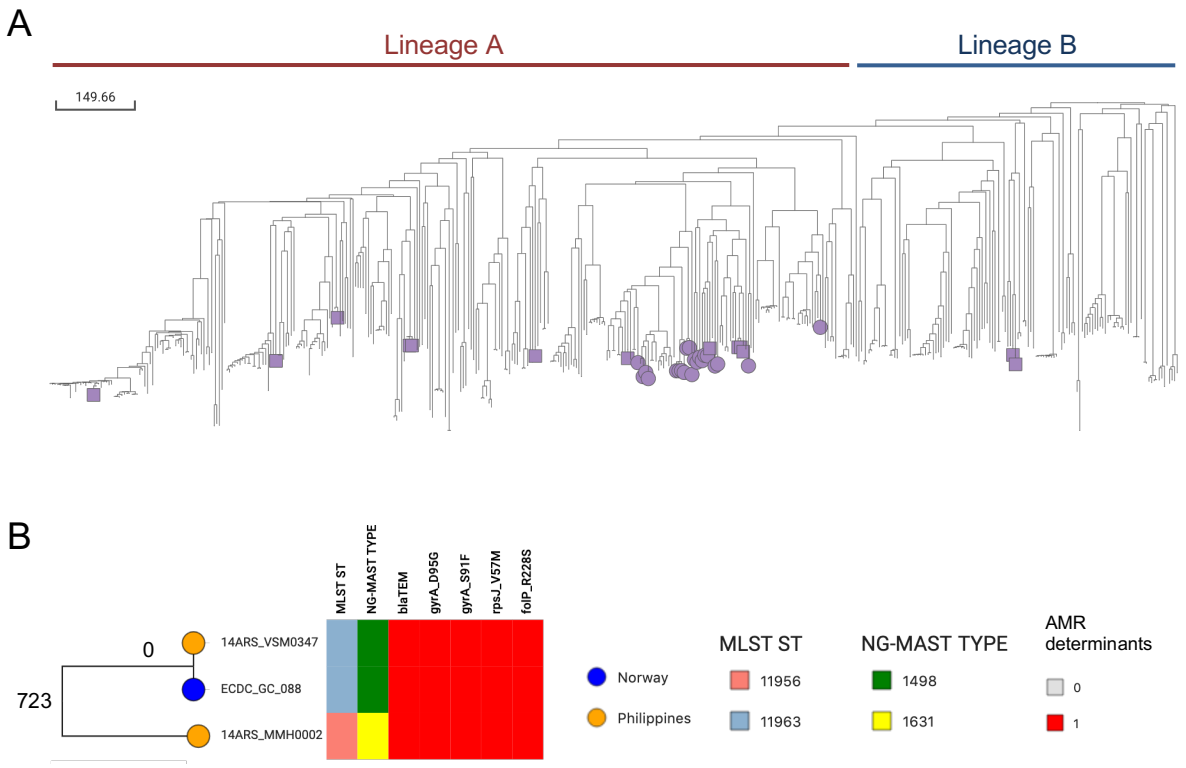

Figure 3. Global context of *N. gonorrhoeae* from the Philippines. A) Tree of 440 genomes from this study and a recent global collection, inferred with Pathogenwatch from 23444 variant sites in 1543 core genes. Purple nodes indicate genomes from the Philippines from this study (circles) or the global collection (squares). The data are available at https://pathogen.watch/collection/t5ja1h5pd4be-see-and-sequence-ngo-global-context. **B)** Detail of the subtree of closely related genomes from the Philippines (orange nodes) and Norway (blue node), inferred with Pathogenwatch. The numbers on the nodes indicate the pairwise SNP differences between isolates. The ST, NG-MAST type and presence of AMR determinants are indicated as blocks next to the tree. The full collection is available at https://pathogen.watch/collection/73stpaz0nqc7-see-and-sequence-ngo-european-context.