Antimicrobial-resistant *Neisseria gonorrhoeae* in Europe in 2020 compared with in 2013 and 2018: a retrospective genomic surveillance study

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Summary

Background Regular quality-assured whole-genome sequencing linked to antimicrobial resistance (AMR) and patient metadata is imperative to elucidate the shifting gonorrhoea epidemiology, both nationally and internationally. We aimed to examine the gonococcal population in the European Economic Area (EEA) in 2020, elucidate emerging and disappearing gonococcal lineages associated with AMR and patient metadata, compare with 2013 and 2018 whole-genome sequencing data, and explain changes in gonococcal AMR and gonorrhoea epidemiology.

Methods In this retrospective genomic surveillance study, we analysed consecutive gonococcal isolates that were collected in EEA countries through the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) in 2020, and made comparisons with Euro-GASP data from 2013 and 2018. All isolates had linked AMR data (based on minimum inhibitory concentration determination) and patient metadata. We performed whole-genome sequencing and molecular typing and AMR determinants were derived from quality-checked whole-genome sequencing data. Links between genomic lineages, AMR, and patient metadata were examined.

Findings 1932 gonococcal isolates collected in 2020 in 21 EEA countries were included. The majority (81·2%, 147 of 181 isolates) of azithromycin resistance (present in 9·4%, 181 of 1932) was explained by the continued expansion of the *Neisseria gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) clonal complexes (CCs) 63, 168, and 213 (with *mtrD*/*mtrR* promoter mosaic 2) and the novel NG-STAR CC1031 (semi-mosaic *mtrD* variant 13), associated with men who have sex with men and anorectal or oropharyngeal infections. The declining cefixime resistance (0·5%, nine of 1932) and negligible ceftriaxone resistance (0·1%, one of 1932) was largely because of the progressive disappearance of NG-STAR CC90 (with mosaic *penA* allele), which was predominant in 2013. No known resistance determinants for novel antimicrobials (zoliflodacin, gepotidacin, and lefamulin) were found.

Interpretation Azithromycin-resistant clones, mainly with *mtrD* mosaic or semi-mosaic variants, appear to be stabilising at a relatively high level in the EEA. This mostly low-level azithromycin resistance might threaten the recommended ceftriaxone-azithromycin therapy, but the negligible ceftriaxone resistance is encouraging. The decreased genomic population diversity and increased clonality could be explained in part by the COVID-19 pandemic resulting in lower importation of novel strains into Europe.

Funding European Centre for Disease Prevention and Control and Örebro University Hospital.

Introduction

In the European Economic Area (EEA; including the 27 EU countries plus Iceland, Liechtenstein, and Norway), gonorrhoea incidence increased from 7·8 per 100 000 population to 31·6 per 100 000 population from 2008 to 2019.[1] In 2020, national lockdowns and social restrictions due to the COVID-19 pandemic resulted in a decrease in gonorrhoea incidence in the EEA to 9·5 per 100 000 population.[1] Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is common and is compromising gonorrhoea treatment in EEA, as observed worldwide.[2] Resistance to every antimicrobial used for empirical therapy, including the first-line ceftriaxone and azithromycin, has emerged.[2] International spread of a ceftriaxone-resistant clone (FC428)[3] and sporadic isolates with ceftriaxone plus high-level azithromycin resistance were documented in 2021–22.[2,4]

Since 2009, the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) has presented annual, quality-assured AMR data linked with metadata (demographic, epidemiological, and clinical data) of individuals with gonorrhoea in the EEA.[5–10] Euro-GASP molecular surveys were performed; in 2009–10 (using *N gonorrhoeae* multi-antigen sequence typing [NG-MAST]),[7] 2013,[8] and 2018 (both using whole-genome sequencing).[9] Briefly, in 2009–10,[7] NG-MAST genogroup 1407 (G1407; mostly *N gonorrhoeae* sequence typing for antimicrobial

Lancet Microbe 2024; 5: e478–88

Published Online April 10, 2024 https://doi.org/10.1016/S2666-5247(23)00370-1

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Research in context

Evidence before this study

Gonorrhoea remains a public health concern globally, and antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* has made gonorrhoea a difficult infection to treat. AMR surveillance is essential worldwide. Where feasible, whole-genome sequencing should support AMR surveillance through: description of *N gonorrhoeae* population dynamics and AMR (or antimicrobial-susceptible) lineages, clades, or clones; description of national and international transmission of the *N gonorrhoeae* lineages and strains; and detection of AMR determinants and molecular prediction of AMR. We searched PubMed using the terms "*Neisseria gonorrhoeae*" OR "gonorrhoea" with "genome sequencing" for papers published in English between Jan 1, 2000, and Sept 29, 2022. Whole-genome sequencing of *N gonorrhoeae* has mainly been used to investigate molecular epidemiology at national or local levels in, for example, the USA, Canada, Argentina, Brazil, the UK, China, Japan, Viet Nam, and Australia. The majority of these studies have examined selected national AMR isolates (with few isolates sequenced in many studies) or the spread of few AMR strains or local outbreaks. With the exception of our whole-genome sequencing studies from 2013 (1054 isolates from 20 European Economic Area [EEA] countries) and 2018 (2375 isolates from 26 EEA countries), no large, international, whole-genome sequencing studies of consecutive or selected gonococcal isolates have been published.

Added value of this study

We report whole-genome sequencing data for 1932 *N gonorrhoeae* isolates cultured in 2020 in 21 EEA countries, linked to phenotypic AMR and gonorrhoea patient metadata (demographic, epidemiological, and clinical), and compare with the whole-genome sequencing data from 2013 and 2018. We elucidate that the main European *N gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) clonal complex (CC) 63/CC168 with mosaic *mtrD*/*mtrR* promoter (also currently found in many other parts of the world) causing low-level resistance to azithromycin, based on minimum inhibitory concentrations (MICs), appears to be stabilising and that the main clone (NG-STAR CC90 with mosaic *penA-34*) causing resistance to cefixime (and decreased susceptibility to ceftriaxone) during the recent decade is progressively being eradicated, which explains the AMR fluctuations. We also describe the expansion of the novel NG-STAR CC1031 with semi-mosaic *mtrD* variant 13, resulting in resistance or decreased susceptibility to azithromycin, associated with men who have sex with men and extragenital (oropharyngeal or anorectal) infections. We describe the current prevalence of AMR determinants and their associations with AMR for currently recommended therapeutic antimicrobials and antimicrobials in the pipeline (eg, zoliflodacin and gepotidacin), and we show that AMR or increased antimicrobial MICs can, in the majority of cases, be adequately predicted using whole-genome sequencing. Furthermore, we report an updated genomic baseline of the gonococcal population in the EEA. Finally, we report evidence of decreased genomic diversity and increased clonality in the gonococcal population, which could be a consequence of the COVID-19-associated national lockdowns and restrictions, including travel restrictions, in 2020.

Implications of all the available evidence

We report the third survey (of 21 countries) using whole-genome sequencing linked to AMR and patient metadata in an international programme for European surveillance of gonorrhoea and gonococcal AMR. AMR and antimicrobial-susceptible gonococcal strains are transmitted within and between countries; accordingly, improved international AMR surveillance, including whole-genome sequencing, is imperative. Whole-genome sequencing in conjunction with AMR and patient metadata (demographic, epidemiological, and clinical) explains the international and national fluctuations in AMR and spread of gonococcal strains (AMR and antimicrobial-susceptible), their associations with patient groups, and the dynamics in gonorrhoea epidemiology. Azithromycin resistance, mainly caused by two clones carrying *mtrD*/*mtrR* promoter mosaic 2 or semi-mosaic *mtrD* variant 13, has stabilised at a relatively high level in the EEA, which could threaten the use of ceftriaxone–azithromycin dual therapy. However, the majority of azithromycin-resistant isolates showed a low-level resistance and, due to the lack of a clinical azithromycin resistance breakpoint, it is unclear whether these isolates are treatable with the recommended azithromycin 2 g dose. For the development of molecular assays predicting azithromycin resistance, it is important to note that the majority of azithromycin resistance is currently caused by *mtrD*/*mtrR* promoter mosaic 2 or semi-mosaic *mtrD* and not 23S rRNA target mutations, which were previously the main cause of azithromycin resistance. The increasing ceftriaxone and cefixime susceptibility and absence of known resistance determinants for novel antimicrobials (zoliflodacin, gepotidacin, lefamulin) are encouraging. Continued research to identify new determinants associated with resistance to azithromycin, extended-spectrum cephalosporins, rapid point-of-care tests predicting AMR and informing individualised treatment, and future gonorrhoea treatment options remains crucial. Finally, novel effective, affordable, and accessible treatment options for gonorrhoea and ideally a gonococcal vaccine remain imperative.

resistance [NG-STAR] clonal complex [CC] 90), associated with men who have sex with men (MSM), accounted for most cefixime resistance. Consequently, ceftriaxone–azithromycin replaced cefixime for empirical gonorrhoea therapy in Europe and improved management, especially for MSM, was emphasised in the 2012 European gonorrhoea guideline.[11] In 2013, after the introduction of ceftriaxone–azithromycin as first-line therapy,[11] cefixime resistance declined due to decreasing prevalence of NG-MAST G1407, by then more associated with heterosexual people.[8] In 2018,[9] susceptibility to ceftriaxone and cefixime had continued to increase, mostly because of the continued decrease of

	Number of isolates in Euro-GASP 2020, n	Number of isolates in genomic survey, n (%)	Azithromycin resistance, n (%)*	Ciprofloxacin resistance, n (%)*	Cefixime resistance, n (%)*	Ceftriaxone resistance, n (%)*
Austria	245	69 (28·2%)	9 (13·0%)	38 (55·1%)	0	0
Belgium	159	149 (93·7%)	25 (16·8%)	83 (55·7%)	3 (2·0%)	1 (0·7%)
Cyprus	2	0	..	..	..	..
Czech Republic	116	75 (64·7%)	3 (4·0%)	18 (24·0%)	0	0
Denmark	110	108 (98·2)	1 (0·9%)	52 (48·1%)	0	0
Estonia	2	2 (100·0%)	0	2 (100·0%)	0	0
Finland	203	76 (37·4%)	1 (1·3%)	37 (48·7%)	0	0
France	200	146 (73·0%)	12 (8·2%)	90 (61·6%)	0	0
Germany	200	150 (75·0%)	24 (16·0%)	90 (60·0%)	0	0
Greece	100	80 (80·0%)	1 (1·3%)	72 (90·0%)	0	0
Hungary	80	78 (97·5%)	18 (23·1%)	51 (65·4%)	1 (1·3%)	0
Iceland	42	39 (92·9%)	3 (7·7%)	15 (38·5%)	0	0
Ireland	104	99 (95·2%)	2 (2·0%)	63 (63·6%)	0	0
Italy	100	72 (72·0%)	2 (2·8%)	46 (63·9%)	0	0
Malta	5	0	..	..	..	..
Netherlands	332	153 (46·1%)	11 (7·2%)	83 (54·2%)	0	0
Norway	450	74 (16·4%)	4 (5·4%)	45 (60·8%)	1 (1·4%)	0
Poland	23	20 (87·0%)	7 (35·0%)	10 (50·0%)	0	0
Portugal	110	78 (70·9%)	11 (14·1%)	43 (55·1%)	0	0
Slovakia	108	102 (94·4%)	2 (2·0%)	50 (49·0%)	0	0
Slovenia	168	71 (42·3%)	10 (14·1%)	61 (85·9%)	0	0
Spain	232	144 (62·1%)	10 (6·9%)	77 (53·4%)	0	0
Sweden	200	147 (73·5%)	25 (17·0%)	92 (62·6%)	4 (2·7%)	0
Total	3291	1932 (58·7%)	181 (9·4%)	1118 (57·9%)	9 (0·5%)	1 (0·1%)

Antimicrobial resistance was determined after retesting of isolates with discrepant results. Euro-GASP aims to collect at least 100 isolates per country and year. Countries that cannot obtain 100 isolates within the official collection window (Sept 1 to Nov 30) include isolates beyond the official window. Euro-GASP isolates reflect the gonococcal antimicrobial resistance situation in the region,[5] but the low number of isolates in some countries is a major concern regarding representativity. Euro-GASP=European Gonococcal Antimicrobial Surveillance Programme. *Phenotypic resistance among the isolates included in the Euro-GASP 2020 genomic survey.

Table 1: **Phenotypic antimicrobial resistance of *Neisseria gonorrhoeae* isolates and isolates included in the Euro-GASP 2020 genomic survey,[6] by country**

NG-MAST G1407. However, azithromycin resistance had substantially increased due to the international expansion of a novel gonococcal lineage (NG-MAST G12302; NG-STAR CC168/63).[9] These findings contributed to revised gonorrhoea treatment in Europe, with ceftriaxone high-dose (1 g) monotherapy recommended in certain circumstances or settings as an alternative to the ceftriaxone (1 g)–azithromycin (2 g) dual therapy.[12] Accordingly, Euro-GASP molecular surveys have enhanced the gonorrhoea and gonococcal AMR surveillance, nationally and internationally, and informed surveillance, public health interventions, and management guidelines.

Since the Euro-GASP 2018 whole-genome sequencing survey,[9] cefixime resistance has further decreased and almost disappeared among MSM, whereas azithromycin and ciprofloxacin resistance has increased among MSM but decreased among heterosexual people.[6] These changes in the Euro-GASP gonococcal AMR profiles, the revised European treatment recommendations,[12] and the COVID-19 pandemic in 2020 resulting in less international travel and changes in sexual behaviours, suggest that the European gonococcal population might have changed.

We analysed whole-genome sequencing results on gonococcal isolates from Euro-GASP countries in 2020 linked to quality-assured AMR data and patient metadata to explain recent changes in Europe. We identified emerging and disappearing genomic lineages and their association with AMR and patient metadata, predicted AMR (currently used antimicrobials and candidates for future use, specifically zoliflodacin and gepotidacin), and monitored circulating lineages through comparisons with the Euro-GASP whole-genome sequencing surveys from 2013 and 2018.[8,9]

Methods

Study design and isolates

In the Euro-GASP 2020 survey,[6] 3291 gonococcal isolates linked to patient metadata were collected in 23 EEA countries between Jan 4 and Dec 31, 2020. Of these, we selected isolates for the present genomic study from 21 EEA countries (table 1, appendix 1 pp 1–2, 10). Quality-assured whole-genome sequencing data linked to AMR data and patient metadata were obtained for the majority of selected isolates (table 1, appendix 1 p 10). One isolate per gonorrhoea episode was included in accordance with Euro-GASP priority rules (appendix 1 p 2).

See Online for appendix 1

All countries were approved in the Euro-GASP external quality assessment for antimicrobial susceptibility testing.[13] Isolates from 16 countries (76%) were tested for AMR in a decentralised manner (in country), and isolates from five countries (24%) were tested centrally

For more on **EUCAST breakpoints** see https://www.eucast.org/clinical_breakpoints

(appendix 1 p 1).[6] Minimum inhibitory concentration (MIC) was determined for ceftriaxone, cefixime, azithromycin, and ciprofloxacin, as described previously.[5–10] European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (version 14.0) were applied.

All gonococcal isolates were cultured and preserved as part of routine diagnostics (standard care), and isolates or data were submitted to the European Centre for Disease Prevention and Control through the Euro-GASP surveillance programme with no patient identifiable information. Accordingly, no separate ethical approval was required.

Genomic sequencing and analyses

Total DNA was extracted from pure cultures using a QIAsymphony instrument (Qiagen, Hilden, Germany). Whole-genome sequencing using Illumina paired-end technology was performed centrally for 15 (71%) of 21 countries (appendix 1 pp 2–3) and in a decentralised manner in six (29%) countries. Raw sequencing data were quality checked before phylogenomic inference, assembly, and data processing to obtain molecular typing information (multilocus sequence typing [MLST] and NG-STAR sequence types [STs] and CCs[8,9,14,15]) and genetic AMR determinants.[8,9,15] Association analyses among genotypes and phenotypic AMR or patient epidemiological data were performed.

For details regarding Euro-GASP structure, sampling, AMR (including MIC breakpoints) and genomic analyses, quality assurance, and statistical analyses (including impact on the EEA gonococcal population by the COVID-19 pandemic), see appendix 1 (pp 1–6, 9, 23).

Role of the funding source

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

Results

Of the 3291 gonococcal isolates collected as part of the Euro-GASP 2020 survey,[6] we sequenced 1973 (60·0%) selected isolates from 21 EEA countries (table 1). Of the sequenced isolates, 1932 (97·9%) had whole-genome sequencing data that passed quality checks and complete linked AMR and patient metadata. These 1932 isolates were collected between Jan 4 and Dec 31, 2020.

Of the 1932 patients, sex was reported for 1919 (99·3%); 1607 (83·7%) were male and 312 (16·3%) were female. Age was reported for 1918 (99·3%) of 1932 patients. The median age for female patients was 27 years (IQR 21–39), with younger than 24 years being the most common age group (40·2%, 125 of 311). For male patients, the median age was 32 years (26–41), with 25–34 years being the most common age group (38·3%, 612 of 1596). Both sexual orientation or mode of transmission and sex were reported in 1008 (52·2%) of 1932 patients; 506 (50·2%) were MSM, 304 (30·2%) were heterosexual men, 198 (19·6%) were women, and one (0·1%) patient reported mother-to-child-transmission.

Ciprofloxacin resistance was prevalent (57·9%, 1118 of 1932 isolates; table 1), as in previous years.[5–10] Azithromycin resistance was 9·4% (181 of 1932), compared with 8·0% (191 of 2375) in 2018.[9] However, 93·4% (169 of 181) of the azithromycin-resistant isolates showed low-level resistance (MIC 2–4 mg/L). Seven (0·4%) isolates showed high-level azithromycin resistance (MIC ≥256 mg/L). Cefixime resistance (0·5%, nine of 1932) continued to decrease, from 4·8% (51 of 1054) in 2013,[8] and 1·5% (36 of 2375) in 2018.[9] All nine cefixime-resistant isolates were azithromycin susceptible. Ceftriaxone resistance remained infrequent at only 0·1% (one of 1932) of isolates, compared with 0·5% (five of 1054) in 2013,[8] and less than 0·1% (one of 2375) in 2018.[9] The resistance levels by country are summarised in table 1.

Ciprofloxacin resistance was found in all 21 countries, with a median of 55·4% (IQR 48·9–62·9) and ranging from 24·0% in the Czech Republic (n=75) to 100% in Estonia (n=2). Resistance to azithromycin was observed in 95% (20 of 21) of countries, ranging from 0·9% (one of 108 isolates) in Denmark to 35·0% (seven of 20) in Poland. Cefixime resistance was detected in only four (19%) of 21 countries, compared with 12 (60%) of 20 countries in 2013,[8] and 12 (46%) of 26 countries in 2018,[9] and ranged from 1·3% (one of 78 isolates) in Hungary to 2·7% (four of 147) in Sweden (table 1). All 1118 ciprofloxacin-resistant isolates had the *gyrA* S91F substitution, which was absent in all ciprofloxacin-susceptible isolates (figure 1, appendix 1 pp 11–12).

All azithromycin-resistant isolates (n=181) had at least one known azithromycin resistance determinant (appendix 1 pp 11–12). The isolates with the highest azithromycin MICs had four 23S rRNA gene alleles with the A2045G (MIC ≥256 mg/L, n=7) or C2597T mutations (MIC 2–32 mg/L, n=7). Six isolates with one 23S rRNA C2597T mutated allele (MIC 0·125–4 mg/L, two with MICs >1 mg/L also harbouring *mtrD* mosaic 2),[9,15,16] two isolates with two 23S rRNA C2597T mutated alleles (MIC 0·5 mg/L and 2 mg/L), and two isolates (MIC 4 mg/L and 8 mg/L) with three 23S rRNA C2597T mutated alleles were also observed (appendix 1 pp 11–12). 190 and 392 isolates had semi-mosaic and mosaic *mtrD* variants, respectively (appendix 1 pp 7–8, 13, 22). The most common semi-mosaic *mtrD* was variant 13 (n=104), which was the only semi-mosaic *mtrD* variant frequently associated with azithromycin resistance (40·4%, 42 of 104 isolates)—ie, in isolates without 23S rRNA mutations. 384 isolates had a mosaic *mtrD* variant (appendix 1 pp 13, 22) but no 23S rRNA mutations, of which 114 isolates showed low-level azithromycin resistance (MIC 2–8 mg/L) and 270 remained susceptible (MIC <0·064–1 mg/L). The majority of these 114 azithromycin-resistant isolates (89·5%, 102 of 114) formed a single lineage constituted by NG-STAR CC63/168/213, which mainly contained *mtrD*/*mtrR* promoter mosaic 2,[9,15,16] and was found in 19 (90·5%) EEA countries (figures 1–3). In total, 18 different mosaic *mtrD*

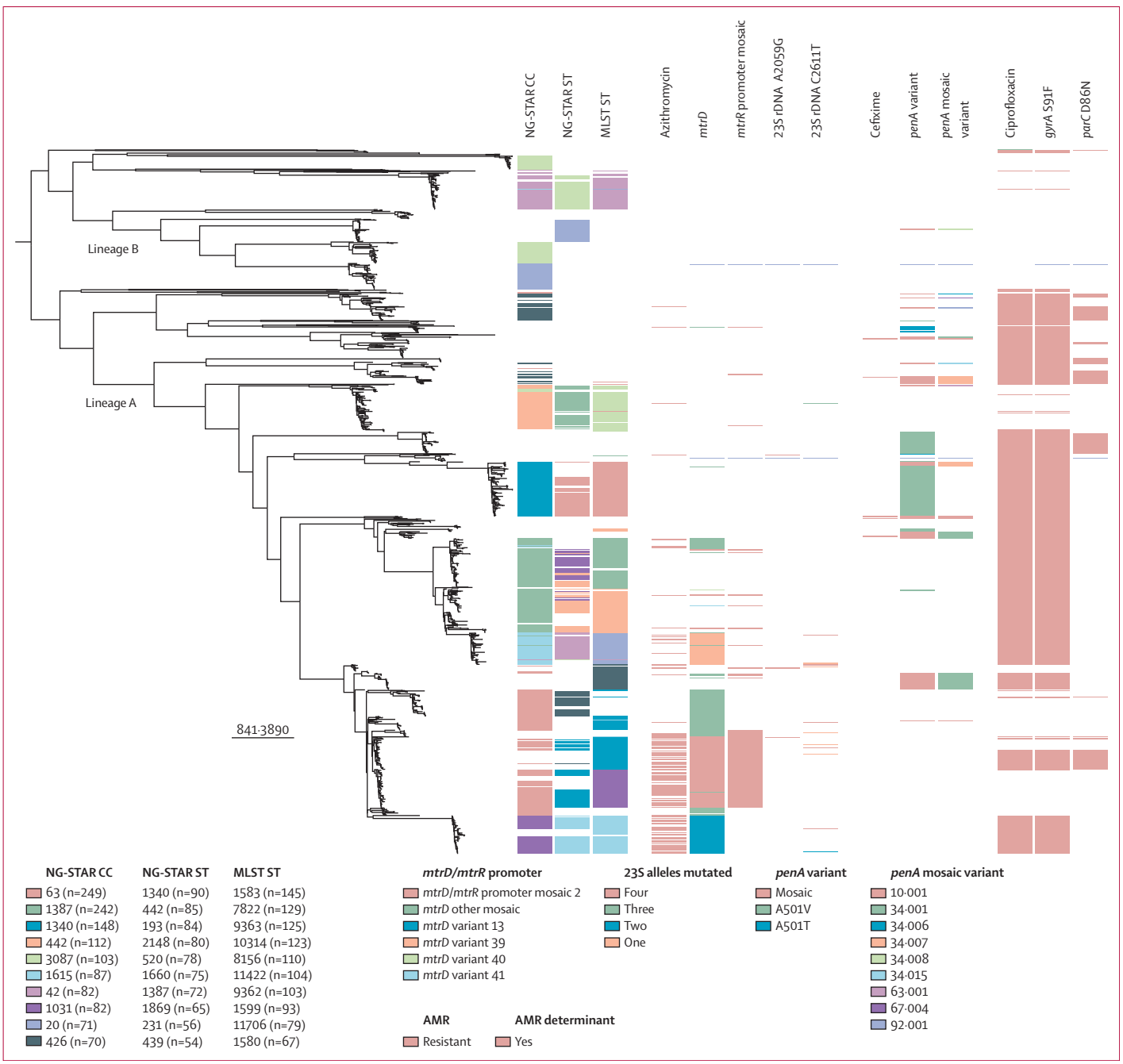

Figure 1: **Main *N gonorrhoeae* molecular CCs, STs, and genomic lineages in the EEA in 2020**
The figure shows a midpoint rooted phylogenetic tree of 1932 *N gonorrhoeae* isolates from 21 EEA countries in 2020. The first block of columns shows the assignment of each isolate to the three typing schemes: NG-STAR CC, NG-STAR ST, and MLST ST. Only the ten most common CCs or STs are shown for visualisation purposes. The following blocks represent phenotypic AMR data followed by the main AMR determinants for each of azithromycin, cefixime, and ciprofloxacin. AMR=antimicrobial resistance. CC=clonal complex. EEA=European Economic Area. MLST=multilocus sequence typing. *N gonorrhoeae=Neisseria gonorrhoeae.* NG-STAR=*N gonorrhoeae* sequence typing for antimicrobial resistance. ST=sequence type.

variants were found, with the majority of isolates belonging to *mtrD*/*mtrR* promoter mosaic 2 (n=201; appendix 1 pp 13, 22).[9,15,16] Semi-mosaic *mtrD* variant 13 isolates belonged to NG-STAR CC1031 and CC1818, both emerging in 2020 (figure 2, appendix 1 pp 13–15, 17, 18). All azithromycin-resistant isolates lacking 23S rRNA mutations and mosaic *mtrD*[9,15,16] had semi-mosaic *mtrD* variant 13 plus mosaic *mtrE* variant D (n=42) or semi-mosaic *mtrD* variant 39 (n=5) or a disrupted *mtrR* (n=1; appendix 1 pp 11–12). Of note, 30 isolates (1·6%) contained *mtrD*/*mtrR* promoter mosaic 2[9,15,16] and were susceptible to azithromycin; however, 13 of these isolates had the two-base-pair deletion

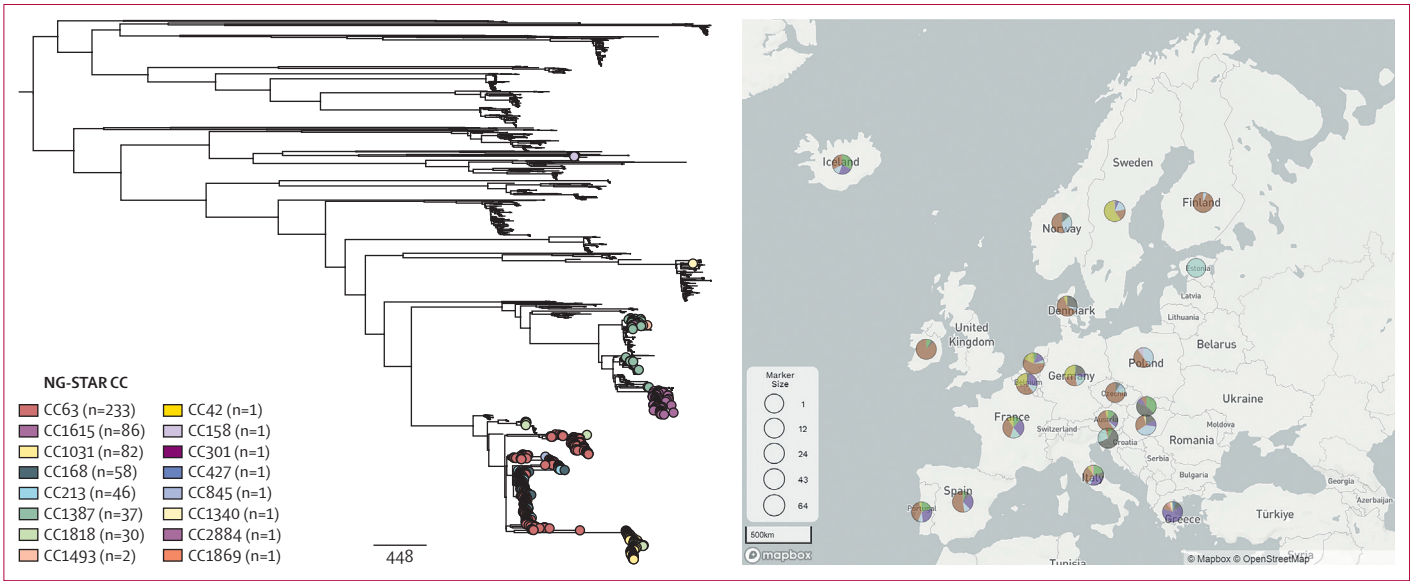

Figure 2: **Distribution of *N gonorrhoeae* isolates in the EEA carrying a mosaic or semi-mosaic *mtrD* sequence in 2020**

The figure shows a phylogenetic tree of 1932 Euro-GASP isolates. Coloured nodes show the distribution of NG-STAR CCs carrying mosaic and semi-mosaic *mtrD* variant. The European map shows pie charts with the proportion of NG-STAR CCs; the size of the pie chart reflects the number of isolates from each country. Map data from © OpenStreetMap. CC=clonal complex. EEA=European Economic Area. Euro-GASP=European Gonococcal Antimicrobial Surveillance Programme. *N gonorrhoeae*=*Neisseria gonorrhoeae*. NG-STAR=*N gonorrhoeae* sequence typing for antimicrobial resistance.

in a GC dinucleotide repeat in *mtrC* that can increase azithromycin susceptibility (MIC 0·032–0·064 mg/L).[17] The remaining 17 azithromycin-susceptible isolates with *mtrD*/*mtrR* promoter mosaic 2[9,15,16] had MICs of 0·064–0·5 mg/L. In total, the GC base-pair deletion in *mtrC* that can increase azithromycin susceptibility[17] was found in 105 isolates, including 18 with mosaic *mtrD* (MIC 0·032–0·064 mg/L), three with semi-mosaic *mtrD* (MIC <0·064 mg/L), and one with a 23S rDNA C2597T mutation in two alleles (MIC 0·5 mg/L). Ten isolates were lacking 23S rRNA mutations and mosaic or semi-mosaic *mtrD* but carried the *rplD* G70D alteration;[15] however, all of these isolates were azithromycin susceptible (appendix 1 pp 11–12).

All nine cefixime-resistant isolates (MIC 0·25–0·5 mg/L) had a mosaic *penA* allele (figure 1, appendix 1 pp 11–12): mosaic *penA*-10.001 (n=6), mosaic *penA*-34.001 (n=2), or mosaic *penA*-34.007 (n=1). However, 112 cefixime-susceptible isolates also harboured a mosaic *penA* allele: *penA*-34 (n=101, MIC 0·032–0·125 mg/L), *penA*-10.001 (n=4, MIC 0·064–0·125 mg/L), *penA*-63.001 (n=3, MIC ≤0·016 mg/L), *penA*-92·001 (n=3, MIC 0·032–0·064 mg/L), and *penA*-67.004 (n=1, MIC 0·032 mg/L; figure 1). The only ceftriaxone-resistant isolate (MIC 0·25 mg/L; cefixime MIC 0·25 mg/L; azithromycin MIC 1 mg/L) carried mosaic *penA*-34.001. No isolates carried other potential ceftriaxone-resistance mutations (eg, *rpoB* P157L, G158V, or R201H, or *rpoD* D92–95 deletion or E98K).[15]

Regarding novel antimicrobials, no known *gyrB*, *gyrA*, or *rplC* target resistance determinants for zoliflodacin, gepotidacin, or lefamulin, respectively, were observed.[18–22] However, 216 (11·2%) of 1932 isolates carried the *parC* D86N mutation that predisposes to gepotidacin resistance emergence.[18,19]

MLST, NG-STAR STs, and NG-STAR CCs across countries are shown in appendix 1 (pp 20–21). 128 MLST STs (30 with more than ten isolates), 318 NG-STAR STs (38 with more than ten isolates), and 80 NG-STAR CCs (26 with more than ten isolates) were found. NG-STAR CCs were assigned to 1925 (99·6%) of 1932 isolates. The three most prevalent NG-STAR CCs were CC63 (12·9%, 249 of 1932), CC1387 (12·5%, 242 of 1932), and CC1340 (7·6%, 148 of 1932; table 2, figures 1, 3, appendix 1 pp 14, 17–18). NG-STAR CC63 has been prevalent in previous Euro-GASP whole-genome sequencing surveys (figure 3, appendix 1 pp 14, 17–18) but since 2013,[8] new NG-STAR STs have been emerging within the CC63 cluster, mainly NG-STAR ST193 and ST2885, both of which have *mtrD* and *mtrR* mosaic structures. NG-STAR ST193 and ST2885, not present in 2013,[8] emerged in 2018 (ST193 1·8%, 42 of 2375; ST2885 0·4%, ten of 2375),[9] and had substantially expanded in 2020 (ST193 4·3%, 84 of 1932; ST2885 1·1%, 21 of 1932). Both of these NG-STAR STs are likely to expand to create separate NG-STAR CCs in the near future. NG-STAR CC1031 (4·2%, 82 of 1932) is an emerging CC closely related to CC1818 and CC1340 (appendix 1 pp 17–18) and mainly consisting of NG-STAR ST1660. All CC1031 isolates had semi-mosaic *mtrD* variant 13 with 47·6% azithromycin resistance. CC1031 was found in nine countries, with the highest proportions in Sweden (40·2%, 33 of 82), Belgium (19·5%, 16 of 82), and Denmark (14·6%, 12 of 82; appendix 1 p 15). NG-STAR CC1387 and CC1340 have also steadily increased from no isolates in 2013[8] to 242 (12·5%) and 148 (7·6%) isolates, respectively, in 2020 (table 2, figure 3, appendix 1 pp 14, 17–18). NG-STAR CC1340 was conserved in 2018, with 100% of isolates carrying *penA* A501V (ST1340),[9] but in 2020 CC1340 also included mosaic

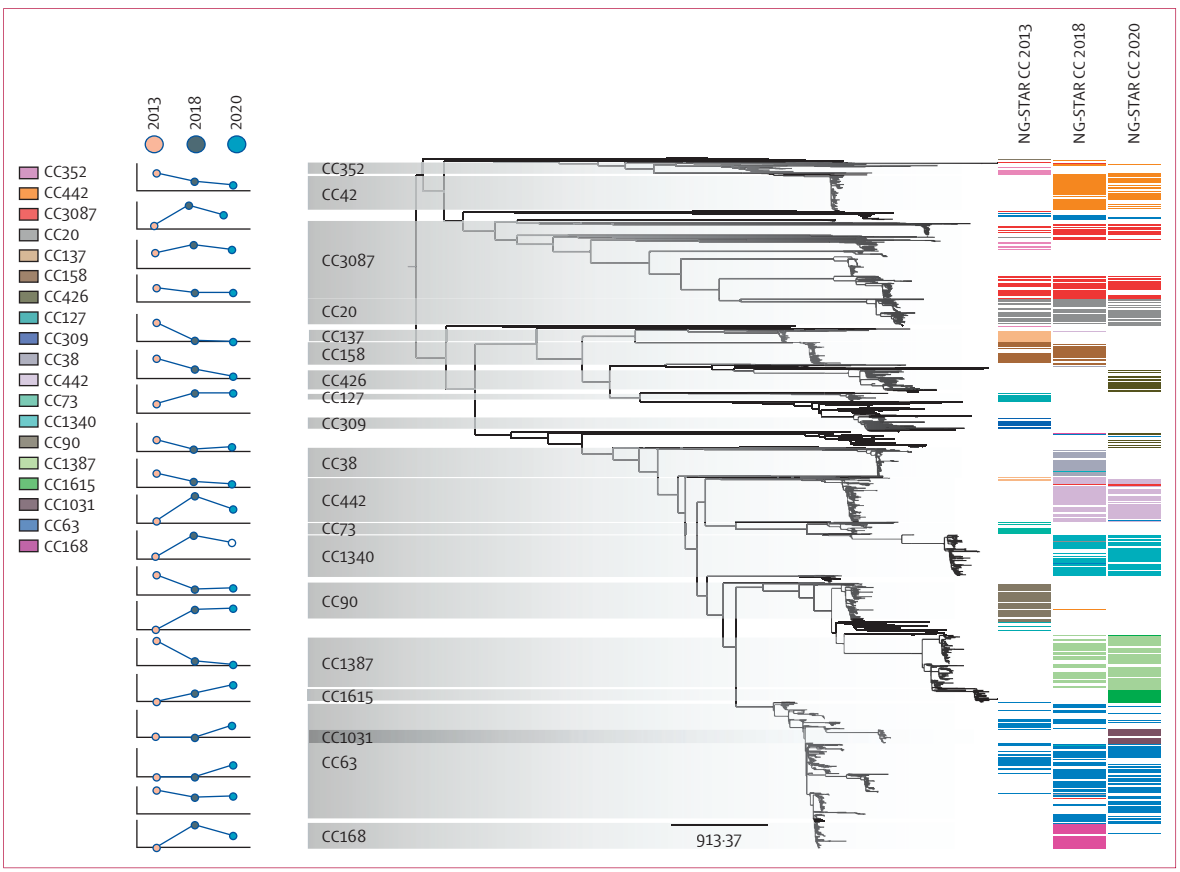

Figure 3: **Trends in NG-STAR CC proportions in the *N gonorrhoeae* population in the EEA in 2013 (n=1053),[8] 2018 (n=2374),[9] and 2020 (n=1932)**
The figure shows a phylogenetic reconstruction of 5359 *N gonorrhoeae* isolates from the EEA. NG-STAR CCs are highlighted as they appear in the midpoint rooted tree. The columns represent the ten most common NG-STAR CCs from 2013,[8] 2018,[9] and 2020. On the left, charts for each CC show the shift in proportion over the three timepoints. CC=clonal complex. EEA=European Economic Area. *N gonorrhoeae=Neisseria gonorrhoeae*. NG-STAR=*N gonorrhoeae* sequence typing for antimicrobial resistance.

penA allele (7·4%) in the novel NG-STAR ST4536 (figure 1, appendix 1 pp 17–18). Similar to NG-STAR CC63, CC1387 included many novel NG-STAR STs that have mosaic *mtrD* and *mtrR* sequences and the proportion of isolates carrying mosaic *mtrD* and *mtrR* sequences within CC1387 substantially increased from 0·6% (one of 165) in 2018[9] to 14·0% (34 of 242) in 2020. NG-STAR CC90 (NG-MAST genogroup G1407), which caused most cefixime resistance in previous Euro-GASP molecular surveys,[7–9] was the most abundant in 2009–10 (23·3%, 248 of 1066)[7] and 2013 (18·2%, 192 of 1054),[8] but it has decreased substantially to only 51 (2·1%) of 2375 isolates in 2018[9] and 18 (0·9%) of 1932 in 2020 (table 2, figure 3, appendix 1 pp 14, 17–18).

Compared with the Euro-GASP 2013[8] and 2018[9] whole-genome sequencing surveys, NG-STAR CCs and isolates with mosaic *penA* alleles have decreased, whereas prevalence of isolates with mosaic and semi-mosaic *mtr* have increased (appendix 1 pp 17–18). In 2013, few NG-STAR CCs (mostly CC90 and CC63) represented a high proportion of isolates,[8] whereas in 2018, the gonococcal population was more diverse and spread across numerous CCs.[9] In 2020, the CC distribution suggested a shift towards a gonococcal population with less genomic diversity (figure 3, appendix 1 pp 14, 17–18), which is supported by a decrease in the proportion of unique MLST and NG-STAR STs from 2018 to 2013 (appendix 1 p 19, appendix 2). This shift could also have been affected by the COVID-19 pandemic. Of note, Tajima's neutrality test (D) suggested that the gonococcal population in most EEA countries with high COVID-19 stringency index has not evolved in a neutral manner (appendix 1 pp 9, 23). The decreased diversity in the gonococcal population can be observed in the negative value of Tajima's D, and the decreasing nucleotide diversity and number of segregating sites in almost all countries with high COVID-19 stringency index.

The largest NG-STAR CC (CC63) was more often associated with MSM (odds ratio [OR] 2·94 [95% CI 1·99–4·32], corrected p value [p]<0·0001), reconfirmed with multivariate analysis (coefficient 0·81, SE 0·24, Z-test p=0·0007), and anorectal infections (OR 2·31 [95% CI 1·65–3·22], p<0·0001). The second and third largest CCs (CC1387 and CC1340) were also more often found in men (CC1387: OR 1·97 [95% CI 1·25–3·24], p=0·016; CC1340: 4·12 [1·92–10·55], p=0·0002), MSM (CC1387: 1·83 [1·23–2·75], p=0·018; CC1340: 3·25 [1·87–5·88], p=0·0001), and, in the case of CC1387, in anorectal

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For more on the **COVID-19 stringency index** see https://ourworldindata.org/covid-stringency-index

	Patients, n	Patient age in years, mean (range)	Patients with known sex, n	Male patients, n (%)	Patients with known sexual orientation, n (male patients, n)	Heterosexual patients, n (%)	Men who have sex with men, n (%)	Most common NG-STAR ST (n)	Most common MLST (n)
CC63	249	38·2 (4–64)	247	219 (88·7%)	145 (132)	41 (28·3%)	104 (71·2%)	ST193 (84)	ST11422 (82)
CC1387	242	34·0 (16–64)	241	218 (90·5%)	128 (113)	46 (35·7%)	80 (62·5%)	ST2184 (80)	ST7822 (117)
CC1340	148	37·6 (0–64)	146	139 (95·2%)	78 (72)	19 (24·4%)	57 (73·1%)	ST1340 (90)	ST1583 (140)
CC442	112	38·5 (16–70)	112	96 (85·7%)	62 (50)	32 (51·6%)	30 (48·4%)	ST442 (85)	ST8156 (102)
CC3087	103	30·0 (10–54)	103	67 (65·0%)	52 (24)	47 (90·4%)	4 (7·7%)	ST568 (32), ST729 (32)	ST8135 (35)
CC1615	1615	35·3 (11–58)	87	77 (88·5%)	48 (41)	16 (33·3%)	32 (66·7%)	ST1869 (65)	ST11706 (76)
CC1031	82	36·6 (18–62)	81	80 (98·8%)	55 (54)	5 (9·1%)	49 (89·1%)	ST1660 (75)	ST9362 (82)
CC42	82	36·9 (16–64)	81	70 (86·4%)	44 (39)	31 (70·5%)	13 (29·5%)	ST520 (78)	ST1599 (72)
CC20	71	31·2 (14–67)	71	45 (63·4%)	48 (29)	41 (85·4%)	7 (14·6%)	ST20 (26)	ST11990 (36)
CC426	70	34·2 (18–60)	68	57 (83·8%)	33 (25)	16 (48·5%)	15 (45·5%)	ST427 (16)	ST8143 (28)
CC1818	68	33·3 (17–54)	68	63 (92·6%)	20 (19)	4 (20·0%)	15 (75·0%)	ST1818 (38)	ST1580 (45)
CC231	59	30·0 (16–57)	58	31 (53·4%)	37 (15)	30 (81·1%)	5 (13·5%)	ST231 (56)	ST7359 (57)
CC168	58	34·3 (14–60)	57	53 (93·0%)	22 (20)	11 (50·0%)	11 (50·0%)	ST168 (40)	ST9363 (56)
CC38	55	36·4 (17–71)	55	47 (85·5%)	25 (17)	16 (64·0%)	9 (36·0%)	ST38 (21)	ST38 (7827)
CC213	46	36·5 (19–57)	45	41 (91·1%)	20 (16)	8 (40·0%)	12 (60·0%)	ST213 (22)	ST11422 (21)
CC427	41	36·4 (18–79)	41	35 (85·4%)	34 (29)	18 (52·9%)	11 (32·4%)	ST2537 (12)	ST7363 (23)
CC1096	37	38·0 (19–63)	37	30 (81·1%)	22 (16)	11 (50·0%)	11 (50·0%)	ST1873 (27)	ST1588 (28)
CC127	27	32·2 (19–53)	27	15 (55·6%)	13 (7)	13 (100·0%)	0	ST1463 (8)	ST1901 (16)
CC73	25	36·9 (18–71)	25	22 (88·0%)	4 (2)	3 (75·0%)	1 (25·0%)	ST73 (7)	ST11984 (14)
CC352	22	27·9 (18–49)	22	11 (50·0%)	11 (4)	11 (100·0%)	0	ST84 (16)	ST8163 (9), ST11975 (9)
CC520	19	35·8 (21–71)	18	16 (88·9%)	11 (11)	2 (18·2%)	9 (81·8%)	ST2152 (14)	ST1599 (19)
CC90	18	33·0 (17–59)	18	11 (61·1%)	12 (7)	12 (100·0%)	0	ST90 (14)	ST1901 (16)
CC158	16	26·9 (16–46)	16	12 (75·0%)	4 (1)	4 (100·0%)	0	ST158 (8)	ST7363 (14)
CC199	14	31·4 (19–63)	14	14 (100·0%)	7 (7)	4 (57·1%)	3 (42·9%)	ST1816 (9)	ST10314 (8)
CC124	13	32·4 (20–48)	13	11 (84·6%)	10 (9)	8 (80·0%)	2 (20·0%)	ST124 (6)	ST1901 (11)
CC891	11	30·0 (18–58)	11	10 (90·9%)	9 (9)	1 (11·1%)	8 (88·9%)	ST1530 (11)	ST11370 (11)

Data are shown by NG-STAR CC. NG-STAR=*Neisseria gonorrhoeae* sequence typing for antimicrobial resistance. MLST=multilocus sequence typing. CC=clonal complex. ST=sequence type. Euro-GASP=European Gonococcal Antimicrobial Surveillance Programme.

Table 2: **Characteristics of patients infected with the most common NG-STAR CCs (more than ten isolates), and the most common MLST and NG-STAR STs within these NG-STAR CCs, in the Euro-GASP 2020 genomic survey**

infections (1·68 [1·19–2·38], p=0·0029). In the multivariate analysis, CC1387 remained associated with men (coefficient 0·50, SE 0·25, Z-test p=0·045) and CC1340 with men (coefficient 1·06, SE 0·41, Z-test p=0·010) and MSM (coefficient=0·86, SE 0·31, p=0·0054). MLST ST1583, within NG-STAR CC63, was more often isolated from men (OR 4·02 [95% CI 1·88–10·31], p=0·0007) and MSM (3·12 [1·79–5·66], p=0·0005) than from other groups. The strongest associations with men, MSM, and pharyngeal or anorectal sites were observed for the novel CC1031 (men: OR 16·28 [95% CI 2·82–651·82], p=0·0002; MSM: 10·73 [4·25–34·81], p<0·0001; pharyngeal site of infection: 3·79 [1·56–8·36], p=0·0159; anorectal site of infection: 3·59 [2·06–6·20], p=0·0001). In the multivariate model, CC1031 remained strongly associated with MSM (coefficient 1·81, SE 0·51, Z-test p=0·0004). NG-STAR CC42 was positively associated with men and MSM in 2018,[9] but less associated with MSM in 2020 (univariate analysis: OR 0·40 [95% CI 0·21–0·78], p=0·0377; multivariate analysis: coefficient –1·11, SE 0·39, Z-test p=0·0043; appendix 3 pp 1–3).

See Online for appendix 3

NG-STAR CC63, the largest CC in 2020 and one of the major CCs in NG-MAST G12302 in 2018,[9] and CC168 were associated with azithromycin-resistant isolates (CC63: OR 3·39 [95% CI 2·39–4·82], p<0·0001; CC168: 8·34 [4·84–14·39], p<0·0001). CC213, which also carries a *mtr* mosaic, showed the strongest association with azithromycin resistance (19·46 [10·46–36·22], p<0·0001). Several CCs were also associated with an increased azithromycin MIC (decreased susceptibility; appendix 3 p 4). CC90, one of the major lineages with extended spectrum cephalosporin resistance in previous surveys,[8,9] showed significantly higher MICs of cefixime (9·43 [6·94–12·81], p<0·0001) and ceftriaxone (2·64 [2·22–3·12], p<0·0001) than other larger CCs. Of note, despite the high level of ciprofloxacin resistance in the entire study (57·9%, 1118 of 1932) CC63 was only formed by ciprofloxacin-susceptible isolates (appendix 3 p 4).

Discussion

We report findings of the Euro-GASP 2020 genomic survey in comparison with those from 2018[9] and 2013.[8] In 2020, only one isolate was ceftriaxone resistant and cefixime resistance had continued to decline to 0·5%, whereas azithromycin resistance had slightly increased to 9·4%. The expansion of one main azithromycin-resistant genomic

lineage with *mtrD*/*mtrR* promoter mosaic 2[9,15,16] was observed already in 2018 (NG-STAR CC63/CC168).[9] This expansion continued in 2020 and was found in 19 of 21 participating EEA countries, including novel NG-STAR STs within the lineage. Fortunately, NG-STAR CC63 remains susceptible to most other antimicrobials, including ciprofloxacin, whereas NG-STAR CC168 remains cefixime and ceftriaxone susceptible but is ciprofloxacin resistant. In 2013,[8] the majority of AMR was represented by the predominant NG-STAR CC90 (NG-MAST G1407), which was phylogenomically highly distant from the ancestral lineage.[23] Previous studies have shown that antimicrobial use and subsequent AMR drives gonococcal evolution,[24,25] suggesting that the EEA expansion of NG-STAR CC90 was mainly selected for by wide use of cefixime. In 2018, azithromycin-resistant isolates within NG-STAR CC63 had gradually replaced CC90;[9,26] in 2020, NG-STAR CC63, together with CC1031, which are both very phylogenomically distant from the ancestral lineage, had evolved and were expanding in the EEA. Of note, NG-STAR CC90 (NG-MAST G1407) was associated with MSM in 2009–10,[7] was more associated with heterosexuals in 2013,[8] and was found exclusively in heterosexuals in 2018[9] and 2020. The decline in NG-STAR CC90 and cefixime resistance, the very low level of ceftriaxone resistance, and a ceftriaxone MIC distribution shifting to lower MICs, is reassuring for the continued use of ceftriaxone as first-line gonorrhoea treatment. However, the further spread of NG-STAR CC63, mainly found in MSM in 2020, and similar frequently azithromycin-resistant clones could threaten the use of ceftriaxone–azithromycin dual therapy.[12] Nevertheless, due to the lack of a clinical azithromycin resistance breakpoint, it remains unclear whether gonococcal isolates with *mtrD* mosaic or semi-mosaic variants causing low-level azithromycin resistance (MIC 2–4 mg/L) are treatable with the recommended azithromycin 2 g. It is promising that only 0·1% of isolates in 2018[9] and no isolates in 2020 had concomitant resistance to azithromycin and cefixime.

Azithromycin resistance in gonococci has traditionally been caused by 23S rRNA target mutations acquired by isolates with no or limited phylogenomic relationship and clonal expansion.[8,27] In recent years, recombination in the *mtrRCDE* loci, after acquisition of DNA from commensal *Neisseria* species, was shown to result in low-level azithromycin resistance.[9,15,16] In the present study, only 10·5% of azithromycin-resistant isolates had 23S rRNA target mutations. However, 99·4% of azithromycin-resistant isolates had either 23S rRNA resistance mutations or *mtrD* mosaic or semi-mosaic variants. Nevertheless, 70·4% of isolates with *mtrD* mosaic or semi-mosaic variants remained azithromycin susceptible (ie, despite an increased azithromycin MIC of 0·5–1 mg/L in 75·1%).

The only ceftriaxone-resistant isolate (MIC 0·25 mg/L) in 2020 (female urogenital infection in Belgium) belonged to NG-STAR CC90 and had a mosaic *penA*-34.001 variant, which has been frequently associated with cephalosporin resistance (especially cefixime), and the multidrug-resistant NG-MAST G1407 clone.[7–9,14,15]

No zoliflodacin-resistance mutations in *gyrB*[20,21] or *gyrB* mutations predisposing to resistance development[21] were found, suggesting full susceptibility to zoliflodacin. Of concern, many ciprofloxacin-resistant isolates (11·2% of isolates) contained the *parC* D86N substitution, which predisposes for the emergence of clinical gepotidacin resistance.[18,19] In a global phase 3 randomised controlled clinical trial (ClinicalTrials.gov identifier, NCT03959527), zoliflodacin 3 g showed non-inferiority for treatment of urogenital gonorrhoea when compared with the internationally recommended ceftriaxone 500 mg plus azithromycin 1 g. Full results of this trial will be published in 2024. Recruitment has finished in a phase 3 randomised controlled trial of gepotidacin for treatment of uncomplicated gonorrhoea (NCT04020341).

No spectinomycin resistance mutations (ie, 16S rRNA C1192T or *rpsE* mutations)[15,28,29] were found, which suggests spectinomycin resistance is absent in the EEA and spectinomycin could be an alternative gonorrhoea treatment.[12] Furthermore, no known mutations conferring resistance to gentamicin[30] or the novel antimicrobial lefamulin[22] were found.

In the present Euro-GASP 2020 whole-genome sequencing study, gonococcal isolates were sampled during the COVID-19 pandemic. A comparison between the isolates from the Euro-GASP whole-genome sequencing surveys in 2018[9] and 2020 with regards to genomic variability was made to understand whether COVID-19-associated restrictions could have had any effect on the EEA gonococcal population. The decrease in nucleotide diversity, number of segregating sites, and Tajima's D from 2018[9] to 2020 show decreased genomic diversity and increased clonality in the gonococcal population, which was also supported by the decreased number of unique MLST and NG-STAR STs. These findings could be in part a consequence of COVID-19-associated national lockdowns and social restrictions, including restrictions on travel into the EEA and within EEA countries.[31] Accordingly, the importation of novel gonococcal strains into the EEA from other global regions, and spread between and within individual countries, could have been substantially reduced.

The main limitations of Euro-GASP include the low number and suboptimal representativeness of isolates from some countries, and limited reporting of many epidemiological variables.[5,6,8–10] The selection of one isolate per infection episode could introduce a risk of bias towards associations with infection sites. Nevertheless, Euro-GASP continuously works to overcome these limitations[5,6] and overall, Euro-GASP AMR data was shown to adequately reflect the EEA AMR situation.[5]

In summary, the Euro-GASP gonococcal population in 2020 showed the continued expansion of the NG-STAR CC63 clone with a *mtrD*/*mtrR* promoter mosaic 2[9,15,16] and the emergence of the novel NG-STAR CC1031 with

semi-mosaic *mtrD* variant 13, which together explain more than half of the azithromycin resistance in 2020, associated with MSM and extragenital infections. Furthermore, the emergence of mosaic *mtr* genes in CC1387, the second most common CC in 2020, is concerning. Nevertheless, many isolates with similar *mtr* mosaic sequences remained azithromycin susceptible. Notably, NG-STAR CC90, which is frequently cefixime resistant due to a mosaic *penA* allele, and which was predominant in 2013[8] and 2018,[9] is progressively disappearing. This finding explains the decrease in cefixime resistance and increase in ceftriaxone susceptibility in recent years. Continued research to identify and verify novel determinants associated with resistance to azithromycin, ceftriaxone, and future gonorrhoea treatment options, such as zoliflodacin and gepotidacin,[18–21] remains imperative. Increasing azithromycin resistance could threaten the effectiveness of the current recommended dual treatment for gonorrhoea (ceftriaxone plus azithromycin).[12] However, ceftriaxone-resistant gonococcal isolates, especially isolates with combined ceftriaxone and azithromycin resistance, remain rare in the EEA, indicating that azithromycin in the dual gonorrhoea therapy can currently eradicate any sporadic ceftriaxone-resistant strains. Moreover, most azithromycin-resistant isolates expressed low-level resistance and it remains unknown whether these isolates are treatable with the recommended azithromycin 2 g dose. A clinical azithromycin resistance breakpoint is thus imperative. New gonorrhoea treatment options (eg, zoliflodacin[20,21] and gepotidacin[18,19]) that the Euro-GASP gonococcal population appears to be susceptible to, rapid point-of-care tests predicting AMR and informing individualised treatment,[2,15] and ideally a gonococcal vaccine[32] remain essential.

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Contributors

DG, MJC, MD, SJ, BBl, and MU designed, initiated, and coordinated the study. The European STI network members supplied gonococcal isolates and patient data, and RA, BBe, DAC, DH, KJ, SP, and PS additionally coordinated decentralised whole-genome sequencing in their countries. DG, MD, TU, and SJ performed the main laboratory work. DG analysed and interpreted the data with support from LS-B and MU. DG wrote a first draft of the paper with support from MU. DG, MJC, LS-B, MD, SJ, TU, RA, BBe, DAC, DH, KJ, SP, PS, DMA, BBl, and MU read, commented on, and approved the final manuscript. The first author (DG) and corresponding author (MU) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

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

Acknowledgments

This study was supported by the European Centre for Disease Prevention and Control, the Örebro County Council Research Committee, and the Foundation for Medical Research at Örebro University Hospital, Sweden. Decentralised whole-genome sequencing for Spanish isolates was supported by the Spanish National Centre of Microbiology (ISCIII). LS-B was funded by Generalitat Valenciana (Conselleria de Sanitat Universal i Salut Pública [CDEI-06/20-B] and Conselleria d'Innovació, Universitats, Ciència i Societat Digital [CISEJI/2022/66]), Valencia, Spain; and the Ministry of Science and Innovation (PID2020-120113RA-I00), Madrid, Spain.

Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

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