Whole genome sequencing of *Neisseria gonorrhoeae* reveals transmission clusters involving patients of mixed HIV-serostatus

Joanna Peters[1,2], Fiona Cresswell[3], Lauren Amor[4], Kevin Cole[1,2], Gillian Dean[3], Xavier Didelot[5], Dilrini De Silva[6], David W. Eyre[6], John Paul[1,2]

1. Brighton PHE Collaborative Centre, Royal Sussex County Hospital, Brighton, UK.
2. Microbiology and Infection, Royal Sussex County Hospital, Brighton, UK.
3. Claude Nicol |Centre, Royal Sussex County Hospital, Brighton, UK.
4. Brighton and Sussex Medical School, Falmer, UK.
5. Department of Infectious Disease Epidemiology, Imperial College, London, UK
6. Nuffield Department of Medicine, University of Oxford, Oxford, UK

Corresponding author: Dr John Paul,
Microbiology and Infection, Royal Sussex County Hospital, Eastern Road, Brighton, BN2 5BE.
john.paul@phe.gov.uk 07810 815445

Word count (excluding title page, abstract, references, figures and tables): 2488

Key words:
Gonorrhoea
Neisseria gonorrhoeae
Whole genome sequencing
Sexual behaviour
Sexual networks
HIV-serosorting

Key messages
Combined whole genome sequencing data and patient questionnaire data allows enhanced understanding of gonorrhoea transmission.

Gonorrhoea transmission networks include patients of mixed HIV-serostatus: serosorting may be declining as a practice.

Public health interventions should be designed to minimise the risk of disease transmission associated with use of recreational drugs ("chemsex") and use of geosocial networking applications to facilitate contact with partners.

Legend to Figures and Tables.

Figure 1. Maximum likelihood recombination-corrected phylogenetic tree of 84 genetically distinct infections identified in 83 individuals with whole genome sequencing data and questionnaire data. The tips of the tree are labelled with transmission cluster identifiers (A-Z, a-m) based on transmission clusters identified in [10]. Five clusters with three or more cases with questionnaire data are highlighted. A single isolate for each patient infection is shown.

Figure 2. Maximum likelihood recombination-corrected phylogenetic trees of transmission clusters 1-3. Where more than one sequence was available from a patient, all are shown.

Table 1. Anatomical sites of infection in MSM

Table 2. Patient characteristics and behavioural information for 14 clusters of patients linked by WGS data. *one patient did not report sexual orientation

Supplementary materials

Table S1. NCBI (National Center for Biotechnology Information) short read archive accession numbers for sequences used in this publication with associated patient study numbers, anatomical site details for samples, patient gender and subtype/cluster designations

Abstract

Objectives
Prevention and control of gonorrhoea depends on understanding the nature of sexual networks and risk factors for infection. We aimed to use high resolution typing (whole genome sequencing) of *Neisseria gonorrhoeae* isolates plus patient questionnaire data to gain insights into transmission patterns in a high prevalence setting.

Methods
During a nine month period (July 2014-March 2015) patients diagnosed with gonorrhoea attending sexual health service in Brighton, UK, were invited to provide anonymised detailed information by questionnaire about risk factors for infection. Questionnaire data plus whole genome sequence data from cultured isolates were analysed to yield information about sexual networks and risk factors for infection.

Results
104/149 individuals who consented to participate in the study were culture-positive. 97/104 (93%) were male. 80 self-reported to be men who have sex with men (MSM). 35/104 (34%) of patients were HIV-positive. 51/104 (49%) individuals reported using geosocial networking applications to facilitate contact. Sex under the influence of drugs was reported by 16/34 (46%) of HIV-positive MSM, 17/41 (41%) of HIV-negative MSM and 5/15 (31%) of heterosexuals. WGS data were available for 100 isolates from 83 patients. 55 isolates (66%) belonged to genetically related subtypes involving one or more patients, who could be plausibly linked through recent direct or indirect transmission. Four transmission clusters containing 3-12 individuals were composed of MSM of mixed HIV-serostatus.

Conclusions
We show that data obtained from whole genome sequencing of *Neisseria gonorrhoeae* and enhanced epidemiological data obtained from patient questionnaires are mutually supportive and reveal insights into sexual networks. Our findings suggest that serosorting may have declined as a practice and indicate the importance of designing public health interventions that target infection risks associated with recreational drug use and contact made using geosocial networking applications.

Introduction

Gonorrhoea is of public health concern globally, with 78 million cases occurring annually and increasing rates of antimicrobial resistance[1]. Between 2014 and 2015, the annual incidence rose in England by 11% from 37,100 to 41,193 reported cases[2]. In the USA almost 400,000 cases were reported in 2015, a 12.8% increase from 2014[3]. Gonorrhoea is associated with significant morbidity including pelvic inflammatory disease and tubal factor infertility in women and epididymo-orchitis in men.

Gonorrhoea control depends on effective prevention, reliable diagnostics and prompt antimicrobial treatment of the index patient and recent sexual contacts. Asymptomatic carriage of *Neisseria gonorrhoeae,* especially in the rectum and pharynx, facilitates onward transmission, and therefore, frequent screening is recommended. For high-risk individuals, such as men who have sex with men (MSM), this is recommended every three months[4]. However, despite evidence of increased access and engagement with sexual health services, gonorrhoea incidence is rising, particularly amongst MSM[3]. Several possible drivers may explain this increase. Firstly, the widespread use of online and mobile phone geosocial networking applications to facilitate contact may increase the number of casual sex partners and has been associated with sexually transmitted infection (STI) outbreaks[5,6]. Secondly, "chemsex," which is associated with disinhibitory behaviour and prolonged episodes of condom-less sex with multiple partners, has become increasingly popular[5,6,7]. Thirdly, concern about HIV acquisition may have diminished with increasing availability of HIV pre-exposure prophylaxis[8] and perceptions that effective antiretroviral therapy prevents onward transmission to HIV-negative partners[9].

Understanding the networks within which gonorrhoea is transmitted is of crucial importance for directing public health interventions, where success depends on targeting effective interventions at appropriate individuals. *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) has previously been used to identify strains associated with different populations but lacks the precision to link individuals, whereas whole genome sequencing (WGS) is more discriminatory and reveals networks of individuals infected with genetically related isolates[10,11]. Combined with epidemiological data WGS has the potential to shed light on local and global epidemiology of gonorrhoea.

We aimed to test the feasibility of using WGS data coupled with patient questionnaire data from a sexual health clinic to investigate and characterise transmission networks in a high prevalence setting.

Methods

Setting and participants
Brighton Sexual Health and Contraception centre has approximately 25,000 attendances a year of which 25% are MSM. In a prospective observational study all attendees, over a nine month period (July 2014-March 2015, inclusive), with confirmed gonorrhoea on either nucleic acid amplification test (NAAT; BD ProbeTec, BD, Franklin Lakes, NJ, USA) or microscopy were invited to enter the study and complete a detailed questionnaire (see supplementary materials) on sexual behaviour and lifestyle. Patients with gonorrhoea were treated with ceftriaxone plus azithromycin. Ethical approval was obtained from Brighton and Sussex Research Ethics Committee (14/LO/0435) to collect anonymised data from patients and invite them to complete a questionnaire. The questionnaire was developed in consultation with local practitioners with reference to the British National Survey of Sexual Attitudes and Lifestyles for rapid completion in the clinic to capture key information about infection risk factors. Individual consent for the use of anonymised bacterial isolates was not required.

Samples
Urogenital, rectal or pharyngeal samples were obtained as part of routine sexual health testing. Study isolates of *N. gonorrhoeae* (VCAT selective-agar, Oxoid, Basingstoke, UK) at Royal Sussex County Hospital laboratory underwent WGS as part of a previously published study[10].

Procedures
Laboratory and sequencing procedures have been previously described in detail[10]. Samples were processed in a routine diagnostic laboratory where isolates were identified and antibiotic susceptibilities were determined. A sweep of colonies from culture-positive selective agar plates underwent Illumina (San Diego, CA, USA) WGS. Sequence data were mapped to the *Neisseria gonorrhoeae* NCCP11945 reference genome and variants identified and compared with single nucleotide polymorphisms (SNPs) obtained from maximum likelihood phylogenetic trees, adjusted from the impact of recombination with ClonalFrameML[12]. Sequence data (see supplementary materials) were deposited at the National Center for Biotechnology Information (NCBI). Isolates were assigned to subtypes, related by recent direct or indirect transmission, using a transmission nomogram, described elsewhere[10], taking into account genetic relatedness of isolates as well as sampling dates. The nomogram gives the expected range in genetic difference (single nucleotide polymorphisms, SNPs) between isolates that includes 99% of direct or indirect transmissions for a given time (e.g. 0-11 SNPs for samples taken six months apart). When identifying transmission clusters, all previously sequenced isolates were considered, including those without questionnaire data, however, only isolates from patients with questionnaire data are depicted in the current study. Sequenced isolates from the same patient that were not related by transmission using the transmission nomogram were considered to be distinct infections.

Consenting individuals completed a two-page questionnaire with information about their sexual history, condom use, STI history, use of internet/mobile phone apps to facilitate sex, travel history, recreational drug and alcohol consumption. Data collected were analysed using IBM SPSS Statistics (version 22).

Results
339 patients were NAAT-positive for *N. gonorrhoeae* during the study period. 149 (44%) individuals consented to participate in the study. 45/149 (30%) patients were culture-negative. Questionnaire

data were analysed for the 104/149 (70%) individuals with a positive *N. gonorrhoeae* culture. 97/104 (93%) of patients were male, and 90/104 (87%) were white. Median age was 35 years old (range 20-61 years), and the most represented age group was 25-44yrs (70%). 35/104 (34%) of patients were HIV-positive. Sexual orientation was: MSM, 80/104 (77%); bisexual, 6/104 (6%); heterosexual, 15/104 (14%); unreported, 3/104 (3%). WGS data were available, from a previous transmission study,[10] for 100 isolates from 83/104 (80%) patients.

Sexual behaviour

The median number of sexual partners in the past 3 months was 1 (range: 1-23) for heterosexuals, 5 (range: 1-45) for HIV-positive MSM and 5 for HIV-negative MSM (range 1-30). 51/104 (49%) individuals reported using geosocial networking applications to facilitate contact with partners. Overall, condom usage in the preceding 3 months was reported as: always by 16/86 (19%), sometimes 47 (54%), and never by 18 (21%).

Recreational drug use

Sex under the influence of drugs was reported by 16/34 (46%) of HIV-positive MSM, 17/41 (41%) of HIV-negative MSM and 5/15 (33%) of heterosexuals. Mephedrone and cocaine were frequently cited by MSM and heterosexuals respectively. Ten (6 HIV-positive) MSM reported "slamming" (injecting drugs at the time of sex) in the previous 3 months.

Site of infection

45/104 (43%) individuals had rectal infection, 20/104 (19%) had pharyngeal infection, 53/104 (51%) had urethral infection and 7/104 (7%) had cervical infection. Many individuals were simultaneously infected at multiple sites. Sites of infection in MSM are shown in Table 1.

Table 1. Anatomical sites of infection in MSM

Site	Number of patients
Rectum only	31
Urethra only	30
Throat only	11
Rectum + Urethra	7
Rectum + Throat	4
Rectum + Urethra + Throat	1
Urethra + Throat	2
Total	86

Genomic analysis

From 100 sequenced isolates, we identified 84 genetically distinct infections in 83 patients, caused by 44 distinct genetic subtypes of *N. gonorrhoeae*, where isolates within a subtype were related by direct (person-to-person) or indirect (via ≥1 intermediate source) transmission (Figure 1). 28 subtypes were identified in a single infected patient, and could not be linked to others within this particular data set. The remaining 56 infections (67%) belonged to subtypes represented by 14 clusters of two or more isolates where infected patients could be plausibly linked to one or more others. Patient characteristics and behavioural information for these clusters are given in Table 2.

Table 2. Patient characteristics and behavioural information for 14 clusters of patients linked by WGS data. *one patient did not report sexual orientation

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of patients	12	11	4	3	4	2	2	2	2	2	2	2	2	2
MSM/bisexual: heterosexual	12:0	11:0	1:3	3:0	3:0*	2:0	2:0	2:0	2:0	2:0	2:0	2:0	2:0	2:0
HIV +ve : HIV-ve	6:6	4:7	0:4	2:1	3:1	0:2	0:2	2:0	0:2	0:2	0:2	2:0	2:0	2:0
Reported chemsex	5/12	7/11	1/4	1/3	3/4	1/2	0/2	0/2	2/2	0/2	0/2	1/2	0/2	1/2
Reported mobile phone app use	5/12	6/11	2/4	2/3	3/4	2/2	2/2	2/2	1/2	0/2	1/2	1/2	1/2	2/2
Reported condom-less sex	7/12	8/11	3/4	3/3	3/4	2/2	1/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2

Cluster 1

The largest cluster (Figure 2) comprised 12 MSM, of whom six were HIV-positive and six HIV-negative. Five individuals were infected at more than one anatomical site; rectum (n=9), urethra (n=5) and pharynx (n=3). One individual in this cluster with rectal infection yielded a genetically unrelated strain from a urethral sample and belonged simultaneously to two separate transmission networks. Individual sexual practice was diverse, with the majority practising insertive (9/12) and receptive (10/12) anal intercourse and active (11/12) and receptive fellatio (10/12). 5/12 patients reported chemsex (defined as use of γ-hydroxybutyrate, 4-methylmethcathinone, or methamphetamine to facilitate or enhance sex), including one individual who reported injecting drug use at the time of sex. Condom use was recorded as: always by 4/12, sometimes by 4/12, never by 3/12 and unreported by 1/12. To facilitate contact, 4/12 had visited a sauna, 4/12 attended sex parties (4/12) and 5/12 used mobile phone apps. During the preceding 3 months 7 individuals reported sex outside Brighton: London (5/12), Spain (1/12) and Germany (1/12); the remainder reported sex only in Brighton.

Cluster 2

The second largest cluster (Figure 2) comprised 4 HIV-positive MSM and 7 HIV-negative MSM. Three were infected at more than one anatomical site. Infection sites were: urethra (n=6), rectum (n=5), pharynx (n=3). Sexual practice was diverse with the majority practising receptive (8/11) and insertive (10/11) anal intercourse, and active and receptive fellatio (11/11). Condom use was reported as: always by 3/11, sometimes by 6/11 and never by 2/11. 6/11 reported use of mobile phone apps to facilitate sex, 3/11 had visited saunas and 6/11 had attended sex parties. 7/11 reported chemsex, (including slamming by one individual).

Cluster 3

A smaller cluster (Figure 2) comprised three self-reported heterosexual males and one MSM; all HIV negative with urethral infection. 2/4 individuals reported using mobile phone apps. The heterosexual males reported insertive vaginal intercourse and active and receptive oral sex. The MSM reported insertive and receptive anal and oral intercourse. Chemsex was reported only by the MSM. Condom

use was recorded as: never by 1/4, sometimes by 2/4 and unreported by 1/4. The MSM reported sex in Manchester and the USA.

Two smaller clusters (cluster 4, n=3) and cluster 5, n=4) both comprised individuals of mixed HIV serostatus (Figure 1). Most of these individuals reported mobile phone app use to make contact and chemsex.

Discussion

We used WGS and patient questionnaire data to investigate gonorrhoea transmission in Brighton over a nine-month period. Even within this relatively small study sample, most infections (56/84, 67%) could be plausibly linked to another case through recent direct or indirect transmission. Unlinked cases in our study can be explained by patients who did not consent to provide questionnaire data, culture-negative patients and sources of infection outside Brighton. Some highly mobile individuals have sexual encounters and seek sexual health care in other cities and countries. As reported elsewhere, WGS has demonstrated transmission links between Brighton, other regions of the UK and the US[10], highlighting the need for gonorrhoea control efforts to span regional and national boundaries.

Brighton has a large MSM population and relatively high rates of gonorrhoea and also HIV. We found clusters of plausibly linked gonorrhoea cases that included patients of mixed HIV-serostatus. Although some of our participants may have selected partners according to HIV-status our results provide evidence that serosorting was not consistently practiced. This may be related to lower risk thresholds associated with chemsex[5]. Another explanation is use of HIV pre-exposure prophylaxis (PrEP): a PrEP effectiveness study (PROUD) was in progress in Brighton during the study period. Additionally, individuals may feel less motivated to use a condom with a serodiscordant partner if the HIV-positive partner is receiving antiretroviral therapy (ART). The Partner study reported no linked HIV transmission in HIV serodiscordant couples during 16,400 episodes of sex where the HIV-positive partner received ART and viral load was <200copies/ml[9]. We did not collect data on strategic positioning, which has been reported to be a common practice in highly sexually active HIV-negative MSM[14]. We also found evidence of bridging of infection between heterosexuals and MSM. One cluster (cluster 3) included highly related (2-3 SNPs) isolates from three heterosexual men and one isolate from an MSM. The MSM isolate differed by 9 SNPs from the heterosexual sub-cluster, suggesting the existence of missing links with the heterosexual group.

Our results suggest that WGS of pathogens can be used to support conventional epidemiology in defining and characterising core transmitters[15,16] and allow better targeted and more effective control efforts to reduce transmission. Our approach allows identification of risk factors, one being the use of mobile geosocial networking applications to facilitate contact. Utilising phone apps to transmit prevention messages, STI testing information and to undertake partner notification should be explored. Educational messages and substance misuse services focused on chemsex may also have the potential to reduce frequency of partner change and risk taking behaviour. Public Health England released a briefing document for commissioners and providers of drug and alcohol services containing information about chemsex[17]. New HIV diagnoses in the over 50s have doubled over the last decade[18].

One individual had two distinct *N. gonorrhoeae* strains, with different antimicrobial resistance patterns, in different anatomical sites suggesting they were part of two distinct transmission chains. This is consistent with the previous finding, based on WGS, that 13% of Brighton patients had distinct infections at different anatomical sites[10], and highlights the importance of triple site testing, as well as antimicrobial resistance testing, in MSM.

We aimed to test the feasibility of using WGS data coupled with patient questionnaire data to characterise sexual networks. A limitation is that we did not formally investigate congruence of WGS-defined clusters with partner notification records. Nevertheless, WGS and epidemiological results are broadly mutually supportive. Our findings may not be generalisable to other locations, especially where gonorrhoea occurs more commonly in heterosexual populations. Another limitation is that we obtained both WGS data and questionnaire data from only a subset of patients presenting with gonorrhoea. However, we believe selection bias may be minimal as the demographics of the study population correlate with the demographics of the general population experiencing gonorrhoea in Brighton based on local Genito-Urinary Medicine Clinic Activity Dataset (GUMCAD)[19] data. It is possible that participants may have underreported their risk-taking behaviour, such as slamming, sex parties use of commercial sex workers, which may lead to a reporting bias, although to minimize this the questionnaires were self-administered and anonymous.

This study raises questions about whether HIV serosorting has declined as a practice and why this might be. It has highlighted common risk factors such as chemsex and mobile phone apps to facilitate sex amongst MSM, both of which could be suitable targets for tailored interventions. We also found evidence of bridging between populations: it is plausible that interventions that reduce transmission in MSM may have wider impact in the general population. Genome sequencing is becoming increasingly accessible and offers opportunities to improve the investigation and control of gonorrhoea.

Word count: 2488

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in STI and any other BMJPGL products and sub-licences such use and exploit all subsidiary rights, as set out in our licence http://group.bmj.com/products/journals/instructions-for-authors/licence-forms.

Author contributions:

JPe, FC, GD, DS, DE and JPa designed the study
JPe, FC, LA and GD collected patient data and isolates
KC processed the isolates
XD, DS and DE did bioinformatic analysis
JPe, FC, LA XD, DE and JPa analysed the data
DE prepared figures
JPe, FC, LA, DE and JPa wrote the manuscript which was revised by all authors

References

1. Newman L, Rowley J, Vander Hoorn S, *et al.* Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting.
PLoS One 2015; **10:** e0143304.

2. Anon. Sexually transmitted infections and chlamydia screening in England. *Health Protection Report*. 2016; **10** (22), 27pp.

3. Anon. 2015 Sexually transmitted diseases surveillance. Centers for Disease Control and Prevention. 2015; https://www.cdc.gov/std/stats15/gonorrhea.htm [accessed 20 March 2017].

4. Clutterbuck DJ, Flowers P, Barber T, *et al.* UK national guidelines on safer sex advice. *Int J STD AIDS*. 2012; 23: 381-388.

5. Gilbart V, Simms I, Jenkins C, *et al*. Sex, drugs and smart phone applications: findings from semistructured interviews with men who have sex with men diagnosed with *Shigella flexneri* 3a in England and Wales. *Sex Transm Infect*. 2015; **91**:598-602.

6. Thomas DR, Williams CJ, Andrady U, et al. Outbreak of syphilis in men who have sex with men living in rural North Wales (UK) associated with the use of social media. *Sex Transm Infect*. 2016; 92:359-364.

7. Payne L, Lawrence D, Soni S, et al. Investigating factors for increased gonorrhoea re-infection in men who have sex with men attending a genitourinary clinic: a qualitative study. *Int J STD AIDS*. 2016; Nov 3. pii: 0956462416677916. [Epub ahead of print].

8. Kojima N, Davey DJ, Klausner J. Meta-analysis finds association between pre-exposure prophylaxis to prevent human immunodeficiency virus (HIV) infection and increased risk of sexually transmitted infection acquisition among men who have sex with men. *Open Forum Infect Dis*. 2016.; 3 (suppl. 1): 504.

9. Rodger AJ, Cambiano V, Bruun RN, et al. Sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy. *JAMA*. 2016; 316:171-181.

10. 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: 1295–303.

11. Didelot X, Dordel J., Whittles LK, *et al*. Genomic analysis and comparison of two gonorrhoea outbreaks. *MBio*. 2016; Jun 28;7(3). pii: e00525-16. doi: 10.1128/mBio.00525-16.

12. Didelot X, Wilson DJ. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. *PLoS Comput Biol* 2015; **11:** e1004041.

13. McCormack S, Dunn DT, Desai *et al*. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. *Lancet*. 2016; 387:53-60.

14. Grov C, Rendina HJ, Moody RL, *et al*. HIV serosorting, status disclosure, and strategic positioning among highly sexually active gay and bisexual men. *AIDS patient care STDs*. 1015; 29:559-568.

15. Thomas JC, Tucker MJ. The development and use of the concept of a sexually transmitted disease core. *J Infect Dis*.1996; **174**(suppl 2):S134-43.

16. Yorke JA, Hethcote HW, Nold A. Dynamics and control of the transmission of gonorrhea. *Sex Transm Dis.* 1978; **5**:51-6.

17. Anon. Substance misuse services for men who have sex with men involved in chemsex. PHE publications gateway number: 2015476 November 2015.

18. Mahy M, Autenrieth CS, Stanecki, Wynd S. Increasing trends in HIV prevalence among people aged 50 years and older: evidence from estimates and survey data. *AIDS*. 2014; 28: S453–S459.

19. Anon. (2013). Genitourinary medicine clinic activity dataset (GUMCADv2). Public Health England. [https://www.gov.uk/guidance/genitourinary-medicine-clinic-activity-dataset-gumcadv2]