Europe-wide expansion and eradication of multidrug-resistant Neisseria gonorrhoeae lineages: a genomic surveillance study

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Summary

Background Genomic surveillance using quality-assured whole-genome sequencing (WGS) together with epidemiological and antimicrobial resistance (AMR) data is essential to characterise the circulating Neisseria gonorrhoeae lineages and their association to patient groups (defined by demographic and epidemiological factors). In 2013, the European gonococcal population was characterised genomically for the first time. We describe the European gonococcal population in 2018 and identify emerging or vanishing lineages associated with AMR and epidemiological characteristics of patients, to elucidate recent changes in AMR and gonorrhoea epidemiology in Europe.

Methods We did WGS on 2375 gonococcal isolates from 2018 (mainly Sept 1–Nov 30) in 26 EU and EEA countries. Molecular typing and AMR determinants were extracted from quality-checked genomic data. Association analyses identified links between genomic lineages, AMR, and epidemiological data.

Findings Azithromycin-resistant N gonorrhoeae (8·0% [191/2375] in 2018) is rising in Europe due to the introduction or emergence and subsequent expansion of a novel N gonorrhoeae multi-antigen sequence typing (NG-MAST) genogroup, GI2302 (132 [5·6%] of 2375; N gonorrhoeae sequence typing for antimicrobial resistance [NG-STAR] clonal complex [CC]168/63), carrying a mosaic mtrR promoter and mtrD sequence and found in 24 countries in 2018. CC63 was associated with pharyngeal infections in men who have sex with men. Susceptibility to ceftriaxone and cefixime is increasing, as the resistance-associated lineage, NG-MAST GI407 (51 [2·1%] of 2375), is progressively vanishing since 2009–10.

Interpretation Enhanced gonococcal AMR surveillance is imperative worldwide. WGS, linked to epidemiological and AMR data, is essential to elucidate the dynamics in gonorrhoea epidemiology and gonococcal populations as well as to predict AMR. When feasible, WGS should supplement the national and international AMR surveillance programmes to elucidate AMR changes over time. In the EU and EEA, increasing low-level azithromycin resistance could threaten the recommended ceftriaxone–azithromycin dual therapy, and an evidence-based clinical azithromycin resistance breakpoint is needed. Nevertheless, increasing ceftriaxone susceptibility, declining cefixime resistance, and absence of known resistance mutations for new treatments (zoliflodacin, gepotidacin) are promising.

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Introduction

The 2020 global estimates for gonorrhoea indicated 82 million annual cases among adults.1 Antimicrobial resistance (AMR) in Neisseria gonorrhoeae is threatening gonorrhoea treatment. N gonorrhoeae has developed or acquired resistance to every antimicrobial used for empiric therapy, including the first-line extended-spectrum cephalosporin ceftriaxone and azithromycin.2 Fortunately, inability to cure gonorrhoea with ceftriaxone, in recommended monotherapy or together with azithromycin,2 remains rare.3,4 despite the international spread of a ceftriaxone-resistant clone (FC428) and the prevalent decreased susceptibility or resistance to ceftriaxone and azithromycin internationally.3,4 The European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) monitors AMR in the EU and EEA by analysing annual, quality-assured AMR data in conjunction with epidemiological and clinical data of patients with gonorrhoea.4 Molecular epidemiological characterisation of gonococcal isolates was done in two previous Euro-GASP surveys. First, 1066 Euro-GASP isolates from 2009–10 were genotyped using N gonorrhoeae multi-antigen sequence typing (NG-MAST) and, second, 1054 Euro-GASP isolates from 2013 were subjected to whole-genome sequencing (WGS), which revealed the distribution of particular sequence types and AMR clones across different patient groups. In 2009–10, the major EU and EEA lineage was N gonorrhoeae...
Research in context

Evidence before this study
Gonorrhoea is a major public health problem internationally, and antimicrobial resistance (AMR) in Neisseria gonorrhoeae compromises the effective management and accordingly, the control of gonorrhoea. Phenotypic AMR surveillance is imperative, nationally and internationally. Whole-genome sequencing (WGS) can further support phenotypic AMR surveillance by, for example, elucidation of gonococcal population dynamics and identification of AMR (or antimicrobial susceptible) lineages or clones, and by providing understanding of AMR determinants conferring AMR and molecular prediction of phenotypic AMR. We searched PubMed using the terms “Neisseria gonorrhoeae” OR “gonorrhoea” with “genome sequencing” for papers published in English between Jan 1, 2000, and June 25, 2021. WGS of N gonorrhoeae has mainly been used to investigate molecular epidemiology at national or local level in, for example, the USA, Canada, Argentina, Brazil, the UK, China, Japan, Vietnam, and Australia. Most of these studies have focused on isolates selected because of their AMR (with relatively few isolates sequenced in many studies) or for examining the spread of relatively few AMR strains or local outbreaks (including determining transmission chains). Gonococcal genomes with available metadata from most of these studies are available and can be compared in Pathogenwatch. To our knowledge, except for our smaller study that examined 75 azithromycin-resistant isolates from 17 EU and EEA countries and our 2013 WGS study (1054 consecutive antimicrobial-resistant and antimicrobial-susceptible isolates from 20 EU and EEA countries), there have been no other regional studies that used WGS of selected or consecutive N gonorrhoeae isolates.

Added value of this study
We report WGS data for 2375 gonococcal isolates cultured in 2018 in 26 EU and EEA countries, in conjunction with AMR data and epidemiological data for the patients with gonorrhoea, and compare with N gonorrhoeae multi-antigen sequence typing (NG-MAST) data from 2009–10 and WGS data from 2013 for gonococcal isolates from 20 EU and EEA countries. We describe increasing azithromycin resistance and increasing susceptibility to extended-spectrum cephalosporins in the EU and EEA, and elucidate the reasons for these changes. Increasing low-level azithromycin resistance among gonococci in the EU and EEA is largely due to the introduction and subsequent expansion of a novel NG-MAST genogroup, G12302 (NG-STAR clonal complex [CC]168 or CC63), carrying a mosaic mtrR promoter and mtrO sequence resulting in low-level azithromycin resistance.

multi-antigen sequence typing (NG-MAST) genogroup 1407 (G1407), associated with decreased susceptibility and resistance to extended-spectrum cephalosporins and men who have sex with men (MSM). This lineage threatened the recommended empirical cefixime monotherapy in the EU and EEA until 2012, when cefixime monotherapy was replaced by dual therapy (ceftriaxone plus azithromycin). In 2013, the NG-MAST G1407 incidence had substantially decreased and its association switched from MSM to heterosexual groups. Since the change in therapy recommendation, the incidence of resistance to cefixime and ceftriaxone has decreased, but the level of azithromycin resistance has increased, illustrating that recommended treatments impact on the gonococcal...
population dynamics, together with other factors—eg, antimicrobial overuse and misuse, changes in sexual behaviour in or between sexual networks, and expansion or eradication of specific gonococcal lineages.4,5

WGS proved in the 2013 survey6 valuable for detailed surveillance of gonorrhoea by providing a genomic baseline of the EU and EEA gonococcal population, in conjunction with associated AMR and epidemiological data, and by informing public health and associated interventions. WGS provided a more ideal resolution and accuracy compared with other typing schemes.4 WGS allows the identification of high-risk or AMR clones, transmission chains or outbreaks, known and novel AMR determinants, and new targets for therapy, vaccines, and diagnostics, including molecular tests for AMR prediction.

In this study, we analysed WGS results on 2375 N gonorrhoeae isolates from 26 EU and EEA countries in 2018 together with quality-assured AMR data and epidemiological and clinical information from the patients with gonorrhoea. We identified novel genomic lineages and their association with AMR and patient metadata, predicted AMR, and monitored existing high-risk lineages through a longitudinal comparison with the Euro-GASP 2013 WGS7 and 2009–10 NG-MAST7 surveys.

Methods

Euro-GASP sampling and antimicrobial susceptibility testing

In Euro-GASP 2018, 1299 gonococcal isolates linked to metadata of patients were collected in 27 EU and EEA countries, mostly from Sept 1 to Nov 30, 2018.8 2653 (80.4%) of 3299 isolates from 26 countries were available for this study and for 2375 (72.0%) of 3299, quality-controlled genomic data linked to AMR and metadata were obtained (table 1). One isolate per infection episode was included in accordance with sampling priority rules (appendix 1 pp 1–6).

All countries were part of the annual Euro-GASP external quality assessment (EQA).9 19 (73%) of 26 countries did decentralised AMR testing in their country while isolates from 7 (27%) of 26 countries were tested centrally at UK Health Security Agency (UKHSA, London, UK) or Örebro University Hospital (ÖUH, Örebro, Sweden). Minimum inhibitory concentration (MIC) gradient strip tests or agar dilution were done for ceftriaxone, cefixime, azithromycin and ciprofloxacin as previously described.4 Current (v11.0) breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied. AMR testing was repeated for some isolates with discrepancies between phenotypic and genotypic resistance (appendix 1 pp 8–9).

Genomic sequencing and analyses

Total DNA was extracted from pure cultures using a QIAasympnomy instrument (Qiagen, Hilden, Germany) at UKHSA and ÖUH, frozen and sent to the Wellcome Sanger Institute (UK) for WGS. Raw genomic data was quality-checked, assembled and processed to obtain molecular typing information (NG-MAST, multilocus sequence typing [MLST], and N gonorrhoeae sequence typing for antimicrobial resistance [NG-STAR]) and to detect genetic AMR determinants. Association analyses among the genotypes in each country and the phenotypic AMR or patient epidemiological data was performed. For details regarding WGS, quality checks, molecular typing, bioinformatics and statistical analyses, see appendix 1.

Role of the funding source

The funders had no role in study design, data collection, analysis, interpretation, or report writing.
Results

The gonococcal isolates from 26 EU and EEA countries with WGS data and patient metadata linked to AMR passing quality check represented 72·0% (2375 of 3299) of the Euro-GASP 2018 isolates (table 1). Of these gonorrhoea patients, 2343 (98·7%) of 2375 reported sex, of which 1992 (85·0%) of 2343 were men and 351 (15·0%) of 2343 were women. Age was reported for 2319 (97·6%) of 2375 patients, and the largest age group was 25–34 years (872 [37·6%] of 2319) followed by 24 years or younger (660 [28·5%] of 2319). The median age for women was 24 years (IQR 21–32), with 24 years or younger (181 [51·7%] of 350 women with information about sex and age) being the largest group. For men, the median age was 30 years.

For the Euro-GASP 2018 see https://pathogen.watch/collection/eurogasp2018

Figure 1: Concordance between antimicrobial minimum inhibitory concentrations (phenotypes) and genetic determinants of AMR (genotypes, single or in combination) in the Euro-GASP 2018 genomic dataset

Minimum inhibitory concentrations (mg/L) of four key antibiotics showed by the combinations of known genetic AMR determinants observed in 2375 Neisseria gonorrhoeae isolates. X-axis shows the AMR determinants (black dots linked with a solid black line to indicate their presence in combination) and y-axis the minimum inhibitory concentrations (coloured dots, where size of dot indicates number of isolates). Violins are coloured to indicate different combinations of AMR determinants. Dashed horizontal lines represent the epidemiological cut-off for azithromycin and clinical resistance breakpoints for ciprofloxacin, cefixime, and ceftriaxone established by the European Committee on Antimicrobial Susceptibility Testing. AMR=antimicrobial resistance. Euro-GASP=European Gonococcal Antimicrobial Surveillance Programme.
with this mtr mosaic remained azithromycin susceptible (MIC=0–0.032–1 mg/L). These 133 azithromycin-resistant isolates formed a single lineage constituted by mainly NG-MAST G12302 isolates (figure 2), which was found in 24 EU and EEA countries (figure 3; appendix 1 pp 11, 20). Some azithromycin-resistant isolates carried mutations in the mtrR promoter (n=26), mtrR (n=25), or the rplD G70D (n=4) mutation, or any combination of these (figure 1). The GC deletion in mtrR that increases antimicrobial susceptibility was found in 17 isolates, of which 13 had the mtr mosaic14–19 mentioned here (MICs=0–0.032–0.125 mg/L), and four had only a mosaic mtrR (MICs=0–0.32–0.5 mg/L). None of them had any 23S rDNA azithromycin resistance mutations. 24·1% (46 of 191) of azithromycin-resistant cases were explained by considering only 23S rDNA resistance mutations and 69·6% (133 of 191) by considering only the mtr mosaic (appendix 1 pp 11, 18–19).14,15
The only ceftriaxone-resistant isolate (MIC=0–5 mg/L; azithromycin MIC=0–25 mg/L) carried the mosaic penA 60.001, described in the internationally spreading ceftriaxone-resistant clone FC428.1 This strain was genomically similar to FC428 and only 131 single nucleotide polymorphisms (SNPs)-distant (49 loci) in the cgMLST scheme (appendix 1 pp 22–24), compared with over 1800 SNPs to other isolates in the same clade. All 36 cefixime-resistant isolates (MICs=0–25–2 mg/L) carried a mosaic penA (figure 1): 25 isolates carried mosaic penA 10.001 (MICs=0–0.25–0.5 mg/L), nine mosaic penA 34.001 (MIC=0–0.25 mg/L), 1 mosaic penA 60.001 (MIC=2 mg/L) and 1 mosaic penA 171.001 (MIC=0–25 mg/L). Notably, 114 cefixime-susceptible isolates also carried a mosaic penA allele. Of those, 85 carried different variants of penA 34 (MICs=0–0.032–0.125 mg/L), 25 penA 10.001 (MICs=0–0.032–0.125 mg/L), 2 penA 188.001 (MIC=0–0.064 mg/L), 1 penA 63.001 (MIC=0–0.032 mg/L), and 1 penA 171.001 (MIC=0–0.125 mg/L; figure 2).
No known target resistance mutations for the new potential treatment options zoliflodacin or gepotidacin were found.17–19
MLST, NG-MAST, and NG-STAR sequence types across countries are shown in appendix 1 (pp 14–15, 24–25). 189 MLST (34 with >10 isolates), 419 NG-STAR (33 with >10 isolates) and 273 NG-MAST (30 with >10 isolates) sequence types were detected (figure 2, table 2). The most abundant sequence types in the 2375 isolates were MLST sequence type(ST)9363, found in 221 (9·3%) isolates, NG-STAR ST442, in 162 (6·8%) isolates, and NG-MAST ST11461, in 112 (4·7%) isolates (table 2). Of the 2375 isolates, 1592 (67·0%) isolates were assigned to an NG-MAST genogroup and 2329 (98·1%) to an NG-STAR CC (appendix 1 pp 10, 14–15). Using an Analysis of Molecular Variance, most of the genotypic variation was found within countries (96·9%) rather than between them (3·0%). No significant population structure was detected by randomising population structure (p=0·55). The three most abundant NG-MAST genogroups were G12302 (132 [5·6%] of 2375; 89 of
Figure 2: Main Neisseria gonorrhoeae molecular sequence types and antimicrobial-resistant lineages in the EU and EEA in 2018
Phylogenetic reconstruction of 2375 N. gonorrhoeae isolates from 26 European countries from 2018. Main NG-MAST genogroups (G) and NG-STAR CCs are highlighted in different colours. First block of columns shows the assignment of each isolate to the three typing schemes: MLST, NG-MAST, and NG-STAR, including NG-MAST genogroups and NG-STAR CCs. Only types with more than 30 isolates are shown for visualisation purposes. The blocks represent AMR data in the form of SIR for azithromycin, cefixime, and ciprofloxacin followed by the main genetic resistance determinants for each. MtrD mosaic alleles represent eight different haplotypes found for the amino acid sequence of the mtrD gene in this dataset. AMR=antimicrobial resistance. CC=clonal complex. MLST=multi locus sequence typing. NG-MAST=N. gonorrhoeae multi-antigen sequence typing. NG-STAR=N. gonorrhoeae sequence typing for antimicrobial resistance. SIR=susceptible, intermediate (susceptible, increased exposure), resistant.
them NG-STAR CC168 and 35 CC63), G5441 (132 [5·6%] of 2375; 120 CC442) and G11461 (128 [5·4%] of 2375; 123 CC42), which all increased by 5% or more compared with previous Euro-GASP surveys\(^7,8\) (figure 2; appendix 1 pp 24–25, appendix 2 pp 1–2). NG-MAST ST12302 (mostly MLST ST9363 and NG-STAR ST168) is an emerging clone that was not found in the previous surveys (table 2),\(^7,8\) whereas the second major sequence

Figure 3: Distribution of the most prevalent NG-STAR CCs and NG-MAST genogroups in Europe in 2018 compared to 2009–10\(^7\) and 2013\(^8\) expansion of NG-MAST G12302 and remission of G1407

(A) Proportion of isolates assigned to the top three NG-STAR CCs (in each country, and ‘Other’ includes all other CCs in the specific country) found in the Euro-GASP 2018 genomic study in each participating country. (B) Proportion of isolates assigned to the top three NG-MAST genogroups (in each country, and ‘Other’ includes all other genogroups in the specific country), found in the Euro-GASP 2018 genomic study in each participating country. (C) Left panel shows the increase of 5% in frequency of NG-MAST G12302 from 2013 to 2018 (reaching 5·6% [132/2375] of all isolates in the present Euro-GASP 2018 WGS survey) mostly due to the substantial expansion of NG-MAST ST12302. Other NG-MAST STs within this genogroup, including the second major ST3935 were part of G4822 in the Euro-GASP 2013 survey\(^8\) and absent in 2009–10. \(^7\) Right panel shows the sharp decrease in frequency of NG-MAST G1407 with resistance or decreased susceptibility to cefixime in the three Euro-GASP surveys, which decreased by >14% from 2013\(^8\) to 2018 (reaching 2·1% [51/2375] of all isolates in the present Euro-GASP 2018 WGS survey). Notably, the frequencies between the molecular study time points (2009–10,\(^7\) 2013\(^8\) and 2018) are unknown, and only drawn to illustrate possible changes over time.

CC=clonal complex.

Euro-GASP=European Gonococcal Antimicrobial Surveillance Programme.

NG-MAST=Neisseria gonorrhoeae multi-antigen sequence typing.

NG-STAR=N gonorrhoeae sequence typing for antimicrobial resistance.
type in G12302, ST3935 (mostly MLST ST9363 or ST11422 and NG-STAR ST193), was found within G4822 in 2013. Genogroup G1407 (CC90) was the most abundant in 2009–10 (248 [23·3%] of 1066) and 2013 (78 [7·5%] of 1054), but only included 51 (2·1%) of 2375 isolates in 2018, showing a significant decrease (figure 3B, C). NG-MAST ST1407 (MLST ST1901 and NG-STAR ST90) also significantly decreased; 15·6% (166 of 1066) in 2009–10, 7·4% (78 of 1054) in 2013, and 0·8% (19 of 2375) in 2018 (appendix 2 pp 1–2). The four major NG-STAR CCs found in 2375 isolates in 2018 were NG-STAR CC63 (270 [11·4%]), CC442 (219 [9·2%]), CC1340 (166 [7·0%]) and CC1387 (165 [6·9%]). These results contrast with a recent study that mostly included data on isolates from Euro-GASP 2013 and where CC90 was the major complex (194 [18·0%] of 1075) followed by CC63 (166 [15·4%] of 1075). In 2018, 90 (3·8%) of 2375 isolates clustered in CC90, also supporting the 14% decrease in this AMR lineage as calculated for the associated NG-MAST G1407.

Of the three largest NG-MAST genogroups, G12302 did not show any significant association with an age group, gender, sexual orientation, or site of infection.
this genogroup, was associated with MSM (odds ratio [OR] 1·8 [95% CI 1·3–2·5], p<0·0001), men (OR 2·4 [1·5–3·9], p<0·0001), and pharyngeal (OR 2·2 [1·5–3·2], p<0·0001) infections (appendix 2 p 7, 10). Both NG-MAST G5441 and G1461 were associated with men, and G11461 also with MSM (OR 4·9 [2·6–9·6], p<0·0001; appendix 2 p 5). NG-MAST ST11461 was the major sequence type found in 2018 (table 2) and was associated with men and MSM (OR 4·3 [2·2–8·4], p<0·0001; appendix 2 p 3). The major NG-STAR CCs associated with NG-MAST G5441 (CC442) and G1461 (CC42) also supported an association with men and MSM (appendix 2 p 7, 10). NG-MAST G1407 was associated with MSM in 2009–10 and heterosexuals in 2013. In 2018, G1407 (CC90), was exclusively found in heterosexual patients and was associated with women (OR 2·9 [1·6–5·3], p<0·0001; appendix 2 p 6, 9).

The gonococcal lineages with the strongest association with azithromycin resistance were within NG-MAST G12302 (OR 102·6 [61·6–170·1], p<0·0001), including the two major sublineages characterised by NG-MAST ST12302 (MLST ST9363, NG-STAR CC168) and NG-MAST ST3935 (MLST ST9363 or ST11422, NG-STAR CC63; appendix 2 p 11). Cefixime-resistant isolates were strongly associated with NG-MAST G13876 (OR 452·1 [109·8–2342·7], p<0·0001), NG-STAR CC348, and several others, including NG-MAST G1407 (OR 14·5 [5·3–39·6], p<0·0001) (MLST ST1901, NG-STAR CC90). Resistance to ciprofloxacin was associated with several lineages, including STs of the three schemes exclusively formed by ciprofloxacin-resistant isolates. An increased MIC of ceftriaxone was associated with several sequence types, with the top lineage being NG-STAR ST38 (OR 18·2 [13·1–24·9], p<0·0001; MLST ST7827, NG-STAR CC38, and NG-MAST G10386). NG-MAST G13876 (CC348) and G1407 (CC90) were also associated with an increased ceftriaxone MIC (appendix 2 p 11).

Discussion

We report the genomic analysis of the Euro-GASP 2018 survey in the EU and EEA in comparison with the 2013 genomic and 2009–10 NG-MAST surveys. Since 2013, cefixime resistance has declined from 4·8% (51 of 1054) to 1·5% (36 of 2375) and azithromycin resistance increased from 6·7% (71 of 1054, using the 23S rDNA mutations sporadically acquired by isolates with limited phylogenetic relationship. Recombination in the loci encoding the MtrRCDE efflux pump have also been proven to confer resistance to azithromycin. Here, we explain the genetic basis of azithromycin resistance for N gonorrhoeae, azithromycin resistance has frequently been associated with 23S rDNA mutations and presence of N lactamica-like mosaic 2 mtrR promoter and mtrD sequences. Many isolates (80 [37–6%] of 213) with identical or similar mosaic mtrR promoter and mtrD sequences remained azithromycin susceptible; however, most of these isolates had an increased azithromycin MIC close to the epidemiological cutoff value (65 [81·3%] of 80; MIC=0–5–1 mg/L). The mosaic mtrR promoter and mtrD sequences found in this collection were similar to those found in other studies. N lactamica-like mosaic 2 being the most frequent mtr mosaic in Europe. This emerging lineage was recently described in a study that combined worldwide public N gonorrhoeae genomic data and has been reported in several countries. The rplD G70D mutation in the 50S ribosomal protein L4 is another azithromycin resistance determinant. We found rplD G70D in 34 isolates but only four (11·8%) of 34 were azithromycin resistant and three (75·0%) of these four isolates had...
Articles

Inhibitor whose main target is GyrB. Zoliflodacin-in phase 3 RCT is zoliflodacin, a topoisomerase II inhibitor which is promising new treatment for uncomplicated gonorrhoea where available.

To the new topoisomerase II inhibitor gepotidacin, additionally predispose for the development of resistance to the new topoisomerase II inhibitor gepotidacin, which are both in phase 3 RCTs and for promising new treatment for uncomplicated gonorrhoea (ceftriaxone plus azithromycin) but for resistant clone, carrying a mosaic penA, that was predominant in previous Euro-GASP molecular surveys, is progressively disappearing, which largely elucidates the decrease in cefixime resistance and increase in ceftriaxone susceptibility in recent years. The spread of the emerged azithromycin-resistant clone could threaten the effectiveness of the current recommended dual treatment for gonorrhoea (ceftriaxone plus azithromycin) but fortunately, cases of ceftriaxone resistance in the EU and EEA are scarce and combined ceftriaxone and azithromycin resistance is exceedingly rare (so azithromycin might eradicate the occasional ceftriaxone-resistant strains). Furthermore, most azithromycin-resistant isolates have low-level azithromycin resistance (MIC=2–4 mg/L) and it remains unknown whether these MICs translate into clinical resistance when using azithromycin 2 g treatment (an evidence-based clinical recommendation). However, it has been recently shown that the overall AMR prevalence reported by Euro-GASP appropriately reflects the AMR situation in the EU and EEA. Moreover, increased numbers of representative isolates remain crucial in Euro-GASP, which is also a continuous work of Euro-GASP. Furthermore, the selection of one isolate per patient or infection episode through a sampling hierarchy could have slightly biased conclusions regarding associations with infection sites. Finally, in the present Euro-GASP 2018 WGS study some isolates were not available or viable for confirmatory WGS and phenotypic AMR testing (including several of the discrepant ciprofloxacin-resistant isolates).

In summary, the EU and EEA gonococcal population in 2018 shows the emergence and subsequent expansion of an azithromycin-resistant clone (NG-MAST G12302), which largely explains the increase in azithromycin resistance during recent years, associated with pharyngeal infections among MSM (NG-STAR CC63), and carrying mosaic mtrR promoter and mtrD sequences. Nevertheless, many isolates with similar mosaic mtrR promoter and mtrD sequences remained azithromycin susceptible, despite an increased azithromycin MIC. Continued research to identify and verify novel determinants associated with resistance to azithromycin and other current and future gonorrhoea treatment options remains imperative. Contrarily, NG-MAST G1407 (NG-STAR CC90), a frequently extended-spectrum cephalosporin-resistant clone, carrying a mosaic penA, that was predominant in previous Euro-GASP molecular surveys, is progressively disappearing, which largely elucidates the decrease in cefixime resistance and increase in ceftriaxone susceptibility in recent years. The spread of the emerged azithromycin-resistant clone could threaten the effectiveness of the current recommended dual treatment for gonorrhoea (ceftriaxone plus azithromycin) but fortunately, cases of ceftriaxone resistance in the EU and EEA are scarce and combined ceftriaxone and azithromycin resistance is exceedingly rare (so azithromycin might eradicate the occasional ceftriaxone-resistant strains). Furthermore, most azithromycin-resistant isolates have low-level azithromycin resistance (MIC=2–4 mg/L) and it remains unknown whether these MICs translate into clinical resistance when using azithromycin 2 g treatment (an evidence-based clinical resistance breakpoint is required). Ultimately, new gonorrhoea treatments such as zoliflodacin or gepotidacin, which are both in phase 3 RCTs and for which the EU and EEA gonococcal population appears susceptible, will be crucial. The Euro-GASP genomic surveys show that WGS is essential to complement epidemiological and AMR information for quality-assured molecular epidemiology and surveillance of gonococci, including effective molecular AMR prediction, locally, nationally, and internationally.
Article

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Contributors

LSB, MJC, GS, DMA, and MU designed, initiated, and coordinated the study. The European STI network members supplied gonococcal isolates and patient data. MD, SJ, DG, and NS did the main laboratory work. LSB and corresponding authors (DMA and MU) provided comments on, and approved the final manuscript. The first author (LSB) with support by MU wrote a first draft of the paper. LSB analysed and interpreted the data with support by MD, MJC, SJ, and patient data. MD, SJ, DG, and NS did the main laboratory work. MD, SJ, DG, and NS did the main laboratory work. MD, SJ, DG, and NS did the main laboratory work. LSB was funded by Conselleria de Sanitat Universal i Salut Pública, University Hospital, and grants from Wellcome (098051 and 099202).

Declaration of interests

We declare no competing interests.

Data sharing

Most data collected and analysed in this study are included in the main paper or appendixes. However, remaining datasets can be made available from the corresponding author after publication on reasonable request.

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