SHORT REPORT

Ethnically diverse urban transmission networks of *Neisseria gonorrhoeae* without evidence of HIV serosorting

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ABSTRACT

Objective We aimed to characterise gonorrhoea transmission patterns in a diverse urban population by linking genomic, epidemiological and antimicrobial susceptibility data.

Methods *Neisseria gonorrhoeae* isolates from patients attending sexual health clinics at Barts Health NHS Trust, London, UK, during an 11-month period underwent whole-genome sequencing and antimicrobial susceptibility testing. We combined laboratory and patient data to investigate the transmission network structure.

Results One hundred and fifty-eight isolates from 158 patients were available with associated descriptive data. One hundred and twenty-nine (82%) patients identified as male and 25 (16%) as female; four (3%) records lacked gender information. Self-described ethnicities were: 51 (32%) English/Welsh/Scottish; 33 (21%) white, other; 23 (15%) black/British/black African/black, other; 12 (8%) Caribbean; 9 (6%) South Asian; 6 (4%) mixed ethnicity; and 10 (6%) other; data were missing for 14 (9%). Self-reported sexual orientations were 82 (52%) men who have sex with men (MSM); 49 (31%) heterosexual; 2 (1%) bisexual; data were missing for 21 (14%) individuals. Twenty-two (14%) patients were HIV positive. Whole-genome sequence data were generated for 151 isolates, which linked 75 (50%) patients to at least one other case. Using sequencing data, we found no evidence of transmission networks related to specific ethnic groups (p=0.64) or of HIV serosorting (p=0.35). Of 82 MSM/bisexual patients with sequencing data, 45 (55%) belonged to clusters of ≥2 cases, compared with 16/44 (36%) heterosexuals with sequencing data (p=0.06).

Conclusion We demonstrate links between 50% of patients in transmission networks using a relatively small sample in a large cosmopolitan city. We found no evidence of HIV serosorting. Our results do not support assortative selectivity as an explanation for differences in gonorrhoea incidence between ethnic groups.

INTRODUCTION

International travel complicates gonorrhoea transmission and exacerbates the spread of antibiotic resistance.1 Technologies such as whole-genome sequencing (WGS) have the potential to revolutionise gonorrhoea diagnosis and treatment in sexual health clinics where repeat attendance is not guaranteed. Previous WGS studies in Brighton, UK, identified transmission networks in a predominantly white British population of men who have sex with men (MSM), revealing clusters with mixed HIV serostatus and links between Brighton, London and the USA.2,3 Among diverse urban populations, STI incidence differences have been reported between ethnic groups.4 Such differences have been explained by assortative sexual mixing patterns.5 The targeting of public health efforts requires understanding of transmission networks.

Our objective was to combine WGS and epidemiological data to improve our understanding of gonorrhoea transmission networks and antimicrobial resistance in the cosmopolitan population of London.

METHODS

Setting and participants

We undertook a retrospective, observational study of unselected patients attending sexual health clinics during two sampling periods (from 20 May 2013 to 16 August 2013 and from 3 December 2013 to 21 March 2014) at Barts Health NHS Trust, London, UK, serving a diverse population with >100,000 attendances per year of which around 10% are MSM. Genital and extragenital isolates were collected, according to sexual history, from symptomatic and asymptomatic patients as part of routine care. Information was retrieved regarding: symptoms, age, sex, ethnicity, sexual orientation, HIV and hepatitis B serostatus, recreational/innovative drug use, number of sexual partners in the last 3 months, sex abroad and nucleic acid amplification test (NAAT) results.

Antimicrobial susceptibility

Susceptibilities were determined at either of two laboratories, the Royal Sussex County Hospital, Brighton and the London School of Hygiene and Tropical Medicine, using European Committee on Antimicrobial Susceptibility Testing and British Society for Antimicrobial Chemotherapy methods, respectively. Breakpoints used to categorise samples as susceptible and resistant were: azithromycin:
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≤0.25 and >0.5; cefixime: ≤0.125 and >0.125; ciprofloxacin:
≤0.03 and >0.06; penicillin: ≤0.06 and >1; and tetracycline:
≤0.5 and >1 mg/L.8 Descriptive analyses and Fisher’s exact test
were undertaken to examine relationships between antimicro-
bial susceptibilities and risk factors.

Whole-genome sequencing
One isolate per patient was sequenced. Isolates labelled ‘BR−’
underwent WGS on the Illumina HiSeq platform as described
previously.2 Isolates labelled ‘LN−’ underwent Illumina MiSeq
2×251 bp paired-end sequencing as per manufacturer’s protocol.
Sequence data were processed using a bioinformatic pipeline,
described previously.2 Briefly, sequence reads were mapped to
the Neisseria gonorrhoeae NCCP11945 reference genome, and
high-quality variants were identified. Samples with >70% of
the reference genome identified were included in the study. Samples
were compared using maximum likelihood phylogenies,7 after
correction for recombination using ClonalFrameML.8 Sequences
are available on the NCBI SRA under BioProject PRJNA522696.

RESULTS
Demographic data
Data were available for 158 patients. One hundred and twenty-
ine (82%) identified as male and 25 (16%) as female; data were
available for four patients. One hundred and twenty-three
(78%) individuals were born in Europe (96 (61%) in the UK), 6
(4%) Africa, 6 (4%) South America, 5 (3%) South Asia, 5 (3%)
South-East Asia/Australia and 4 (3%) in the Caribbean (data
available for nine patients). Self-reported recorded ethnicities
were: 51 (32%) English/Welsh/Scottish; 33 (21%) white, other;
23 (15%) black British/black African/black, other; 12 (8%)
Caribbean; 9 (6%) South Asian; 6 (4%) mixed; and 10 (6%)
other groups (14 data unavailable).

Patients reported their sexual orientation as: 82 (52%) MSM,
49 (31%) heterosexual and 2 (1%) bisexual (data unavailable
for 25 individuals). Regarding HIV status, 22 (14%) were HIV
positive (16 on treatment) and 109 (69%) were HIV negative;
data unavailable for 27). One hundred and twenty-two (77%)
were hepatitis B negative and 2 (1%) had hepatitis B coinfection
(available for 34 patients). Data on recreational drug use was
available for 24 patients: 11 (46%) reported use and 13 (54%)
denied use. No intravenous drug use was recorded.

The number of sexual partners within the last 3 months varied
from 1 to 60: 27 patients (17%) reported one partner, 31 (20%)
two partners, 16 (10%) 3–4 partners, 9 (6%) 5–9 partners and
15 (9%) ≥10 partners (data unavailable for 60 (38%)). Partner
notification occurred for at least 94 (59%) individuals: infor-
mation was not available for 42 (27%) and notification did not
occur for 22 (14%) patients. Twelve (8%) reported sex abroad.
One isolate per patient was analysed from the following anatom-
ical sites: 76 (48%) urethral, 29 (18%) rectal, 11 (7%) cervical,
9 (6%) pharyngeal and 33 (21%) not specified. NAAT testing
results were available for 109 culture-positive patients; three
were NAAT negative.

Antimicrobial susceptibility data
Antimicrobial susceptibility results were as follows: azithro-
mycin: susceptible 96 (61%), intermediate 7 (4%), resistant 1
(1%) and not available 54 (34%); ciprofloxacin: susceptible 104
(66%), intermediate 1 (1%), resistant 37 (23%) and not available
16 (10%); and tetracycline: susceptible 36 (23%), intermediate
19 (12%), resistant 35 (22%) and not available 68 (43%). All 156
tested isolates were ceftriaxone susceptible (data not available
for two). There was no demonstrable relationship between anti-
microbial susceptibility for any antibiotic and sexual orientation
(Fisher’s exact test: p≥0.49 across all antibiotics) or number of
partners (p≥0.43).

Whole-genome sequencing
WGS data were successfully generated for 151/158 (96%)
isolates. After mapping a median (IQR) range 89.1% (87.7%–
89.6%) (77.8–90.6%) of the NCCP11945 reference genome
was identified. We applied our previously described transmis-
sion nomogram to link cases related by possible direct person-
to-person transmission or indirect transmission via one or more
intermediate cases. Cases were considered genetically linked
if the number of single nucleotide polymorphisms (SNPs)
between them fell within the 99% prediction interval for the
number expected between transmitted cases based on the time
between sampling. Of 151 cases, 76 (50%) were not linked
to another case, 30 (20%) belonged to potential transmission
pairs, 39 (26%) triplets and 6 cases (4%) formed the largest
cluster (Figure 1). Overall, 105 distinct genetic subtypes of N.
gonorrhoeae were observed. The largest cluster spanned June
2013 to March 2014 and consisted of four MSM, one hetero-
sexual male and a male with undisclosed sexual orientation.
All six men were born in Europe (four in the UK) and from
varying ethnic groups: white, South Asian and mixed ethnicity.
One was HIV positive (treated); another had chronic hepatitis
B infection.

Figure 1 shows all sequenced isolates with patient informa-
tion. We investigated for evidence of genetic clustering of cases
by reported sexual orientation (as a positive control), HIV
serostatus and ethnicity. We considered the number of pairs of
cases within genetic clusters who shared the same characteris-
tic and compared this with the distribution when we randomly
permuted the characteristic labels 1000 times. Forty-one
within-pair clusters had the same reported sexual orientation
and seven had different orientations; 21 pairs had missing
data. Under the permuted model, representing the distribu-
tion expected by chance, many more within-cluster pairs had
different sexual orientations, median 23 pairs (95% CI 14 to
30); that is, there is strong evidence for clustering by sexual
orientation as the number of observed pairs with different
sexual orientations was much lower than the lower bound of
the permutation CI.

However, there was no evidence of HIV serosorting beyond
that expected by chance: 34 potential transmission pairs had the
same HIV serostatus and 12 pairs differed; data were missing
for 23 pairs. This compares with 17 discordant pairs (95% CI
10 to 23, ie, containing 12) under the random model. All HIV-
positive patients with available data identified as MSM (n=21)
or bisexual (n=1). Similarly, within the limits of the power of the
study, there was no evidence for clustering by ethnic group, 15
pairs shared the same ethnic group, 45 a different ethnic group,
and data were missing for nine pairs. Forty-five (95% CI 36 to
52) discordant pairs expected were under the random permuta-
tion model.

Overall proportions of cases involved in any cluster were
similar by ethnic group (Fisher’s exact test: p=0.64) and HIV
serostatus (p=0.33). Forty-five out of 82 (53%) MSM or
 bisexual clients with sequencing data were in genetic clusters of
≥2 cases, compared with 16/44 (36%) of clients identifying as
heterosexual (p=0.06). Fifty-six per cent (14/25) patients not
recording their sexuality were part of potential transmission
clusters.

2 Dave J, et al. Sex Transm Infect 2019;0:1–4. doi:10.1136/sextrans-2019-054025

Figure 1 shows all sequenced isolates with patient informa-
tion.
Figure 1  Phylogeny of 151 Neisseria gonorrhoeae isolates from London, May 2013–March 2014. Tips with coloured triangles represent potential transmission clusters, based on the genetic distance and time between isolates. The colouring of each transmission cluster is arbitrary. The right-hand panels show associated epidemiological data regarding HIV serostatus, sexual orientation and self-reported ethnic group. MSM, men who have sex with men.

DISCUSSION

Using combined WGS and epidemiological data, we investigated gonorrhoea in a diverse urban population. Previous studies demonstrated variation in gonorrhoea incidence within different ethnic groups. We detected no evidence of clustering by ethnicity. Therefore, our findings do not support the hypothesis that infection rate differences between ethnic groups can be explained by assortative sexual mixing. However, our study was limited by the relatively small sample size, with only 151 isolates sequenced.

We demonstrated increased linkage of cases among MSM, and we detected clusters involving MSM of mixed HIV serostatus, as reported previously and contrary to the findings of a study using samples from 2004. We demonstrate bridging events between different ethnicities and sexual orientation. We found less case clustering than in Brighton. This may reflect less complete sampling, as London is served by multiple clinics and less clustering of cases in the non-MSM population.

Our results show evidence of transmission in heterosexuals and in MSMs of different ethnicity. As in Brighton, we found evidence of gonorrhoea transmission involving patients of mixed HIV serostatus. Decreased concern regarding HIV risks may be contributing to increased condomless sex and gonorrhoea transmission. The impact on transmission networks of the wider availability of HIV pre-exposure prophylaxis since this study could be investigated using WGS in future.

Handling editor  Henry John Christiaan de Vries
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Acknowledgements  We thank Glenn Phiri for administrative support.
Contributors  Designed the study: JD, JP, DWE, RS and AW. Collected patient data and isolates: AW and JP. Processed the isolates: VFM and KC. Performed bioinformatic analysis: RS and DWE. Analysed the data: JD, JP, RS, FW and DWE. Prepared the figure: DWE. Wrote the manuscript: JD, JP, DWE, FW and RS. Revision of manuscript: all authors.
Funding  The research was funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in healthcare-associated infections and antimicrobial resistance at the University of Oxford in partnership with Public Health England (PHE) (HPRU-2012-10041) and the NIHR HPRU in Modelling Methodology at Imperial College London (HPRU-2012-10080) in partnership with PHE. DWE is a Big Data Institute Robertson Fellow.
Competing interests  None declared.
Patient consent for publication  Not required.
Provenance and peer review  Not commissioned; externally peer reviewed.
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