Sustained transmission of high-level azithromycin-resistant \textit{Neisseria gonorrhoeae} in England: an observational study

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Summary

Background Between Nov 3, 2014, and Feb 24, 2017, 70 cases of high-level azithromycin-resistant (HL-AziR; minimum inhibitory concentration [MIC] ≥256 mg/L) \textit{Neisseria gonorrhoeae} were reported from across England. Whole-genome sequencing was done to investigate this outbreak to determine whether the ongoing outbreak represented clonal spread of an HL-AziR \textit{N gonorrhoeae} strain identified in Leeds. We also wanted to elucidate the molecular mechanisms of azithromycin resistance in \textit{N gonorrhoeae} in the UK.

Methods In this observational study, whole-genome sequencing was done on the HL-AziR \textit{N gonorrhoeae} isolates from England. As comparators, 110 isolates from the UK and Ireland with a range of azithromycin MICs were also sequenced, including eight isolates from Scotland with azithromycin MICs ranging from 0·12 mg/L to 1·00 mg/L that were \textit{N gonorrhoeae} multi-antigen sequence type 9768 (ST9768), which was the sequence type initially responsible for the outbreak. The presence of mutations or genes associated with azithromycin resistance was also investigated.

Findings 37 of the 60 HL-AziR isolates from England belonged to ST9768, and were genetically similar (mean 4·3 single-nucleotide polymorphisms). A 2059A→G mutation was detected in three or all four alleles of the 23S rRNA gene. Five susceptible ST9768 isolates had one mutated 23S rRNA allele and one low-level resistant ST9768 isolate had two mutated alleles.

Interpretation Sustained transmission of a successful HL-AziR clone was seen across England. Mutation 2059A→G was found in isolates with lower azithromycin MICs. Azithromycin exposure might have provided the selection pressure for one or two mutated copies of the 23S rRNA gene to recombine with wild-type copies, leading to three or four mutated copies and the HL-AziR phenotype. HL-AziR could emerge in isolates with low azithromycin MICs and eliminate the effectiveness of azithromycin as part of dual therapy for the treatment of gonorrhoea.

Funding Public Health England.

Introduction In 2012, WHO estimated that 78·3 million new cases of gonorrhoea had arisen worldwide that year.\(^1\) Untreated infection can result in pelvic inflammatory disease, ectopic pregnancy, and infertility, and can increase the risk of HIV transmission and acquisition. \textit{Neisseria gonorrhoeae}, the causative pathogen of gonorrhoea, has developed resistance to successive classes of antibiotics in the past few decades;\(^2\) and few antimicrobials remain effective in its treatment. Extended-spectrum cephalosporins such as ceftriaxone are last-line treatment options for \textit{N gonorrhoeae} because no new antimicrobials are currently available. In an attempt to delay the accumulation of resistance, many countries introduced dual antimicrobial therapy with an extended-spectrum cephalosporin plus azithromycin as first-line treatment. In 2011, the British Association of Sexual Health and HIV changed their gonorrhoea treatment guidelines to recommend dual therapy with single dose ceftriaxone (500 mg intramuscularly) in combination with a single oral dose of azithromycin (1 g).\(^3\)

Public Health England detected an outbreak of high-level azithromycin-resistant \textit{N gonorrhoeae} (HL-AziR; minimum inhibitory concentration (MIC) ≥256 mg/L) in Leeds, northern England, in March, 2015.\(^4\) Before this outbreak, HL-AziR \textit{N gonorrhoeae} had been a rare phenotype observed only sporadically in England. Since then there have been ongoing reports of HL-AziR \textit{N gonorrhoeae} cases from across England. This presents a substantial threat to front-line dual therapy for gonorrhoea because it renders the azithromycin component ineffective.

We previously described the use of whole-genome sequencing in the investigation of seven cases from the beginning of this outbreak in Leeds.\(^5\) All seven isolates characterised by whole-genome sequencing were \textit{N gonorrhoeae} multi-antigen sequence typing (NG-MAST)\(^6\) sequence type 9768 (ST9768) and were virtually identical on whole-genome sequencing core genome comparison (zero or one single-nucleotide polymorphisms [SNPs]). All of the isolates showed mutation 2059A→G (\textit{Escherichia coli} numbering) in all four alleles of the 23S rRNA gene, conferring high-level resistance to azithromycin.

The outbreak first emerged with 16 cases diagnosed in residents of Leeds between Nov 3, 2014, and Oct 9, 2015. A local incident control team in Leeds was formed to coordinate the response. An alert to clinicians was issued...
Research in context

Evidence before this study
We searched PubMed for articles published in English on or before June 30, 2017, with the terms “Neisseria gonorrhoeae” or “gonorrhoea” with “azithromycin”, and “Neisseria gonorrhoeae” or “gonorrhoea” with “sequencing” or “molecular epidemiology”. High-level azithromycin-resistant (HL-AziR) *N gonorrhoeae* has been observed sporadically in the UK and elsewhere, with occasional small clusters reported. To our knowledge, no reports of sustained transmission have been published on the scale described in this paper. Additionally, previous studies describe the national molecular epidemiology of *N gonorrhoeae*, but do not describe the real-time use of whole-genome sequencing as part of an outbreak investigation.

Added value of this study
This report provides evidence of sustained transmission of *N gonorrhoeae* with the HL-AziR phenotype on a national scale. Whole-genome sequencing was a valuable tool for understanding the spread of resistance; the single-nucleotide polymorphism phylogeny provided a level of discrimination beyond that of *N gonorrhoeae* multi-antigen sequence typing in terms of determining which samples were likely to be linked by recent transmission. Mutation 2059A→G was detected in the 23S rRNA genes of isolates with low azithromycin minimum inhibitory concentrations, including isolates that were susceptible; the phylogeny suggested that the HL-AziR isolates emerged from susceptible isolates.

Implications of all the available evidence
Whole-genome sequencing analysis of *N gonorrhoeae* provides a discriminatory typing method, which can be used to investigate and inform outbreak control strategy in real-time. Widespread, sustained transmission of a successful HL-AziR clone and the finding that high-level azithromycin resistance can emerge from low-level resistance is of great concern, and has implications for the long-term viability of azithromycin as part of dual therapy for the treatment of gonorrhoea.

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Methods

Data collection
For this observational study, enhanced case data were collected retrospectively. Data collected included gender, age, sexual orientation, risk factors for infection, site of infection, symptoms, co-infections, treatment, test of cure, number of partners, and partner outcome. This was done either by Public Health England contacting each sexual health clinic by phone to gather case details directly from clinical staff with responsibility for case management, or by use of an online survey tool, whereby case data were entered directly by clinical staff from each sexual health clinic.

Ethics approval was not required because data were collected as part of infectious disease outbreak management. Public Health England has permission to handle data related to public health surveillance, outbreak management, and reference laboratory activities under Regulation 3 of the Health Service (Control of Patient Information) Regulations 2002.

*Neisseria gonorrhoeae* isolates
All primary diagnostic laboratories in England were requested to send all *N gonorrhoeae* isolates found to be resistant to azithromycin (either by disc diffusion or gradient strip methodology) to Public Health England for confirmation. Isolates were confirmed as *N gonorrhoeae* by MALDI-TOF (Bruker, Coventry, UK) and azithromycin MICs (mg/L) were confirmed by Etest (bioMérieux, Basingstoke, UK) on GC agar (BD, Oxford, UK) supplemented with 1% Vitox (Oxoid, Basingstoke, UK), as stated in the manufacturer’s instructions. EUCAST clinical breakpoints were used to report azithromycin resistance (MIC >0.5 mg/L). High-level azithromycin resistance was defined as an MIC of 256 mg/L or higher. Ceftriaxone MICs were also determined using the same methodology, with the EUCAST clinical breakpoint used to define resistance as an MIC higher than 0–125 mg/L.

Of the 70 confirmed cases of HL-AziR *N gonorrhoeae* occurring across England between Nov 3, 2014, and Feb 24, 2017, seven were previously sequenced and were included in the analysis; whole-genome sequencing was attempted on the remaining 63 isolates.
To provide context to the whole-genome sequencing data, the genomes of 110 additional isolates were sequenced: ten HL-AziR isolates identified between January, 2015, and February, 2017, from outside of England (one from Wales, four from Scotland, four from Northern Ireland, and one from Ireland); 19 HL-AziR isolates from previous clusters from 2004 to 2007 in England (n=5) and Scotland (n=14);10 16 HL-AziR isolates from the reference service or the Gonococcal Resistance to Antimicrobials Surveillance Programme from 2009 to 2016; 28 isolates of NG-MAST genogroup-1407 (azithromycin MICs 0·03–1·00 mg/L); 27 isolates with a range of azithromycin MICs (1·0–36·0 mg/L); two intermediate azithromycin isolates (0·5 mg/L) from Leeds; and eight isolates from Scotland of NG-MAST ST9768 with azithromycin MICs ranging from 0·12 to 1·00 mg/L (seven isolated between April, 2014, and October, 2014, and one from February, 2016).

**Whole-genome sequencing and antimicrobial genotyping**

We mapped short read data from the isolates to a close reference sequence (WHO P),11 determined using the mash algorithm,12 and produced variant call files.13 We filtered and processed the variant call files to produce an alignment, from which we removed recombination using the Gubbins algorithm (version 2.0.0);14 we then

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**Figure 1:** Maximum likelihood phylogeny of azithromycin resistant and susceptible isolates from the UK and Ireland, 2004–17

Data derived from an alignment of single-nucleotide polymorphism variants from 170 samples without recombination removal applied (small inset). The annotated phylogeny is a monophyletic subclade of 101 samples from the larger phylogeny. In this phylogeny, recombination was removed using Gubbins. Isolates are labelled: year of isolation-month of isolation_unique identifying number. MSM=men who have sex with men. NG-MAST=Neisseria gonorrhoeae multi-antigen sequence typing. *mtrR (pR) mutations include G45D mutation in MtrR and mtrR promoter deletion.

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produced a maximum likelihood tree in conjunction with a rapid bootstrap analysis.\textsuperscript{15} We did a Bayesian time-measured phylogenetic analysis using BEAST, determined the NG-MAST sequence types, and analysed the presence of mutations or genes that have previously been associated with azithromycin resistance (appendix). We also compared the sequences with international HL-AziR \textit{N gonorrhoeae} whole-genome sequences from published studies,\textsuperscript{21–18} which were either available within the short read archive or acquired directly from the corresponding author.\textsuperscript{19}

**Role of the funding source**

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

Most of the initial cases identified in Leeds were young (16–20 years) and all were heterosexual. Over time, the majority of cases were reported from London, including 20 cases in men who have sex with men (MSM), among whom four were known to be bisexual. 43 (61\%) of 70 cases were known to be symptomatic at presentation. 46 (66\%) were positive from a single site (predominantly genital) and 17 (24\%) were positive from more than one site (23 [33\%] were positive from pharyngeal specimens: seven women and 16 men). Information on site (or sites) of infection was not available for seven cases. 18 (26\%) of the 70 cases had co-infection with chlamydia at the time of first attendance. The isolates were all susceptible to ceftriaxone (MICs of 0·008–0·032 mg/L). Most patients were treated empirically with dual therapy before susceptibility testing results were available and no confirmed treatment failures occurred.

Partner notification had limited success: of a total of 139 partners reported, only 28 (20\%) were known to have been successfully contacted. Of the 26 partners with a confirmed test result, 20 (77\%) tested positive for \textit{N gonorrhoeae}. Particularly in the MSM population in London, patients reported multiple anonymous partners (n=63) and it was not possible to verify if any were successfully contacted and tested. The patients did not provide information to allow clear links to be established between them, with the exception of nine couples and two sets of four linked individuals. Description of a clearly linked sexual network was not possible, which gives an indication of the number of undiagnosed cases involved in transmission.

Of the 70 HL-AziR \textit{N gonorrhoeae} isolates referred from England between Nov 3, 2014, and Feb 24, 2017, ten isolates could not be retrieved or failed sequencing. Seven isolates had been sequenced previously and were all ST9768.\textsuperscript{1} In-silico NG-MAST for the remaining isolates revealed ten sequence types: 649 (seven cases), 2475 (four cases), 5543 (one case), 7573 (one case), 9768 (30 cases), 13124 (two cases), 13125 (one case), 13377 (three cases), 14110 (one case), and 14308 (three cases). The majority of isolates from England (37 of the 60 cases for which whole-genome sequencing was successful) were ST9768.

A phylogeny based on whole-genome SNPs was produced (appendix). Within the phylogeny, three clades were defined to describe the isolates from the 2014–17 period, which included the additional non-outbreak isolates as described in the methods (isolates were labelled: year of isolation-month of isolation_unique identifying number; figure 1). Clade 1 consisted of the ST9768 isolates and the ST14308 isolates. Clade 3 consisted of isolates closely related to clade 1, and all were ST649. Clade 2 consisted of isolates with a more distant common ancestor to that of clades 1 and 3 and had a mixture of sequence types, including some (5 of 16) of ST649. Four of the HL-AziR \textit{N gonorrhoeae} isolates referred during the outbreak (sequence types 7573, 13124 [two cases], and 14110) were unrelated to the rest of the isolates and are not shown in the large phylogeny. The number of SNPs observed between any two isolates from the epidemiologically linked couples or the two sets of linked individuals ranged from zero to three. Isolates of ST9768 were all genetically similar, with a mean distance of 4·3 SNPs. When one outlying sample (sample 2016-02_73, azithromycin susceptible) was excluded, the mean SNP difference between clade 1 isolates was 4–0 SNPs, with a maximum SNP difference between any two isolates of 14 SNPs. All of these ST9768 isolates shared a recent common ancestor indicative of recent transmission. The three ST14308 isolates were of a lineage directly derived from the ST9768 lineage (bootstrap value of 100). They were all from Leeds and were isolated at the end of 2016, a year after the previous Leeds isolates. Three HL-AziR isolates from Scotland and the single Welsh isolate were also ST9768 and fell within clade 1, but those from Northern Ireland and Ireland, and three from Scotland were different sequence types and were in the phylogenetically distinct clade 2. The mean SNP difference between clade 2 isolates was 18–8 (range 0–36); and between clade 3 isolates was 3–4 (range 0–7). The minimum difference between any clade 2 isolate and any isolate within clade 1 was 14 SNPs, and between any clade 2 isolate and any isolate within clade 3 was 18 SNPs. The isolates in clades 1 and 3 were closer because the minimum difference between any clade 1 and any clade 3 isolate was seven SNPs. Ten unique SNPs separated clade 2 from both clades 1 and 3 each, and six unique SNPs separated clade 1 and clade 3. Analysis using BEAST (figure 2; appendix) suggested that the time to the most recent common ancestor for the three clades was around 6·4 years (95\% CI 5–8). The time to the most recent common ancestor for clades 1 and 3 was 4·3 years (3–5–3).
Figure 2: BEAST analysis of a subclade of samples isolated from the UK in 2004–17

The BEAST analysis is of an alignment of single-nucleotide polymorphism variants from a subclade of samples described in this Article. Posterior probabilities are indicated by the colour of the branches, which are coloured to represent confident (blue) through to low confidence (red). The clades described within this Article are highlighted using three shaded blocks and are labelled to the right of the sample names. The samples highlighted in green are the azithromycin-susceptible isolates from Scotland in 2014. The scale on the phylogeny represents years in the past (from right to left) since the most recent isolate (February, 2017). Isolates are labelled: year of isolation-month of isolation_unique identifying number.
Almost all of the HL-AziR isolates had the 2059A→G mutation (E coli numbering) either in three or all four alleles of the 23S rRNA gene, apart from one isolate (2015-07_101) which had two mutated alleles. Unfortunately, sequencing could not be repeated to confirm this finding because the sample was lost due to an archiving error. The eight isolates from Scotland (2014-04_61, 2014-04_62, 2014-04_63, 2014-06_64, 2014-06_65, 2014-08_66, 2014-10_67, 2016-02_73) that were NG-MAST ST9768 with azithromycin MICs

Figure 3: Maximum likelihood phylogeny of high-level azithromycin-resistant Neisseria gonorrhoeae collected from Canada, Europe, and the USA, compared with the UK samples
(A) Produced from an alignment of single-nucleotide polymorphism variants derived from all samples described in this Article (inset of figure 1) and Neisseria gonorrhoeae samples that were reported as high-level azithromycin-resistant from the public short read archive. Because of the diversity of the samples, a procedure to remove recombination was not done. (B) Only those UK samples present in the monophyletic clade descending from a common ancestor shown in the large phylogeny in figure 1 are included. In this phylogeny, recombination was removed using Gubbins. The UK clade with NG-MAST type 9768 (clade 1) is highlighted with a shaded box. Isolates are labelled: year of isolation-month of isolation_unique identifying number. *Specifically from the monophyletic clade described in figure 1, with clade 1 shown in yellow and the rest in blue.
ranging from 0·12 mg/L to 1·00 mg/L were in some cases within zero SNPs of the ST9768 HL-AziR isolates, suggesting the 23S rRNA copy number evolution occurred more rapidly than the accumulation of neutral SNPs. Five susceptible isolates (MIC of 0·25 mg/L) had one mutated 23S rRNA allele and one low-level resistant isolate (MIC of 1·0 mg/L) had two mutated alleles. Two susceptible isolates did not have any mutated alleles. The phylogeny provided evidence that the HL-AziR isolates were descendants of the low-level azithromycin-resistant isolates, which were in turn, descendants of the susceptible isolates. The position of sample 2016-02_73 within the phylogeny is the only sample not congruent with this suggested scenario, but the bootstrap value for this node is low. The BEAST phylogeny generated using the same sequence data (figure 2; appendix) grouped this isolate with the other susceptible isolates, although the posterior probabilities for this section of the tree were low. All of the HL-AziR isolates also carried a G45D mutation in mtrR, but none had the 261IC→T mutation in the 23S rRNA gene or any mutations in other genes associated with macrolide resistance.

Comparison with the few international HL-AziR *N gonorrhoeae* whole-genome sequences available within the short read archive showed that the UK HL-AziR *N gonorrhoeae* cases reported from 2014 onwards were distinct from those reported elsewhere (figure 3; appendix).

**Discussion**

This is the first report of sustained transmission of a clonal outbreak of HL-AziR *N gonorrhoeae* over several years. Previously the HL-AziR phenotype has been observed sporadically and in small clusters in the UK and elsewhere.\(^5\, 10, 16, 20–22\) Our study included the largest number of HL-AziR isolates sequenced to date; we found that they clustered into three phylogenetic clades and were distinct from the majority of the samples with low-level azithromycin resistance. We have also shown that high-level resistance can emerge from susceptible strains or strains with low-level resistance in a short amount of time.

The molecular clock of *N gonorrhoeae* needs to be understood to predict the likelihood of transmission. However, the SNP phylogeny was difficult to interpret due to the little context available with regard to other English samples because *N gonorrhoeae* is not routinely sequenced as part of Public Health England’s surveillance programme. Data from De Silva and colleagues\(^6\) suggest that within a 12 month period the mean number of expected substitutions per genome is four, with an upper 95% confidence limit of 14. Within clade 1, the earliest sample was from April, 2014, and the latest sample was February, 2017—a period of 2 years 10 months. The isolates of ST9768 differed by fewer than 14 SNPs, which was close to the mean of four SNPs per genome per year. Together with our BEAST analysis, this finding supports the conclusion that these isolates shared a common ancestor indicative of very recent transmission.

By contrast, the isolates from clade 2 differed from all isolates in clade 1 by at least 14 SNPs. There are isolates within clade 2 with similar isolation dates to those in clade 1 from the early part of the outbreak period. This finding, together with the topology of the phylogeny and BEAST analysis, suggests that clades 1 and clades 2 are unlikely to be part of the same recent transmission chain but shared a common ancestor 6·5 years ago. These lineages might have evolved from a UK-derived common ancestor and have been circulating in the UK population, or might be due to separate introductions of an internationally successful clone that diversified to produce the extant variation observed in clades 1 and 2. The minimum distance between any two isolates in clades 1 and 3 was seven SNPs. Considering that the earliest sample in clade 3 was from April, 2016, and the most ancestral part of clade 1 was from late 2014, we believe that the common ancestor probably diverged into two clonal expansions, one of which manifested as the successful ST9768 clade and the other as clade 3, but that we have not detected cases in the intervening period.

The SNP phylogeny gave a level of discrimination beyond that of NG-MAST in terms of determining which isolates were likely to be linked by recent transmission— for example, NG-MAST ST649 was found throughout the phylogeny. A ST649 HL-AziR *N gonorrhoeae* cluster was identified in the UK in 2007,\(^9, 10\) and this sequence type has been associated with HL-AziR *N gonorrhoeae* internationally.\(^16, 20–22\) The ST9768 clone might be a descendant of one of the lineages of the globally successful HL-AziR ST649. Interestingly, the Scottish ST9768 isolates, which were susceptible or demonstrated low-level resistance to azithromycin, were found within the HL-AziR ST9768 clade. The phylogeny showed that the HL-AziR ST9768 isolates were descendants of the low-level azithromycin-resistant isolates, which were in turn, descendants of the susceptible isolates. However, the maximum likelihood and BEAST phylogenies both suggest that the susceptible ST9768 Scottish isolates originated from an azithromycin-resistant parent but became susceptible through reversion of mutant alleles of the 23S rRNA gene to wild type, either through back mutation or recombination. This change might occur if HL-AziR is associated with a fitness cost. We hypothesise that azithromycin exposure might then have provided selection pressure for the one or two mutated alleles to recombine with wild-type copies without the mutation. Alternatively, azithromycin exposure might have induced additional 2059A→G mutations in the wild-type alleles. Either scenario would lead to three or four mutated copies, which would confer the HL-AziR phenotype, and has been shown to occur in the laboratory.\(^20\) However, without more comprehensive sampling, particularly of isolates from England, we cannot know this for certain. Azithromycin is used in the treatment of other sexually
transmitted infections, particularly chlamydia; as such, concurrent undiagnosed *N gonorrhoeae* could be exposed to subtherapeutic levels of azithromycin during the treatment of these other infections and select for resistance. Additionally, inappropriate azithromycin monotherapy for *N gonorrhoeae* could lead to treatment failure by providing selection pressure for the development of resistance.

We found that most HL-AziR *N gonorrhoeae* isolates had the 2059A→G mutation in all four copies of the 23S rRNA genes. Low-level azithromycin resistance (MICs 1.0–32.0 mg/L) is commonly associated with a 2611C→T 23S rRNA gene mutation. Whole-genome sequencing of 75 azithromycin-resistant isolates from Europe\(^6\) identified the 2611C→T mutation in two to four alleles of the 23S rRNA gene in isolates with MICs ranging from 4 mg/L to 8 mg/L, and in all four alleles of isolates with MICs of 16–32 mg/L. The 2059A→G mutation was detected in all four alleles of isolates with MICs of 256 mg/L or higher (n=4), but not in isolates with lower MICs. Additionally, mutations in *mtrR* and its promoter, leading to overexpression of the MtrCDE efflux pump, occurred in isolates across the whole MIC range. A Canadian study,\(^7\) which included five isolates with HL-AziR, had similar findings. In both studies, the resistant isolates clustered clonally into distinct lineages, but the HL-AziR isolates were found across the phylogenetic tree. By contrast, a US study\(^1\) found that isolates with azithromycin resistance were more diverse and showed less clonal expansion than the European and Canadian studies. Only two isolates had HL-AziR, both with four alleles with the 2059A→G mutation.

The UK HL-AziR isolates from 2014 onwards were distinct from those seen previously in the UK, from the international HL-AziR *N gonorrhoeae* sequences available from the studies that we have previously described,\(^1\) and also from a cluster of seven cases of HL-AziR *N gonorrhoeae* reported in Hawaii in 2016. A retrospective prevalence study\(^2\) from Hangzhou, China, in 2012, found HL-AziR in 21 of 118 isolates tested, suggesting that HL-AziR might be widespread in this region. These isolates belonged to seven different NG-MAST sequence types, although some of the sequence types were more than 99% similar;\(^2\) these were different sequence types to those found in our study. HL-AziR might lead to a fitness cost, which could explain why this phenotype has previously been seen only sporadically or in non-sustained clusters. We do not know why sustained transmission of ST9768 has occurred in England; perhaps compensatory mutations are necessary for this HL-AziR phenotype to be present. Further research to investigate this hypothesis is needed.

In our study, cases from both heterosexual and MSM populations were seen across the phylogenetic tree, providing evidence of transmission between MSM and heterosexual networks on several occasions during the course of this relatively short time period. This suggests that there might be greater fluidity between sexual networks than previously supposed, which, in view of the large numbers of partners reported in both populations in this outbreak, has implications for infection control. It also suggests that when resistance emerges in one population it can soon spread to the other. Of particular concern is that 80% of contacts were not traceable, which could mean a substantial burden of unidentified cases given that 77% of contacts who were traced were found to be positive.

Fortunately, there were no confirmed treatment failures in any of these cases in this study, probably because the isolates were all susceptible to ceftriaxone. The cluster of seven cases of HL-AziR *N gonorrhoeae* reported in Hawaii in 2016\(^6\) is concerning because these isolates also showed decreased susceptibility to ceftriaxone (MIC 0.125 mg/L). It is reassuring that all of the patients in Hawaii were successfully treated with 250 mg ceftriaxone plus 1 g azithromycin, and there have been no further cases.

Given that the prevalence of low-level azithromycin resistance (MICs >0.5 mg/L) among culture-positive detected cases in England is now 5%,\(^7\) there is concern that HL-AziR *N gonorrhoeae* might arise more frequently if a proportion of the low-level resistant isolates already harbour an 2059A→G mutation in a single allele. Even low-level azithromycin resistance is likely to render the azithromycin component of dual therapy ineffective. In a Japanese study,\(^8\) treatment failures in men with gonococcal urethritis treated with an extended-release 2 g azithromycin single dose were associated with azithromycin MICs of higher than 0.5 mg/L. Additionally, the in-vitro MIC of azithromycin does not necessarily correlate with clinical treatment outcome, and treatment failures occur in patients with isolates identified as azithromycin susceptible in the laboratory.\(^9\)

In conclusion, sustained transmission of a successful HL-AziR *N gonorrhoeae* clone has been observed across England. Whole-genome sequencing provided characterisation of an outbreak substantially beyond that achieved by NG-MAST. Whole-genome sequencing also progressed our understanding of the emergence and mechanisms of resistance, particularly the finding that high-level resistance can emerge from low-level resistance. Dual therapy for gonorrhoea using azithromycin with ceftriaxone is clearly under threat and we might not be able to rely on azithromycin to protect ceftriaxone. Surveillance of resistance, regular review of treatment guidelines, and detection of treatment failures are crucial towards keeping gonorrhoea as a treatable infection in the future.

**Contributors**

HF prepared the manuscript with input from MC, GH, SP, NW, and AU. AU and US prepared the figures. All other authors contributed to the review of the final manuscript. MC managed the laboratory work and assisted with the analysis. NM prepared the cultures for whole-genome sequencing. US, RM, and AU developed and performed the bioinformatic analysis. HF and AU analysed the data. HF and GH...
coordinated the national outbreak control team. SP, AW, and CS performed data collection, analysis, and interpretation for epidemiological information for the outbreak cases. KT and JS managed the Scottish Reference service and identified ST9768 isolates in their laboratory.

Declaration of interests
Public Health England’s Antimicrobial Resistance and Healthcare Associated Infections Reference Unit has received financial support for conference attendance, lectures, research projects, or contracted evaluations from numerous sources, including Accelerate, Achaogen, Allerega, Amplex, AstraZeneca, Basilea, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, the BSAC, Cepheid, Check-Points, Cubist Pharmaceuticals, Department of Health, European Centre for Disease Prevention and Control, Enigma Diagnostics, Food Standards Agency, GlaxoSmithKline, Henry Stewart Talks, IHMA, Kalidex, Melinta, Merck Sharp & Dohme, Meiji, Mobidiag, Momentum Biosciences, Nordic, Norgine, Rempex, Roche, Rokitan, Smith & Nephew, Trius, VenatoRx, and Wockhardt. HF is a member of the Scientific Advisory Board for Discuva. All other authors declare no competing interests.

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