1　**Utility of whole genome sequencing in assessing and enhancing partner notification of**

2　*Neisseria gonorrhoeae* **infection**

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46   **<u>Short Summary</u>**

47   In a sexual health clinical setting, we demonstrate the feasibility and utility of whole genome

48   sequencing as a tool to measure the performance of and to improve partner notification.

49 **Abstract**

50

51 **Background**

52 Gonorrhea is a sexually transmitted infection of global concern. We investigated whole genome

53 sequencing (WGS) as a tool to measure and enhance partner notification (PN) in gonorrhea

54 management.

55

56 **Methods**

57 Between May-November 2018, all *N. gonorrhoeae* isolated from patients attending Leeds Sexual

58 Health, UK, underwent WGS. Reports listing sequences within 20 single nucleotide

59 polymorphisms (SNPs) of study isolates within a database containing select isolates from April 1

60 2016 to November 15 2018 were issued to clinicians. The proportion of cases with a potential

61 transmission partner identified by PN was determined from patient and PN data. WGS reports

62 were reviewed to identify additional cases within ≤6 SNPs and verified for PN concordance.

63

64 **Results**

65 380 isolates from 377 cases were successfully sequenced; 292 had traceable/contactable partners

66 and 69 (18%) had a potential transmission partner identified by PN. Concordant PN and WGS

67 links were identified in 47 partner pairs. Of 308 cases with no transmission partner by PN, 185

68 (60%) had a case within ≤6 SNPs; examination of these cases' PN data identified seven partner

69 pairs with previously unrecognized PN link, giving a total of 54 pairs; all had ≤4 SNP

70 differences. WGS clusters confirmed gaps in partner finding, at individual and group levels.

71 Despite the clinic providing sexual health services to the whole city, 35 cases with multiple

72 partners had no genetically related case, suggesting multiple undiagnosed infections.

73

74 **Conclusions**

75 WGS could improve gonorrhea PN and control by identifying new links and clusters with

76 significant gaps in partner finding.

77

78 **Key Words:**

79 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

103   months for each case was obtained, including name, gender, type and date of last sex, and partner

104   contact information if available. Patients diagnosed with gonorrhea were asked to return for test

105   of cure 14 days after treatment. Information on whether reported partners attended a sexual

106   health service was documented at this visit. Those diagnosed with gonorrhea >= four weeks from

107   initial diagnosis, with distinct dates of symptoms if applicable, were considered as two infection

108   episodes.

109

110   <u>Isolation of *N. gonorrhoeae*</u>

111   Samples were cultured and tested for antimicrobial susceptibility at Leeds Teaching Hospitals

112   NHS Trust microbiology laboratory as per local protocols (Supplementary Appendix).

113

114   <u>Whole genome sequencing and data reporting</u>

115   Isolates from every culture-positive case during the study period underwent WGS at University

116   of Leeds, plus 335 historical isolates from Leeds collected during 2016-2017 (July – September

117   annually) as part of the Public Health England Gonococcal resistance to antimicrobials

118   surveillance programme. In patients with more than one positive sample from different sites,

119   typically only the first accessioned was submitted for sequencing. Details on DNA extraction,

120   sequencing, and bioinformatics are provided in the Supplementary Appendix. WGS data were

121   used to generate one report per study isolate, containing the isolate's sample identifier,

122   sequencing quality parameters, and sample identifiers for all sequences within 20 SNPs to date

123   (Supplementary Figure 1). Reports were issued to LSH in weekly batches, with a target

124   turnaround of 14 days from sample collection, determined to be a clinically reasonable

125   timeframe since patients diagnosed with gonorrhea would return 14 days after for follow-up and

126 test of cure. Data from reports were examined weekly at LSH and formally analyzed at the end

127 of the study period (Supplementary Appendix and Supplementary Figure 2).

128

129 PN analysis

130 Clinical data was collected on all cases associated with WGS reports, including demographics,

131 details of infection, and PN data (Supplementary Appendix). To assess PN effectiveness, we

132 analysed partners reported by each case. Reported partners were first classified as traceable (if

133 index cases stated they were able to contact them or if enough information was given to enable a

134 provider referral) or untraceable. Among traceable partners, attendance was classified as verified

135 (if they attended LSH or had a clinician-verified attendance elsewhere), unverified (if index case

136 reported partner attendance which could not be confirmed), or no known attendance.

137

138 We calculated the nationally established auditable outcome measure for gonorrhea PN in the

139 United Kingdom, defined as the number of all contacts of the index case who attended a service

140 within four weeks of the first PN discussion, targeting 0.4 contacts per index case in large

141 conurbations or 0.6 contacts elsewhere(5). Additionally, we determined the proportion of cases

142 with a potential transmission partner identified by PN, i.e. those with a partner with a

143 culture/NAAT confirmed diagnosis of gonorrhea. For couples reporting each other, only one

144 case was counted as having an identified transmission partner, as one partner had to have

145 acquired the infection from a third person.

146

147 WGS analysis

148   We examined WGS data for PN-linked cases to confirm concordance. Genetic distances between

149   isolates from known partners with a culture positive diagnosis were recorded to estimate the

150   genetic distance expected following presumed direct transmission. Combining our findings and

151   the observed number of SNPs between couples with epidemiologically confirmed direct contact

152   from previous studies(12) we classify potential direct transmissions as pairs within ≤6 SNPs. For

153   other cases linked by WGS reports (i.e. within 20 SNPs), but not PN, to determine if

154   transmission was plausible, either directly or indirectly through a third party, we applied a

155   published nomogram for *N. gonorrhoeae*(12). The nomogram categorizes any given pair of

156   isolates as "transmission supported" or "transmission not supported" based on the time and

157   number of SNPs between them. Thus, all pairs from WGS reports were classified as: not linked

158   by nomogram, linked by nomogram but not by PN, and linked by both nomogram and PN.

159   Among pairs linked by nomogram but not by PN, we examined PN data in more detail to search

160   for any unidentified potential direct transmission events. We further examined WGS links

161   between isolates from the same patient when they had more than one infection episode.

162

163   Ethics

164   As no patient-identifiable data was used outside the usual clinical team and sequencing

165   performed on routinely cultured samples, this study was conducted as an NHS service evaluation

166   of WGS as an alternative to previously used typing methods (e.g. NG-MAST), and was therefore

167   exempt from requiring ethical approval using the Health Research Authority guidance tool.

168

169   **Results**

170 During the study period, 474 cases of gonorrhea were diagnosed; cultures were performed on

171 455. The 385 positive isolates were submitted for WGS, five were excluded (one not *N.*

172 *gonorrhoeae*, 4 contaminated culture plates); thus, 380 isolates were successfully sequenced and

173 WGS reports generated (sample list in Supplementary Table 2). These originated from 362

174 patients (median age 23 years) with 377 infection episodes (15 patients had two infection

175 episodes). Cases and diagnosed partners are included in these numbers. Although most patients

176 had only one isolate submitted, one patient had three identical isolates sequenced from different

177 anatomical sites from the same infection episode, and another had two identical isolates from

178 different sites from the same infection episode; one sequence per infection episode was retained

179 for analysis. Another two patients had two isolates submitted from different sites on the same

180 day; these revealed genetically unrelated isolates (4699 and 3919 SNPs different), and were

181 counted as distinct infection episodes. Thus, 377 sequences were analyzed. There were 118 cis-

182 females, one trans-woman, and 243 cis-males. Among females, the majority (116/118, 98%)

183 were female heterosexuals; two were women who have sex with men and women (WSMW).

184 Among males, nearly half (119/243, 49%) were MSM, 22 (9%) were men who have sex with

185 men and women (MSMW), and 102 (42%) were male heterosexuals. One patient was a

186 transgender woman who has sex with men. Amongst males, infections were primarily urethral

187 (168/254, 66%), followed by rectal (62/254, 24%) and pharyngeal (24/254, 9%). Amongst

188 females, most were urogenital infections (114/122, 93%). All isolates were susceptible to

189 ceftriaxone; 14 were resistant, and 29 had intermediate susceptibility, to azithromycin. Overall,

190 319 (84%) isolates were successfully sequenced within 14 days, and 246(65%) had WGS reports

191 sent to LSH within 14 days of sample reception. Reasons for delayed WGS results included

192 numbers of samples exceeding weekly capacity (12-16 isolates), isolates missing the scheduled

193   batch due to impurity (requiring sub-culture), delays associated with WGS report generation

194   (software problems, manual interventions), and sub-optimal sequence data. Turnaround times

195   from sample collection to time points in the sequencing and reporting process are presented in

196   Supplementary Table 1.

197

198   <u>Partner notification</u>

199   From 377 episodes 1395 partners were reported, median two per case. Eighty-five cases had only

200   untraceable partners; 292(77%) cases reported at least one traceable partner, providing a total of

201   434 traceable partners (Figure 1).

202

203   Considering performance against national audit standards, 125 partners had verified attendance

204   in Leeds or elsewhere within four weeks of PN discussion (9% of total reported partners),

205   representing 0.33 contacts per index case (national target 0.40). Including 44 partners with

206   unverified attendance, there were 0.44 contacts per index case. By study end, 11 more partners

207   had verified attendance for 0.48 contacts per index case.

208

209   Eighty-five cases had culture-positive verified partners diagnosed at LSH, 12 had verified

210   NAAT-positive but culture-negative partners, and four had verified partners testing positive at

211   another sexual health clinic. Among the 85 cases with culture-positive partners, there were 32

212   mutually reporting couples for which only one partner could be counted; thus, in total, only 69

213   (85+12+4-32) (18%) of the 377 infection episodes had a potential transmission partner identified

214   by PN, with 308 cases with no identifiable transmission partner.

215

216   <u>WGS results for PN-linked cases</u>

217   In examining WGS data for PN-linked cases, we considered the proportion of partner WGS data

218   that was available at the test-of-cure visit for each index case. Of 130 partners with verified

219   attendance in Leeds, 85 were culture-positive and had isolates submitted for sequencing. As over

220   half of reported partners (78/130, 60%) attended before or on the same day as the index case

221   (Table 1) and 53/78(68%) were culture-positive, 45/53(85%) of partner isolates could be linked

222   to their index cases by WGS reports (i.e. within 20 SNPs) at 14 days. The remaining eight

223   partners with isolates cultured before their index cases included two diagnosed before the study

224   and therefore not sequenced, one with a contaminated culture, one with an unrelated isolate

225   (4429 SNPs different), and four with a delay in sequencing. The four partner isolates that were

226   delayed in sequencing were linked to their index cases at a later time when both sequences were

227   available.

228

229   A further 52 partners attended after their index cases; 32 were culture-positive, and 30(94%)

230   were linked by WGS reports (within 20 SNPs). The two non-linked partners were diagnosed

231   after the study end so not captured in the database. Thus, of 85 partners testing culture-positive in

232   Leeds, 79(93%) could be linked by WGS to the cases who reported them. These 79 partners

233   linked to their index cases by both WGS and PN, comprised 64 individuals from 32 mutually

234   reporting couples, and 15 from couples where only one partner reported the other. Among these

235   47 (32 + 15) couples with known sexual contact and presumed direct transmission and available

236   sequence data, all pairs of isolates were between 0-4 SNPs (Figure 3).

237

238   <u>WGS findings across the whole study</u>

239 From the 377 cases analyzed, 266 had linked isolates that were within the 99% prediction

240 interval supporting transmission using a previously published nomogram, and 237 cases had

241 links to isolates within 6 SNPs (Figure 2). Examining the 308 cases with no transmission partner

242 found by PN, 211(69%) had ≥1 plausible direct or indirect transmission partner within the

243 nomogram thresholds and 185(60%) ≥1 plausible direct or indirect transmission partner within

244 ≤6 SNPs. Thus, the majority of cases did not have a transmission partner identified by PN but

245 did have a genetically plausible direct or indirect transmission partner within the *N. gonorrhoeae*

246 infections diagnosed in Leeds.

247   Clinic health advisors were able to use WGS reports to identify seven additional couples

248 with suspected direct transmission, not identified by PN. For example, several cases reported

249 partners without verifiable information (e.g. first name only) for whom confirmation of partner

250 attendance was impossible with available information, but facilitated by WGS. Together with the

251 47 couples linked through PN, a total of 54 couples with presumed direct transmission were

252 identified. All pairs were within 4 SNPs (Figure 3).

253   Fifteen patients had two infection episodes during the study. Three had the same isolate

254 twice with the same reported partners. Five patients reported at least one partner that was the

255 same across episodes, but had genetically unrelated isolates between episodes; this includes one

256 patient who had two genetically unrelated isolates from different anatomical sites on the same

257 day. He reported only one partner. The remaining were all MSM, had different isolates, and did

258 not report the same partners across episodes.

259

260 Sequencing-based clusters

261 Cases related to ≥1 other case(s) within 20 SNPs were clustered into groups to describe the

262 different lineages circulating in Leeds (Supplementary Figure 3). Each cluster contained only

263 genomes with the same multi-locus sequence type (MLST, provided in Supplementary Table 2).

264 322 cases fell into 62 clusters of ≥2 cases, plus 55 singletons. Most clusters (54/62, 87%) had

265 <10 cases, with 34 containing 2-3 cases, and only two containing >20 cases (21 and 31 cases).

266 The eight clusters with >10 cases were mixed in terms of several characteristics (Figure 4). For

267 example, although two major clusters contained primarily MSM, these were mixed with MSMW

268 and heterosexuals. Three clusters included HIV seropositive and seronegative cases. Although no

269 isolates had azithromycin resistance in the two largest clusters, a cluster of 17 cases contained

270 seven cases with azithromycin intermediate resistance. All clusters contained asymptomatic

271 cases, including three with more than half who were asymptomatic.

272   We next combined PN networks with cases linked within ≤6 SNPs to allow us to

273 visualize potential direct transmission events. The vast majority of PN reported partners were

274 not verified, whilst diagnosed cases could be organized into genetically related transmission

275 chains (Figure 5). Tracking the growth of clusters over time permitted us to make observations

276 both at an individual patient level and at a group level. At an individual level, linking PN data

277 and WGS clusters allowed us to identify undiagnosed individuals reported by several index

278 cases: for example, two heterosexual females diagnosed with three infections over four months

279 reported the same male who could not be located within the database.

280   At a group level, emerging epidemiological trends could be identified. For example, one

281 cluster consisted of two heterosexual males with an identical isolate, both of whom reported

282 contact with female sex workers; another contained three heterosexual males with an identical

283 isolate, with one reporting sex worker contact. Another contained a female (sex worker) who

284   reported multiple male partners, but the only other case in the cluster was a heterosexual male

285   who reported two female partners who were not sex workers. Yet another contained eight MSM

286   reporting recent sauna use, including two naming the same sauna. Finally, we noted that of the

287   55 genetic singletons, 35 reported multiple sexual partners. As might be expected, many of the

288   total 189 partners reported by the singletons were untraceable (110, 58%), and were from outside

289   the local area (other countries [68, 36%)], or elsewhere in the UK [21, 11%]).

290

291   **Discussion**

292   Although WGS has been useful to inform public health measures surrounding *N. gonorrhoeae*

293   outbreaks, ours is the first study to evaluate its usefulness in a clinical setting. It is also the first

294   exploration of the clinical utility of WGS for PN as part of routine STI control, where we

295   demonstrate the feasibility of sequencing and reporting to a sexual health clinic. Although WGS

296   confirmed nearly all known links from PN with a sequenced isolate, PN identified potential

297   transmission partners for only a minority (18%) of cases, despite considerable investment in

298   skilled PN services. This was frequently due to the index cases' lack of knowledge of their

299   partners' identities or reluctance to disclose information. WGS also enabled identification of

300   cases of confirmed attendance that could not be verified through PN, therefore enhancing the

301   reported performance of PN. Although the number of verified contacts per index case, 0.33, fell

302   below the national audit standard of 0.4, even had this been met, a majority of index cases would

303   still have undiagnosed partners.

304   WGS offers a potential assay of PN performance and the effectiveness of the clinic in

305   terms of the proportion of all cases diagnosed. For example, 60% of cases with no transmission

306   partner by PN had a closely genetically related case within 6 SNPs. WGS also offers a potential

307   mechanism for directing interventions to key gaps in partner finding. We have shown examples

308   where individual-level focus could be achieved: for the undiagnosed partner reported by several

309   index cases, it would be reasonable to intensify health advisor efforts, and further information

310   gathering, surrounding a potential untreated person. More frequently, groups could be identified:

311   the recognition of an emerging transmission chain involving multiple sauna-attending MSM

312   might prompt intensified screening in addition to the usual outreach services. The example of the

313   female sex worker with multiple related cases could prompt liaison with sex worker outreach

314   projects to sensitively increase efforts to locate her and her partners. A similar approach could be

315   adopted for partners of cases representing genetic singletons, especially when clinical history is

316   consistent with local acquisition. As the clinic serves the whole local population, it appears likely

317   that a large proportion of such cases' partners are undiagnosed. Finally, examination of patients

318   with repeat infections can reaffirm the direction of intervention needed: re-infection from the

319   same partners vs. acquisition from new sources. We did not systematically sequence isolates

320   from each positive anatomical site, assuming most such cases would yield identical isolates.

321   However, out of the four cases with >=1 isolate submitted from different sites on the same day,

322   two revealed genetically unrelated isolates, raising the possibility of more than one transmission

323   partner (one of these cases reported only one partner). This represented an unexpected finding

324   that could have implications for further questioning of the patients.

325   To summarize, periodic review of WGS clusters could inform PN efforts in two main

326   ways. First, one might search for any missed attendances in reported partners with incomplete

327   information. Second, areas requiring intervention can be identified through the examination of

328   clusters and genetic singletons. General epidemiological trends can be followed: we observed

329   evidence of bridging between sexual populations (e.g. MSM and heterosexuals), and mixing of

330   individuals with discrepant HIV sero-status within the same clusters, similarly to other

331   studies(13, 16). Granular trends within clusters, such as increasing rates of asymptomatic or

332   extra-genital infections and antibiotic resistance, can be identified and acted upon rapidly when

333   observed within WGS clusters, which provide evidence for sustained transmission, providing

334   focus and incentive for intervention.

335   Our study also provides further data for improved clinical use of genomic tools such as

336   the nomogram, which provides compatibility with direct or indirect transmission. In our cohort,

337   couples with presumed direct transmission were often within 0-1 SNPs, and all were within 4

338   SNPs of one another. This reflects the fact that most pairs related by recent transmission are

339   more likely to have lower SNP values (Figure 6).

340   WGS implementation in a sexual health setting raises ethical concerns. It is important to

341   recognize that the PN process involves the seeking and use of sensitive information, to which a

342   reported partner cannot provide consent a priori. In this context, WGS represents an adjunctive

343   tool to enhance surveillance and partner finding as used in outbreaks (9). Potentially important

344   issues are that neither partner has consented to links made by WGS, and WGS may also provide

345   indirect links between two individuals via one or more intermediate cases. This is an area that

346   merits formal ethical research and patient and public consultation.

347   Our study has certain limitations. As the first exercise and analysis of its kind, the

348   availability and utility of results within 14 days and SNP threshold used were exploratory. The

349   implementation of weekly analysis with the clinical team had challenges, such as the exact

350   actions that could be taken within ethical boundaries, when a gap in partner finding was

351   identified. However, our work provides a framework on which subsequent clinical

352   implementation efforts can be based, by demonstrating that a periodic examination of WGS

353 clusters and analysis could enhance PN. Cost-effectiveness analysis of implementing such a

354 pipeline should be considered. Finally, despite providing sexual health care to the entire city, our

355 study is a single-centre study that may not be representative of different settings.

356

357 **Conclusion**

358 Against a background of rising gonorrhea infection rates, we emphasise that PN only enables the

359 sources of a minority of cases to be identified and treated. There is an urgent need for novel

360 control interventions. We have demonstrated the feasibility and utility of WGS to confirm PN

361 links, reveal new PN links, and to help clinicians focus in on undiagnosed cases for intervention.

362 With expanding databases and understanding of relationships between genomic and clinical data,

363 the implementation of WGS in sexual health will likely be beneficial to the control of STIs.

364

365 **Declarations**

366 DWE declares lecture fees from Gilead outside the submitted work. IBM has received funding to

367 attend conferences from Techlab, Inc. outside the submitted work. No other author has a conflict

368 of interest to declare.

369

370 **Contributors**

371 MHW, DWE, ASW, JDW, and LYK designed and coordinated the study. IBM and WF

372 performed WGS sequencing and reporting. LYK, LK, and JDW contributed to data

373 collection and management. LYK analyzed the data with support from DWE, JDW,

374 ASW, and MHW. LYK wrote the first draft of the paper and all authors read, commented

375 on, and approved the final manuscript.

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416

417   **Figure 1. Routine partner notification results**

418   Separately submitted

419   Legend: Flowchart of routine partner notification results

420 **Figure 2. Breakdown of all WGS links**

421 Separately submitted

422 Legend: Flowchart of WGS links analysis for study cases

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

424 **transmission**

425 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

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

430   Separately submitted

431   Legend:

432   Eight clusters are represented with each horizontal bar representing a cluster

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

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

435   F Hetero: female heterosexuals

436 **Figure 5. PN and WGS networks**

437 Separately submitted

438 Legend:

439
● Female   ——— WGS link only, within 6 SNPs

440
● Male   ——— PN link only

441
● Transgender   ——— Both WGS and PN link

442

443 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.

444   **Figure 6. Transmission nomogram with bands depicting varying confidence ranges for**

445   **recent transmission event**

446   Separately submitted