Article:
Kong, LY, Wilson, JD, Moura, IB orcid.org/0000-0002-3019-7196 et al. (5 more authors) (2021) Utility of Whole Genome Sequencing in Assessing and Enhancing Partner Notification of Neisseria gonorrhoeae Infection. Sexually Transmitted Diseases, 48 (10). pp. 773-780. ISSN 0148-5717

https://doi.org/10.1097/olq.0000000000001419

Utility of whole genome sequencing in assessing and enhancing partner notification of *Neisseria gonorrhoeae* infection

Ling Yuan Kong MD[1,2‡], Janet D. Wilson MB ChB[1,3], Ines B. Moura PhD[2], Warren Fawley PhD[2,5], Laura Kelly MSc[1,3], A. Sarah Walker PhD[4,6,7*], David W. Eyre BMBCh[4*], Mark H. Wilcox MD[1,2*]

[1]Leeds Teaching Hospitals NHS Trust, UK

[2]Leeds Institute for Medical Research, Faculty of Medicine and Health, University of Leeds, UK

[3]Leeds Sexual Health, UK

[4]Nuffield Department of Medicine, University of Oxford, UK

[5]Public Health England, UK

[6]NIHR Oxford Biomedical Research Centre, University of Oxford, UK

[7]NIHR Health Protection Research Centre in Antimicrobial Resistance and Healthcare Associated Infections, University of Oxford, UK

[‡]Current affiliation:
Division of Infectious Diseases
SMBD Jewish General Hospital
McGill University

*Joint last authors

Corresponding author:

Ling Yuan Kong MD
SMBD Jewish General Hospital, G-200
3755 chemin de la Côte Sainte-Catherine
Montréal, Québec, H3T 1E2, Canada
Email: ling.kong@mcgill.ca
Telephone: (514) 340-8222 ext. 22933
Fax: (514) 340-7546

Summary word count: 29
Abstract word count: 246
Manuscript word count: 3433
Number of references: 16
Number of figures: 6
Number of tables: 1

Conflicts of Interest and Source of Funding: This work was supported by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Healthcare Associated Infections and Antimicrobial Resistance at Oxford University in partnership with Public Health England (PHE) [grant HPRU-2012-10041 and NIHR200915] and the NIHR Biomedical Research Centre, Oxford. DWE is a Robertson Foundation Fellow and an NIHR Oxford BRC Senior Fellow. ASW is an NIHR Senior Investigator. The other authors have no relevant declarations.

Short Summary

In a sexual health clinical setting, we demonstrate the feasibility and utility of whole genome sequencing as a tool to measure the performance of and to improve partner notification.

Abstract

Background

Gonorrhea is a sexually transmitted infection of global concern. We investigated whole genome sequencing (WGS) as a tool to measure and enhance partner notification (PN) in gonorrhea management.

Methods

Between May-November 2018, all *N. gonorrhoeae* isolated from patients attending Leeds Sexual Health, UK, underwent WGS. Reports listing sequences within 20 single nucleotide polymorphisms (SNPs) of study isolates within a database containing select isolates from April 1 2016 to November 15 2018 were issued to clinicians. The proportion of cases with a potential transmission partner identified by PN was determined from patient and PN data. WGS reports were reviewed to identify additional cases within ≤6 SNPs and verified for PN concordance.

Results

380 isolates from 377 cases were successfully sequenced; 292 had traceable/contactable partners and 69 (18%) had a potential transmission partner identified by PN. Concordant PN and WGS links were identified in 47 partner pairs. Of 308 cases with no transmission partner by PN, 185 (60%) had a case within ≤6 SNPs; examination of these cases' PN data identified seven partner pairs with previously unrecognized PN link, giving a total of 54 pairs; all had ≤4 SNP differences. WGS clusters confirmed gaps in partner finding, at individual and group levels.

Despite the clinic providing sexual health services to the whole city, 35 cases with multiple partners had no genetically related case, suggesting multiple undiagnosed infections.

Conclusions

WGS could improve gonorrhea PN and control by identifying new links and clusters with significant gaps in partner finding.

Key Words:

Gonorrhea, partner notification, whole genome sequencing

Introduction

Gonorrhea, a sexually transmitted infection (STI) caused by *Neisseria gonorrhoeae*, has emerged as a global public health concern due to increasing incidence and antimicrobial resistance(1). The worldwide appearance of isolates resistant to ceftriaxone and/or azithromycin highlights the urgent need for effective control measures(2-4). In the United Kingdom, traditional control methods such as partner notification (PN), screening and treatment of asymptomatic persons, and promotion of condom use are standard practice with nationally established guidelines(5, 6); yet gonorrhea rates are increasing(7). Whole genome sequencing (WGS) has been used to characterize *N. gonorrhoeae* lineages, including those with antimicrobial resistance(8), investigate outbreaks(9, 10), predict antimicrobial susceptibility patterns(11), and provide insight into transmission networks(12, 13). Here, we investigate the use of WGS as a tool to measure the performance of PN and to enhance current control of gonorrhea infections in a clinical setting.

Materials and Methods

Clinical setting

This study was conducted between May 1 and November 15 2018 at Leeds Sexual Health (LSH), a clinic with a catchment area of one million people and over 70,000 students(14). Screening, diagnosis and management of gonorrhea and PN for confirmed cases followed national guidelines(5, 6, 15). Anatomical sites were sampled according to sexual history and symptoms. For asymptomatic men who have sex with men (MSM), samples were taken from the urethra, rectum, and pharynx. Samples were taken for culture when patients presented with gonorrhea symptoms, or when asymptomatic cases had positive nucleic acid amplification tests (NAATs), before treatment whenever possible. PN information on sexual contacts within the previous three

months for each case was obtained, including name, gender, type and date of last sex, and partner contact information if available. Patients diagnosed with gonorrhea were asked to return for test of cure 14 days after treatment. Information on whether reported partners attended a sexual health service was documented at this visit. Those diagnosed with gonorrhea >= four weeks from initial diagnosis, with distinct dates of symptoms if applicable, were considered as two infection episodes.

Isolation of *N. gonorrhoeae*

Samples were cultured and tested for antimicrobial susceptibility at Leeds Teaching Hospitals NHS Trust microbiology laboratory as per local protocols (Supplementary Appendix).

Whole genome sequencing and data reporting

Isolates from every culture-positive case during the study period underwent WGS at University of Leeds, plus 335 historical isolates from Leeds collected during 2016-2017 (July – September annually) as part of the Public Health England Gonococcal resistance to antimicrobials surveillance programme. In patients with more than one positive sample from different sites, typically only the first accessioned was submitted for sequencing. Details on DNA extraction, sequencing, and bioinformatics are provided in the Supplementary Appendix. WGS data were used to generate one report per study isolate, containing the isolate's sample identifier, sequencing quality parameters, and sample identifiers for all sequences within 20 SNPs to date (Supplementary Figure 1). Reports were issued to LSH in weekly batches, with a target turnaround of 14 days from sample collection, determined to be a clinically reasonable timeframe since patients diagnosed with gonorrhea would return 14 days after for follow-up and

test of cure. Data from reports were examined weekly at LSH and formally analyzed at the end of the study period (Supplementary Appendix and Supplementary Figure 2).

PN analysis

Clinical data was collected on all cases associated with WGS reports, including demographics, details of infection, and PN data (Supplementary Appendix). To assess PN effectiveness, we analysed partners reported by each case. Reported partners were first classified as traceable (if index cases stated they were able to contact them or if enough information was given to enable a provider referral) or untraceable. Among traceable partners, attendance was classified as verified (if they attended LSH or had a clinician-verified attendance elsewhere), unverified (if index case reported partner attendance which could not be confirmed), or no known attendance.

We calculated the nationally established auditable outcome measure for gonorrhea PN in the United Kingdom, defined as the number of all contacts of the index case who attended a service within four weeks of the first PN discussion, targeting 0.4 contacts per index case in large conurbations or 0.6 contacts elsewhere(5). Additionally, we determined the proportion of cases with a potential transmission partner identified by PN, i.e. those with a partner with a culture/NAAT confirmed diagnosis of gonorrhea. For couples reporting each other, only one case was counted as having an identified transmission partner, as one partner had to have acquired the infection from a third person.

WGS analysis

We examined WGS data for PN-linked cases to confirm concordance. Genetic distances between isolates from known partners with a culture positive diagnosis were recorded to estimate the genetic distance expected following presumed direct transmission. Combining our findings and the observed number of SNPs between couples with epidemiologically confirmed direct contact from previous studies(12) we classify potential direct transmissions as pairs within ≤6 SNPs. For other cases linked by WGS reports (i.e. within 20 SNPs), but not PN, to determine if transmission was plausible, either directly or indirectly through a third party, we applied a published nomogram for *N. gonorrhoeae*(12). The nomogram categorizes any given pair of isolates as "transmission supported" or "transmission not supported" based on the time and number of SNPs between them. Thus, all pairs from WGS reports were classified as: not linked by nomogram, linked by nomogram but not by PN, and linked by both nomogram and PN. Among pairs linked by nomogram but not by PN, we examined PN data in more detail to search for any unidentified potential direct transmission events. We further examined WGS links between isolates from the same patient when they had more than one infection episode.

Ethics

As no patient-identifiable data was used outside the usual clinical team and sequencing performed on routinely cultured samples, this study was conducted as an NHS service evaluation of WGS as an alternative to previously used typing methods (e.g. NG-MAST), and was therefore exempt from requiring ethical approval using the Health Research Authority guidance tool.

Results

During the study period, 474 cases of gonorrhea were diagnosed; cultures were performed on 455. The 385 positive isolates were submitted for WGS, five were excluded (one not *N. gonorrhoeae*, 4 contaminated culture plates); thus, 380 isolates were successfully sequenced and WGS reports generated (sample list in Supplementary Table 2). These originated from 362 patients (median age 23 years) with 377 infection episodes (15 patients had two infection episodes). Cases and diagnosed partners are included in these numbers. Although most patients had only one isolate submitted, one patient had three identical isolates sequenced from different anatomical sites from the same infection episode, and another had two identical isolates from different sites from the same infection episode; one sequence per infection episode was retained for analysis. Another two patients had two isolates submitted from different sites on the same day; these revealed genetically unrelated isolates (4699 and 3919 SNPs different), and were counted as distinct infection episodes. Thus, 377 sequences were analyzed. There were 118 cis-females, one trans-woman, and 243 cis-males. Among females, the majority (116/118, 98%) were female heterosexuals; two were women who have sex with men and women (WSMW). Among males, nearly half (119/243, 49%) were MSM, 22 (9%) were men who have sex with men and women (MSMW), and 102 (42%) were male heterosexuals. One patient was a transgender woman who has sex with men. Amongst males, infections were primarily urethral (168/254, 66%), followed by rectal (62/254, 24%) and pharyngeal (24/254, 9%). Amongst females, most were urogenital infections (114/122, 93%). All isolates were susceptible to ceftriaxone; 14 were resistant, and 29 had intermediate susceptibility, to azithromycin. Overall, 319 (84%) isolates were successfully sequenced within 14 days, and 246(65%) had WGS reports sent to LSH within 14 days of sample reception. Reasons for delayed WGS results included numbers of samples exceeding weekly capacity (12-16 isolates), isolates missing the scheduled

batch due to impurity (requiring sub-culture), delays associated with WGS report generation (software problems, manual interventions), and sub-optimal sequence data. Turnaround times from sample collection to time points in the sequencing and reporting process are presented in Supplementary Table 1.

Partner notification

From 377 episodes 1395 partners were reported, median two per case. Eighty-five cases had only untraceable partners; 292(77%) cases reported at least one traceable partner, providing a total of 434 traceable partners (Figure 1).

Considering performance against national audit standards, 125 partners had verified attendance in Leeds or elsewhere within four weeks of PN discussion (9% of total reported partners), representing 0.33 contacts per index case (national target 0.40). Including 44 partners with unverified attendance, there were 0.44 contacts per index case. By study end, 11 more partners had verified attendance for 0.48 contacts per index case.

Eighty-five cases had culture-positive verified partners diagnosed at LSH, 12 had verified NAAT-positive but culture-negative partners, and four had verified partners testing positive at another sexual health clinic. Among the 85 cases with culture-positive partners, there were 32 mutually reporting couples for which only one partner could be counted; thus, in total, only 69 (85+12+4-32) (18%) of the 377 infection episodes had a potential transmission partner identified by PN, with 308 cases with no identifiable transmission partner.

WGS results for PN-linked cases

In examining WGS data for PN-linked cases, we considered the proportion of partner WGS data that was available at the test-of-cure visit for each index case. Of 130 partners with verified attendance in Leeds, 85 were culture-positive and had isolates submitted for sequencing. As over half of reported partners (78/130, 60%) attended before or on the same day as the index case (Table 1) and 53/78(68%) were culture-positive, 45/53(85%) of partner isolates could be linked to their index cases by WGS reports (i.e. within 20 SNPs) at 14 days. The remaining eight partners with isolates cultured before their index cases included two diagnosed before the study and therefore not sequenced, one with a contaminated culture, one with an unrelated isolate (4429 SNPs different), and four with a delay in sequencing. The four partner isolates that were delayed in sequencing were linked to their index cases at a later time when both sequences were available.

A further 52 partners attended after their index cases; 32 were culture-positive, and 30(94%) were linked by WGS reports (within 20 SNPs). The two non-linked partners were diagnosed after the study end so not captured in the database. Thus, of 85 partners testing culture-positive in Leeds, 79(93%) could be linked by WGS to the cases who reported them. These 79 partners linked to their index cases by both WGS and PN, comprised 64 individuals from 32 mutually reporting couples, and 15 from couples where only one partner reported the other. Among these 47 (32 + 15) couples with known sexual contact and presumed direct transmission and available sequence data, all pairs of isolates were between 0-4 SNPs (Figure 3).

WGS findings across the whole study

From the 377 cases analyzed, 266 had linked isolates that were within the 99% prediction interval supporting transmission using a previously published nomogram, and 237 cases had links to isolates within 6 SNPs (Figure 2). Examining the 308 cases with no transmission partner found by PN, 211(69%) had ≥1 plausible direct or indirect transmission partner within the nomogram thresholds and 185(60%) ≥1 plausible direct or indirect transmission partner within ≤6 SNPs. Thus, the majority of cases did not have a transmission partner identified by PN but did have a genetically plausible direct or indirect transmission partner within the *N. gonorrhoeae* infections diagnosed in Leeds.

Clinic health advisors were able to use WGS reports to identify seven additional couples with suspected direct transmission, not identified by PN. For example, several cases reported partners without verifiable information (e.g. first name only) for whom confirmation of partner attendance was impossible with available information, but facilitated by WGS. Together with the 47 couples linked through PN, a total of 54 couples with presumed direct transmission were identified. All pairs were within 4 SNPs (Figure 3).

Fifteen patients had two infection episodes during the study. Three had the same isolate twice with the same reported partners. Five patients reported at least one partner that was the same across episodes, but had genetically unrelated isolates between episodes; this includes one patient who had two genetically unrelated isolates from different anatomical sites on the same day. He reported only one partner. The remaining were all MSM, had different isolates, and did not report the same partners across episodes.

<u>Sequencing-based clusters</u>

Cases related to ≥1 other case(s) within 20 SNPs were clustered into groups to describe the different lineages circulating in Leeds (Supplementary Figure 3). Each cluster contained only genomes with the same multi-locus sequence type (MLST, provided in Supplementary Table 2). 322 cases fell into 62 clusters of ≥2 cases, plus 55 singletons. Most clusters (54/62, 87%) had <10 cases, with 34 containing 2-3 cases, and only two containing >20 cases (21 and 31 cases). The eight clusters with >10 cases were mixed in terms of several characteristics (Figure 4). For example, although two major clusters contained primarily MSM, these were mixed with MSMW and heterosexuals. Three clusters included HIV seropositive and seronegative cases. Although no isolates had azithromycin resistance in the two largest clusters, a cluster of 17 cases contained seven cases with azithromycin intermediate resistance. All clusters contained asymptomatic cases, including three with more than half who were asymptomatic.

We next combined PN networks with cases linked within ≤6 SNPs to allow us to visualize potential direct transmission events. The vast majority of PN reported partners were not verified, whilst diagnosed cases could be organized into genetically related transmission chains (Figure 5). Tracking the growth of clusters over time permitted us to make observations both at an individual patient level and at a group level. At an individual level, linking PN data and WGS clusters allowed us to identify undiagnosed individuals reported by several index cases: for example, two heterosexual females diagnosed with three infections over four months reported the same male who could not be located within the database.

At a group level, emerging epidemiological trends could be identified. For example, one cluster consisted of two heterosexual males with an identical isolate, both of whom reported contact with female sex workers; another contained three heterosexual males with an identical isolate, with one reporting sex worker contact. Another contained a female (sex worker) who

reported multiple male partners, but the only other case in the cluster was a heterosexual male who reported two female partners who were not sex workers. Yet another contained eight MSM reporting recent sauna use, including two naming the same sauna. Finally, we noted that of the 55 genetic singletons, 35 reported multiple sexual partners. As might be expected, many of the total 189 partners reported by the singletons were untraceable (110, 58%), and were from outside the local area (other countries [68, 36%)], or elsewhere in the UK [21, 11%]).

Discussion

Although WGS has been useful to inform public health measures surrounding *N. gonorrhoeae* outbreaks, ours is the first study to evaluate its usefulness in a clinical setting. It is also the first exploration of the clinical utility of WGS for PN as part of routine STI control, where we demonstrate the feasibility of sequencing and reporting to a sexual health clinic. Although WGS confirmed nearly all known links from PN with a sequenced isolate, PN identified potential transmission partners for only a minority (18%) of cases, despite considerable investment in skilled PN services. This was frequently due to the index cases' lack of knowledge of their partners' identities or reluctance to disclose information. WGS also enabled identification of cases of confirmed attendance that could not be verified through PN, therefore enhancing the reported performance of PN. Although the number of verified contacts per index case, 0.33, fell below the national audit standard of 0.4, even had this been met, a majority of index cases would still have undiagnosed partners.

WGS offers a potential assay of PN performance and the effectiveness of the clinic in terms of the proportion of all cases diagnosed. For example, 60% of cases with no transmission partner by PN had a closely genetically related case within 6 SNPs. WGS also offers a potential

mechanism for directing interventions to key gaps in partner finding. We have shown examples where individual-level focus could be achieved: for the undiagnosed partner reported by several index cases, it would be reasonable to intensify health advisor efforts, and further information gathering, surrounding a potential untreated person. More frequently, groups could be identified: the recognition of an emerging transmission chain involving multiple sauna-attending MSM might prompt intensified screening in addition to the usual outreach services. The example of the female sex worker with multiple related cases could prompt liaison with sex worker outreach projects to sensitively increase efforts to locate her and her partners. A similar approach could be adopted for partners of cases representing genetic singletons, especially when clinical history is consistent with local acquisition. As the clinic serves the whole local population, it appears likely that a large proportion of such cases' partners are undiagnosed. Finally, examination of patients with repeat infections can reaffirm the direction of intervention needed: re-infection from the same partners vs. acquisition from new sources. We did not systematically sequence isolates from each positive anatomical site, assuming most such cases would yield identical isolates. However, out of the four cases with >=1 isolate submitted from different sites on the same day, two revealed genetically unrelated isolates, raising the possibility of more than one transmission partner (one of these cases reported only one partner). This represented an unexpected finding that could have implications for further questioning of the patients.

To summarize, periodic review of WGS clusters could inform PN efforts in two main ways. First, one might search for any missed attendances in reported partners with incomplete information. Second, areas requiring intervention can be identified through the examination of clusters and genetic singletons. General epidemiological trends can be followed: we observed evidence of bridging between sexual populations (e.g. MSM and heterosexuals), and mixing of

individuals with discrepant HIV sero-status within the same clusters, similarly to other studies(13, 16). Granular trends within clusters, such as increasing rates of asymptomatic or extra-genital infections and antibiotic resistance, can be identified and acted upon rapidly when observed within WGS clusters, which provide evidence for sustained transmission, providing focus and incentive for intervention.

Our study also provides further data for improved clinical use of genomic tools such as the nomogram, which provides compatibility with direct or indirect transmission. In our cohort, couples with presumed direct transmission were often within 0-1 SNPs, and all were within 4 SNPs of one another. This reflects the fact that most pairs related by recent transmission are more likely to have lower SNP values (Figure 6).

WGS implementation in a sexual health setting raises ethical concerns. It is important to recognize that the PN process involves the seeking and use of sensitive information, to which a reported partner cannot provide consent a priori. In this context, WGS represents an adjunctive tool to enhance surveillance and partner finding as used in outbreaks (9). Potentially important issues are that neither partner has consented to links made by WGS, and WGS may also provide indirect links between two individuals via one or more intermediate cases. This is an area that merits formal ethical research and patient and public consultation.

Our study has certain limitations. As the first exercise and analysis of its kind, the availability and utility of results within 14 days and SNP threshold used were exploratory. The implementation of weekly analysis with the clinical team had challenges, such as the exact actions that could be taken within ethical boundaries, when a gap in partner finding was identified. However, our work provides a framework on which subsequent clinical implementation efforts can be based, by demonstrating that a periodic examination of WGS

clusters and analysis could enhance PN. Cost-effectiveness analysis of implementing such a pipeline should be considered. Finally, despite providing sexual health care to the entire city, our study is a single-centre study that may not be representative of different settings.

Conclusion

Against a background of rising gonorrhea infection rates, we emphasise that PN only enables the sources of a minority of cases to be identified and treated. There is an urgent need for novel control interventions. We have demonstrated the feasibility and utility of WGS to confirm PN links, reveal new PN links, and to help clinicians focus in on undiagnosed cases for intervention. With expanding databases and understanding of relationships between genomic and clinical data, the implementation of WGS in sexual health will likely be beneficial to the control of STIs.

Declarations

DWE declares lecture fees from Gilead outside the submitted work. IBM has received funding to attend conferences from Techlab, Inc. outside the submitted work. No other author has a conflict of interest to declare.

Contributors

MHW, DWE, ASW, JDW, and LYK designed and coordinated the study. IBM and WF performed WGS sequencing and reporting. LYK, LK, and JDW contributed to data collection and management. LYK analyzed the data with support from DWE, JDW, ASW, and MHW. LYK wrote the first draft of the paper and all authors read, commented on, and approved the final manuscript.

References

1. 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.
2. Lahra MM, Martin I, Demczuk W, et al. Cooperative Recognition of Internationally Disseminated Ceftriaxone-Resistant Neisseria gonorrhoeae Strain. Emerg Infect Dis. 2018;24(4).
3. Eyre DW, Sanderson ND, Lord E, et al. Gonorrhoea treatment failure caused by a Neisseria gonorrhoeae strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. Euro Surveill. 2018;23(27).
4. Poncin T, Merimeche M, Braille A, et al. Two cases of multidrug-resistant Neisseria gonorrhoeae related to travel in south-eastern Asia, France, June 2019. Euro Surveill. 2019;24(36).
5. Society of Sexual Health Advisers**.** Guidance on Partner Notification. London; 2015.
6. British Association for Sexual Health and HIV**.** Standards for the management of sexually transmitted infections (STIs). 2019.
7. Public Health England**.** Sexually transmitted infections and screening for chlamydia in England, 2018. Health Protection Report.
8. Grad YH, Harris SR, Kirkcaldy RD, et al. Genomic Epidemiology of Gonococcal Resistance to Extended-Spectrum Cephalosporins, Macrolides, and Fluoroquinolones in the United States, 2000-2013. J Infect Dis. 2016;214(10):1579-87.
9. Chisholm SA, Wilson J, Alexander S, et al. An outbreak of high-level azithromycin resistant Neisseria gonorrhoeae in England. Sex Transm Infect. 2016;92(5):365-7.

10. Didelot X, Dordel J, Whittles LK, et al. Genomic Analysis and Comparison of Two Gonorrhea Outbreaks. MBio. 2016;7(3).
11. Eyre DW, De Silva D, Cole K, et al. WGS to predict antibiotic MICs for Neisseria gonorrhoeae. J Antimicrob Chemother. 2017;72(7):1937-47.
12. De Silva D, Peters J, Cole K, et al. Whole-genome sequencing to determine transmission of Neisseria gonorrhoeae: an observational study. Lancet Infect Dis. 2016;16(11):1295-303.
13. Williamson DA, Chow EPF, Gorrie CL, et al. Bridging of Neisseria gonorrhoeae lineages across sexual networks in the HIV pre-exposure prophylaxis era. Nat Commun. 2019;10(1):3988.
14. How is the Student Population in Leeds Bosting the Region? Leeds Student Magazine. 2018; November 19 2018.
15. Fifer H, Saunders J, Soni S, Sadiq ST, FitzGerald M**.** 2018 UK national guideline for the management of infection with Neisseria gonorrhoeae. Int J STD AIDS. 2020;31(1):4-15.
16. Town K, Field N, Harris SR, et al. Phylogenomic analysis of Neisseria gonorrhoeae transmission to assess sexual mixing and HIV transmission risk in England: a cross-sectional, observational, whole-genome sequencing study. Lancet Infect Dis. 2020.

Figure 1. Routine partner notification results

Separately submitted

Legend: Flowchart of routine partner notification results

Figure 2. Breakdown of all WGS links

Separately submitted

Legend: Flowchart of WGS links analysis for study cases

Figure 3. SNP distribution for pairs of isolates from couples with presumed direct transmission

Separately submitted

426 Table 1. Partners with verified attendance in Leeds and WGS report links to their index cases
427

	Number of partners with verified attendance in Leeds (n)	Number who tested culture-positive (n)	Partner and index case linked by WGS reports (n, %)	WGS linkage at test of cure visit* (n, %)
Before or on same day as index case attendance	78	53	49 (93%)	45 (85%)
Within four weeks of index case attendance	42	25	23 (92%)	11 (44%)
Four weeks or more after index case attendance	10	7	7 (100%)	--
Total	130	85	79 (93%)	56 (65%)

428 *Test of cure visit usually occurred at14 days from the index case's initial attendance

Figure 4. Patient and infection characteristics of WGS clusters containing more than ten cases

Separately submitted

Legend:

Eight clusters are represented with each horizontal bar representing a cluster

MSM: men who have sex with men only; MSMW: men who have sex with men and women;

M Hetero: male heterosexuals; WSMW: women who have sex with men and women;

F Hetero: female heterosexuals

Figure 5. PN and WGS networks

Separately submitted

Legend:

Large circles denote cases within study cohort; small circles denote historical cases; empty circles denote untraceable partners from PN. A deidentified text version of the network plotted is provided as a Supplementary File.

Figure 6. Transmission nomogram with bands depicting varying confidence ranges for recent transmission event

Separately submitted