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Original article

The recent introduction of mosaic *mtr*-carrying *Neisseria gonorrhoeae* lineages boosts local transmission

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ABSTRACT

Objectives: Gonorrhoea, caused by *Neisseria gonorrhoeae*, is a significant public health challenge due to the rising incidence of antimicrobial resistant (AMR) strains. The Valencian Region, one of the top tourist destinations in Eastern Spain, has witnessed an increase of over 200% in the number of cases in recent years. Here, we aimed to investigate the impact of imported AMR lineages in shaping the local gonococcal population and generating sustained transmission events.
Methods: We analysed 1647 *N. gonorrhoeae* isolates collected in the Valencian Region between 2012 and 2024 with accompanying phenotypic antimicrobial susceptibility and epidemiological data. Genomic data was obtained through high-throughput sequencing and combined with 5894 genomes from national and international isolates. From these, information on typing and genetic AMR determinants was derived. Phylogenomic and statistical inference were used to investigate the local dynamics of this pathogen.
Results: Results revealed high levels of AMR, including 63.6% ($n = 449/706$) ciprofloxacin resistance, 17.7% ($n = 195/1102$) azithromycin resistance, and 8.4% ($n = 66/783$) reduced susceptibility or resistance to ceftriaxone. The two main circulating lineages were NG-STAR CC1615 and CC63, carrying 55.0% ($n = 121/220$) and 26.5% ($n = 45/170$) isolates with a mosaic *mtr* (mosaic *mtrD* and *mtrR* promoter),

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respectively. Phylodynamic analyses identified multiple introductions of AMR lineages into the region leading to sustained transmissions since the 1990s. These lineages significantly carried more isolates with *mtr* mosaics (OR = 4.17 [3.27–5.34]; p 1.26E-33) and phenotypic resistance to azithromycin (OR = 2.22 [1.60–3.06]; p 1.14E-06), among other antimicrobials.

Conclusions: This study highlights the dynamic evolution and dissemination of AMR *N. gonorrhoeae* at the local level, highlighting the role of international mobility, sexual networks, and antibiotic usage in shaping resistance patterns. Enhanced genomic surveillance, with special monitoring of mosaic *mtr*-carrying lineages, together with targeted public health interventions, will be key to curb local and regional spread of resistant gonococcal strains. **Andrea Sánchez-Serrano, Clin Microbiol Infect 2026;■:1**

Introduction

Gonorrhoea, caused by *Neisseria gonorrhoeae*, is a significant global public health threat. Without an appropriate treatment, the sexually transmitted infection caused by this bacterial pathogen can lead to infertility and other sexual and reproductive health problems [1,2]. According to WHO estimates, there were 82.4 million new gonococcal infections worldwide in 2020 among adults [3]. In the European Union/European Economic Area (EU/EEA), the prevalence of gonorrhoea nearly doubled from 2018 to 2022 [4]. This rise has occurred in parallel with an increasing incidence of *N. gonorrhoeae* resistance to every class of antibiotic used for its treatment, threatening available options [5].

Travel has been key in the global expansion of antimicrobial-resistant (AMR) gonococcal lineages, including those carrying ceftriaxone and/or azithromycin resistance [2,6,7,8]. Spain, one of the most visited countries, received over 80 million arrivals in 2024 [9]. The number of gonococcal infections reported in Spain has also doubled in the 2018–2022 period, mirroring the increase in the EU/EEA [4]. The Valencian Region (5.3 million population), located in Eastern Spain, is a major tourist destination and has experienced a marked rise, especially in the summer months [10]. It ranks as the seventh region in Spain with the highest rate of gonococcal infections, with over 200% increase in the number of cases between 2016 and 2022 [11].

A detailed understanding of circulating *N. gonorrhoeae* lineages, their genetic determinants of resistance, and the impact of international introductions is key to prevent the local spread of this pathogen. Here, we apply a genomic epidemiology approach to investigate locally circulating *N. gonorrhoeae* lineages, genetic determinants of resistance and the impact of external introductions on local transmission networks in the Valencian Region between 2012 and 2024. The ultimate aim is to provide a robust baseline for ongoing surveillance to support public health responses against AMR gonorrhoea.

Methods

Study design and sample collection

A total of 2361 *N. gonorrhoeae* isolates were collected from confirmed gonorrhoea cases detected in 13 hospitals throughout the Valencian Region from November 2012 to February 2024. From 2021 all isolates were stored, when possible, while earlier isolates were collected retrospectively. These isolates were provided together with phenotypic antimicrobial susceptibility testing data in the form of MIC for azithromycin, ceftriaxone, cefixime, ciprofloxacin, tetracycline, and benzylpenicillin, as well as anonymized epidemiological data from patients. MICs were interpreted using EUCAST breakpoints (v14.0) as susceptible (S), susceptible with increased exposure (I) or resistant (R) except for azithromycin, where the epidemiological cut-off (ECOFF) was applied. Historical data on laboratory-confirmed gonorrhoea cases reported in the Valencian Region was obtained from local public health databases. The project was approved by the FISABIO ethical committee (Ref. 20201016/04 and 20210730/06). Consent from patients was not needed. For more details see Supplementary Methods.

High-throughput genome sequencing and analysis

Total DNA was extracted from all collected isolates, and 1883 with enough quality and quantity were whole-genome sequenced (Illumina). Raw reads were quality-checked and curated before further processing. After quality filtering, 1647 local isolates were retained for analysis (Table S1). Core genome single nucleotide polymorphisms (SNPs) were identified by reference mapping, followed by recombination masking and phylogenetic reconstruction. Typing information was extracted from the curated assemblies, and the detection of AMR determinants was obtained from clean reads. Public genomes from the EU/EEA, global and national (Spain) genomic surveillance initiatives were included for phylogenetic context, and used to infer external introductions and perform an evolutionary analysis using Bayesian-based time-calibrated phylogenies. More details and references are available in Supplementary Methods.

Results

Local epidemiology and antimicrobial susceptibility

The global rise in gonococcal infections has also been reflected locally in the Valencian Region, where the number of cases of gonorrhoea, mostly resistant to some antimicrobials, has been rising sharply in the last decade (Fig. 1(a)) [11,12]. After data quality control (Fig. S1), 1647 gonococcal genomes were analysed (Fig. 1(b); Table S1). The majority of patients were male, 87.2% ($n = 1386/1590$), with a median age of 30.9 (Fig. 1(c)). Most isolates were obtained from penile/urethral (70.6%, $n = 1013/1435$ with epidemiological information) or cervicovaginal samples (9.5%, $n = 137$).

Antimicrobial resistance was common during the study period: 76.8% of the isolates were phenotypically resistant to tetracycline, 63.6% to ciprofloxacin, 17.7% to azithromycin and 7.3% to benzylpenicillin (87.9% with susceptibility but increased exposure (I) or resistance (R)). Resistance rates for cefixime and ceftriaxone were lower (2.2% and 0.5%, respectively), although decreased susceptibility (defined as MICs between 0.064 and 0.125 mg/L) was noted in 12.9% and 7.9% of the isolates, respectively (Table 1). A

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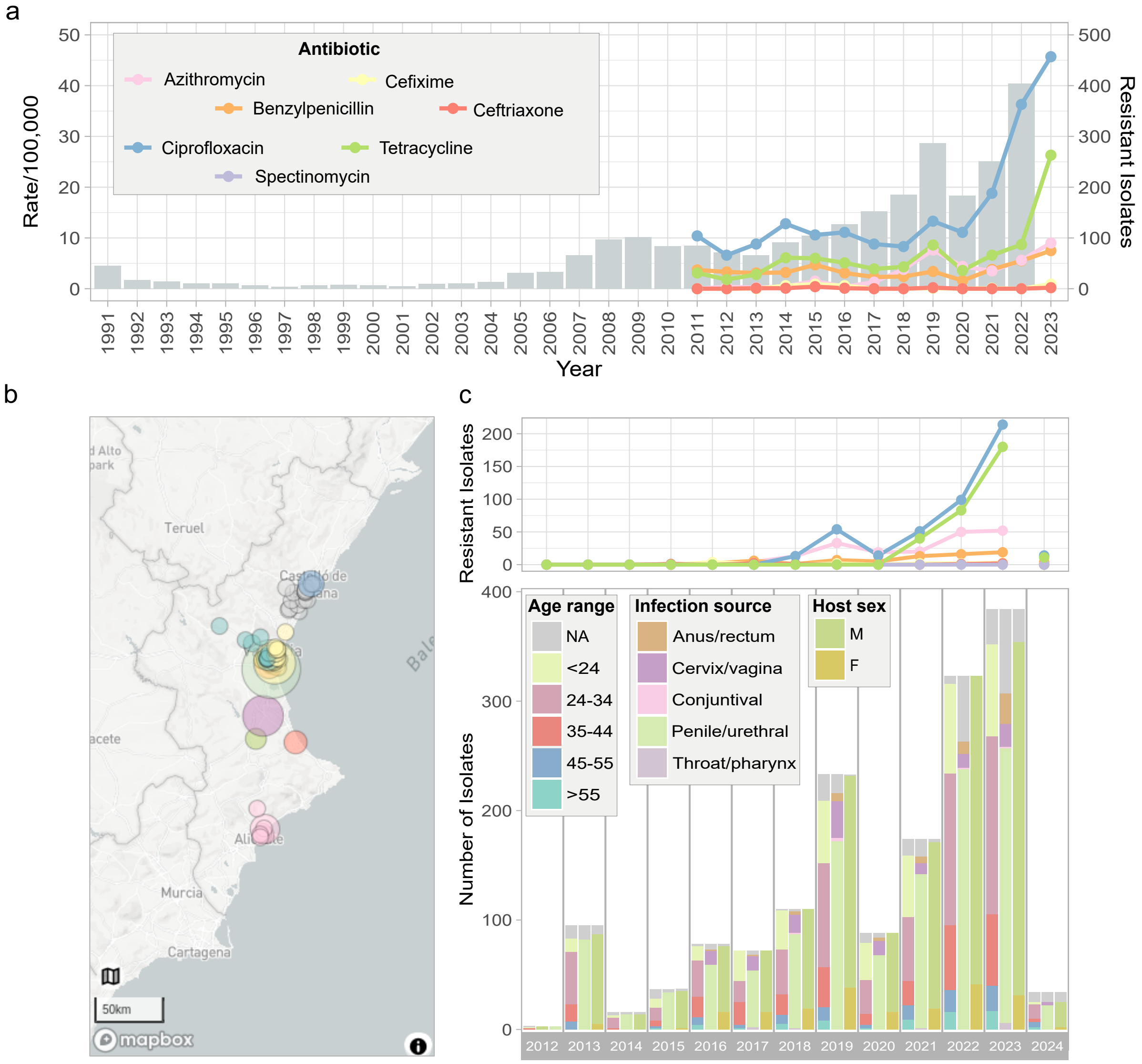

Fig. 1. Gonococcal infections in the Valencian Region, Eastern Spain. (a) Historical incidence of gonococcal infection in the Valencian Region (1991-2023) shown as a bar plot up to the latest report from 2022 [12]. Coloured series show the number of isolates declared as phenotypically resistant to different antimicrobials between January 2011 (when the reporting of this data was made compulsory) and December 2023 (the latest complete year in the study) in the region. (b) Map of the Valencian Region indicating the geographical locations of the health centres where the samples were taken. Marker sizes on the map indicate the relative magnitude of the data for each location. (c) Description of the isolates collected in the Valencian Region for this study from 2012 to 2024 in terms of phenotypic antimicrobial resistance (upper plot) and epidemiological characteristics (in order: age range, infection source and host sex; lower plot).

significant increasing trend in isolates with resistance or decreased susceptibility was found from 2018 to 2023 for ciprofloxacin and benzylpenicillin and a significant decreasing trend for ceftriaxone (Table 1).

High burden of genetic determinants of AMR

Regarding azithromycin resistance (Table 2), a total of 3.6% ($n = 60$) isolates carried mutated copies of the 23S rRNA genes (Fig. 2 and Fig. S2(a)), and 25.0% ($n = 411$) isolates carried a mosaic in the promoter of *mtrR*. Of those, 96.4% ($n = 396$, 24.0% of the total dataset) also carried a mosaic in the *mtrD* gene (this combination will be referred as *mtr* mosaic hereafter). Isolates with an *mtr* mosaic 2 [13] and without 23S rRNA gene mutations had significantly higher MICs than those without this determinant (Table S2). Additionally, 28.9% ($n = 476$) isolates carried other mosaic or semi-mosaic *mtrD* variants [14], 33.6% ($n = 160$) of them together with an *mtrR* promoter mosaic. Of these other *mtrD* variants, four were significantly associated with increased azithromycin MICs after excluding isolates with 23S rRNA gene mutations: mosaic 34, semi-mosaic 13, mosaic 35 and a new mosaic type (named variant 88) (Table S2). This new mosaic type associated with increased azithromycin MICs had only 1 SNP difference compared with *mtrD* mosaic 2. The prevalence of isolates with mosaicism in *mtrD* has significantly increased over time, with 22.1% ($n = 21/95$) in 2013 to 66.7% ($n = 256/384$) in 2023 (Fisher test p 4.05E-15) (Fig. 3(a) and (d)).

Four ceftriaxone-resistant isolates were identified according to EUCAST (Table 1). All of them carried a mosaic in the penicillin-binding protein 2-encoding *penA* gene ($n = 1$ *penA*34.001 and $n = 3$ *penA*10.001; Table 2 and Tables S2 and S3). Cefixime resistance was also predominantly linked to mosaic *penA* alleles ($n = 5$

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Table 1
Phenotypic antimicrobial resistance

Antimicrobial (EUCAST breakpoint or ECOFF)	EUCAST breakpoint/ECOFF	R or DS, n (%)		R or DS, 2018, n (%)	R or DS, 2023, n (%)	2018 vs. 2023; p
Azithromycin	R: MIC >1 mg/L	195/1102 (17.7)		13/100 (13.0)	52/297 (17.5)	0.3496 (n.s.)
Ceftriaxone	R: MIC >0.125 mg/L	4/783 (0.5)	66/783 (8.4)	8/65 (12.3)	3/295 (1.0)	8.07E-05
	DS: 0.064 $\leq$ MIC $\leq$0.125 mg/L	62/783 (7.9)				
Cefixime	R: MIC >0.125 mg/L	15/678 (2.2)	103/678 (15.2)	5/54 (9.3)	12/260 (4.6)	0.1852 (n.s.)
	DS: 0.064 $\leq$ MIC $\leq$0.125 mg/L	88/678 (12.9)				
Ciprofloxacin	R: MIC >0.06 mg/L	449/706 (63.6)		13/40 (32.5)	214/310 (69.0)	1.25E-05
Benzylpenicillin	R: MIC >1 mg/L	74/1007 (7.3)	885/1007 (87.9)	62/82 (75.6)	249/268 (92.9)	6.63E-05
	I: 0.06 < MIC $\leq$1 mg/L	811/1007 (80.5)				
Tetracycline	R: MIC >0.5 mg/L	314/409 (76.8)		40/48 (83.3)	180/239 (75.3)	0.2663 (n.s.)

Proportion of *Neisseria gonorrhoeae* isolates with phenotypic resistance (R) or decreased susceptibility (DS) to six antimicrobials in the Valencian Region during the study period (2012-2024). Values were calculated out of the total number of isolates with MIC data for each category (azithromycin $n = 1102/1647$, 66.9%, $n = 100$ in 2018, and $n = 297$ in 2023; ceftriaxone $n = 783/1647$, 47.5%, $n = 65$ in 2018, and $n = 295$ in 2023; cefixime $n = 678/1647$, 41.2%, $n = 54$ in 2018, and $n = 260$ in 2023; ciprofloxacin $n = 706/1647$, 42.9%, $n = 40$ in 2018, and $n = 310$ in 2023; benzylpenicillin $n = 1007/1647$, 61.1%, $n = 82$ in 2018, and $n = 268$ in 2023; and tetracycline $n = 409/1647$, 24.8%, $n = 48$ in 2018, and $n = 239$ in 2023). Proportions are also provided only for 2018 and 2023, including the statistical significance of the change in proportions (Fisher test). ECOFF, epidemiological cut-off.

Table 2
Genetic determinants of antimicrobial resistance (AMR)

Antimicrobial	Genetic AMR determinant	n (%)
Azithromycin	23S rRNA gene 2045A>G	3 (0.2)
	23S rRNA gene 2597C>T	57 (3.5)
	mtrR promoter mosaic	411 (25.0)
	mtrD mosaic or semi-mosaic	720 (43.7)
	mtr mosaic[a]	396 (24.0)
	RplD G70D	15 (0.9)
Ceftriaxone	PenA I312M, V316P/T, N512Y, and G545S	192 (11.7)
Cefixime	PenA A501T/V	205 (12.4)
	PenA P551S/L	209 (12.7)
	PenA G542S	362 (22.0)
	penA mosaic or semi-mosaic	237 (14.4)
Ciprofloxacin	GyrA S91F	1003 (60.9)
	GyrA D95G	291 (17.7)
	ParC D86N	121 (7.3)
	ParC S87R/I/N	714 (43.4)
Benzylpenicillin	PonA L421P	814 (49.4)
	*bla*TEM-1	121 (7.3)
	*bla*TEM-135	18 (1.1)
Tetracycline	*tetM*	122 (7.4)
	RpsJ V57M	1405 (85.3)
Multiple antimicrobials	*mtrR* promoter -53Adel	413 (25.1)
	MtrR A39T	567 (34.4)
	MtrR G45D	95 (5.8)
	PorB1b G120K	518 (31.5)
	PorB1b A121D/N	510 (31.0)

Major genetic determinants of AMR found in circulating *Neisseria gonorrhoeae* lineages in the Valencian Region during the study period (2012-2024).

[a] *mtr* mosaic: double mosaicism involving the *mtrD* gene and the promoter of *mtrR.*

*penA*10.001 and $n = 2$ *penA*34.001) (Fig. S2(c), Table 2, and Tables S2 and S3). Most isolates with *penA*34.001 and *penA*10.001 showed susceptible phenotypes for ceftriaxone ($n = 40/44$, 90.9%) and cefixime ($n = 29/36$, 80.6%). The decrease in both ceftriaxone and cefixime resistance over the years likely corresponded to the loss of *penA* mosaic alleles, present in 28.2% of the isolates ($n = 22/78$) in 2016 and in 14.8% ($n = 57/384$) in 2023 (Fisher test p 0.0076) (Fig. 3(a)). All of the ciprofloxacin-resistant isolates (63.9%, $n = 449/703$) carried the GyrA S91F substitution (Fig. S2(d)). ParC variants were also found in the dataset, including ParC D86N, which predisposes to gepotidacin resistance [15,16], in 7.3% ($n = 121$) of the isolates (Table 2 and Table S1).

A total of 8.4% ($n = 139$) of isolates carried the *bla*TEM gene (Table 2), and 80.6% ($n = 58/72$ with MIC data) were resistant to benzylpenicillin (Fig. S2(f)). The *tetM* gene was found in 7.4% ($n = 122$) isolates, of which all isolates with MIC information were resistant to tetracycline. The RpsJ V57M mutation was present in 85.3% ($n = 1405$) of the isolates, 82.8% ($n = 309/373$) with a resistant phenotype (Fig. S2(e)). No spectinomycin (16S rRNA or mutations in *rpsE*), zoliflodacin (GyrB D429 or K450 mutations) nor gepotidacin (GyrA A92 mutations) resistance mutations were identified [17].

Major circulating gonococcal lineages carried mtr mosaics

Circulating lineages in the Valencian Region from 2012 to 2024 included 73 NG-STAR CCs (38 with $\geq$5 isolates), 278 NG-STAR STs (61 with $\geq$5 isolates) and 96 MLST STs (35 with $\geq$5 isolates) (Table S4). The diversity of NG-STAR CCs has remained relatively stable from 2016 to 2022, with a slight decrease observed in 2023 (Fig. 3(b)). The most prevalent NG-STAR CC in the dataset were CC1615 (13.6%, $n = 220/1612$ isolates with a CC assigned; mostly NG-STAR ST1969 MLST ST7822 [$n = 92$]) and CC63 (10.5%, $n = 170/1612$; mostly NG-STAR ST2885 MLST ST11422 [$n = 31$]), which were also the most prevalent in 2020 in Europe [18], followed by CC1387 (7.9%, $n = 127$), CC158 (7.3%, $n = 117$) and CC213 (6.8%, $n = 109$) (Table S4).

NG-STAR CC1615 ($n = 220$; Fig. 4) was mostly isolated from males (90.4%, $n = 189/209$), primarily under 35 years old (67.1%, $n = 139/207$) and mostly from penile/urethral infections (Tables S5 and S6). It was significantly associated with increased MICs of azithromycin and ciprofloxacin (Tables S7–S10). In CC1615, 209 isolates (95.0%) carried a mosaic or semi-mosaic in *mtrD*, 121 of those (57.9%; 55.0% of the total) together with *mtrR* promoter mosaic 2. NG-STAR CC63 ($n = 170$; Fig. 4), included 90.4% isolates from males ($n = 151/167$) and individuals $<$35 years old ($n = 120/167$, 71.9%). It was significantly associated with penile/urethral infections (Tables S5 and S6) but not to increased MICs or resistance to key antibiotics (Tables S7–S10). Despite the latter, 93.5% ($n = 159$) isolates carried mosaicism in *mtrD*, and 45 of them (28.3%; 26.5% of the total) also in the promoter of *mtrR* (Table S1).

Sustained transmission driven by imported AMR lineages

A combined phylogeny of 1647 local and 5894 contextual *N. gonorrhoeae* genomes [14,18,19,20,21][14,18,19,20,21] revealed 33 potential lineage introductions into the Valencian Region encompassing 25.9% ($n = 428$) local isolates (Supplementary Methods and Fig. S3). These introductions led to local sustained transmission and were dated using Bayesian inference (Table S11). Detailed examples of these introductions can be observed for I6, I18 and I27 (Fig. 5 and Figs S4–S6). The remaining isolates of the

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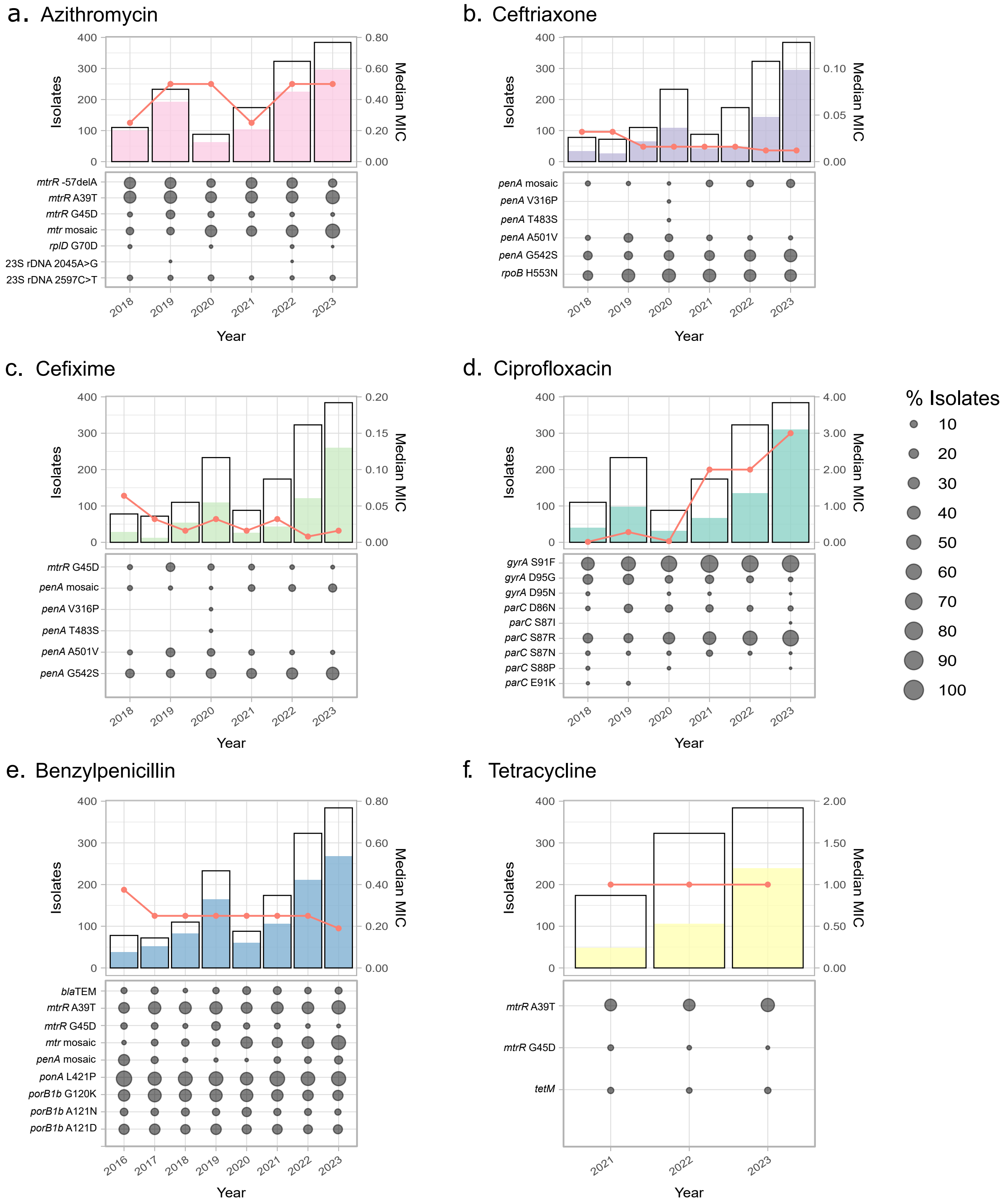

Fig. 2. Trends of phenotypic antimicrobial susceptibility and genetic determinants of antimicrobial resistance (AMR). Temporal evolution in the median MIC and genetic variants associated with AMR to six antibiotics: (a) azithromycin, (b) cefixime, (c) ciprofloxacin, (d) ceftriaxone, (e) benzylpenicillin, and (f) tetracycline. The bar plots indicate the number of isolates with MIC information relative to the total number of isolates collected per year. The red line represents the median MIC per year, and the bubble plots show the proportion of the total isolates carrying each resistance variant per year.

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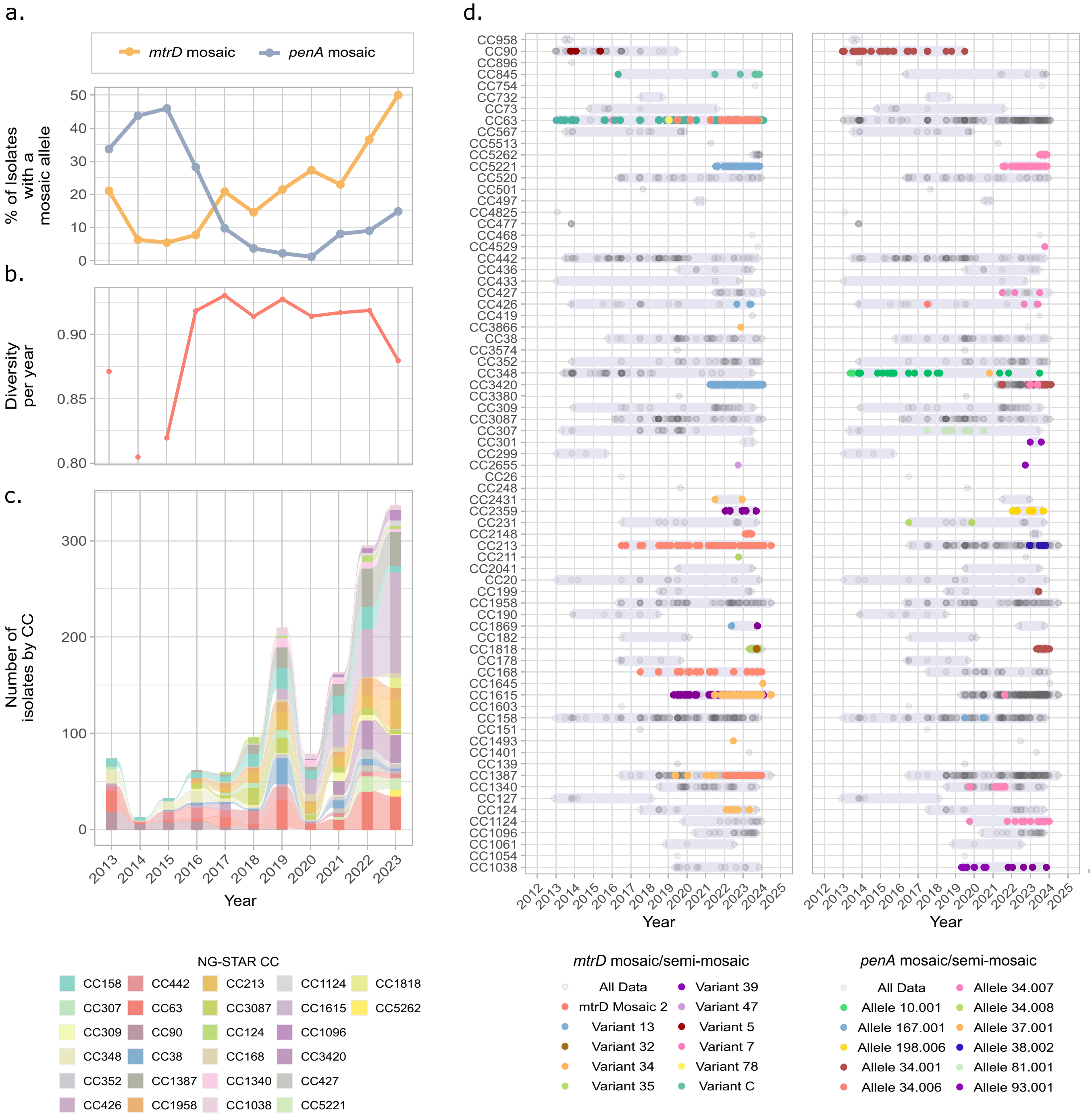

Fig. 3. Dynamics of the gonococcal population in the Valencian Region, Eastern Spain, over time. (a) Percentage of isolates with *mtrD* or *penA* mosaic alleles per year. (b) Diversity of *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) Clonal Complexes (CCs) per year. (c) Number of isolates included in the study by NG-STAR CCs. (d) Temporal distribution of isolates with mosaic alleles in the *mtrD* and/or *penA* genes, categorized by NG-STAR CC. Different colours represent different variants (see legend).

Valencian Region outside these 33 introductions (74.0%, $n = 1476$) represented sporadic cases or nonsustained transmissions.

Of the sustained transmissions, 63.6% (21/33) carried *mtr* mosaics/semi-mosaics or *penA* mosaics (Fig. 5). These imported lineages had a significantly higher burden of mosaic or semi-mosaic *mtr* sequences than nonsustained sporadic cases (Table S12). Eleven out of 33 (33.33%) lineages exclusively involved males, while 22 out of 33 (66.7%) also involved women, although men remained the majority. All these lineages exhibited phenotypic antimicrobial resistance to at least one of the antibiotics included in the study, mainly ciprofloxacin, benzylpenicillin, or tetracycline, although 12 and 5 lineages also carried azithromycin-resistant (8 with *mtr* mosaics) and cephalosporin-resistant (2 with *penA* mosaics) isolates, respectively (Fig. 5). These introduced lineages leading to sustained transmissions included significantly more isolates with phenotypic resistance to azithromycin, ciprofloxacin and tetracycline than isolates from sporadic or non-sustained introductions cases (Table S13). Interestingly, mosaic or semi-mosaic *mtr*-carrying lineages had significantly lower substitution rates than

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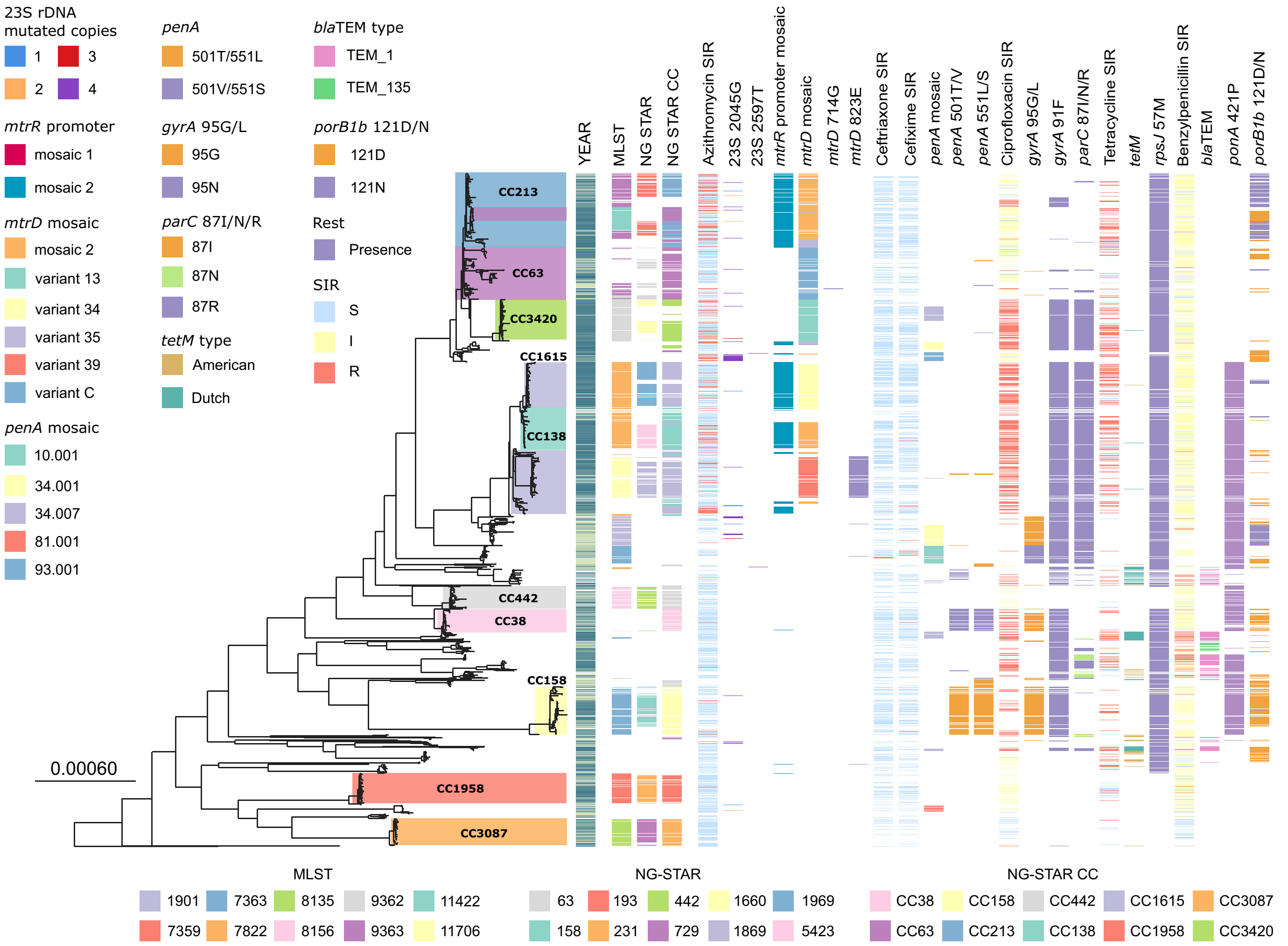

Fig. 4. Phylogenomic reconstruction of the gonococcal population in the Valencian Region, Eastern Spain. Maximum likelihood phylogenetic tree (88,713 single nucleotide polymorphisms [SNPs]) of 1647 *Neisseria gonorrhoeae* isolates from the Valencian Region, the *N. gonorrhoeae* FA1090 reference strain and one *N. meningitidis* genome used for rooting (not shown). The strips show (from left to right): the year were the sample was obtained; the top 10 Multi-Locus Sequence Typing (MLST) sequence types (ST), *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) ST and NG-STAR Clonal Complexes (CC); the azithromycin SIR status according to the EUCAST epidemiological cut-off and the main associated genetic determinants (number of copies of the 23S rRNA gene with the 2045A>G and 2597C>T mutation, mosaic alleles in the *mtrR* promoter and *mtrD* genes ($n > 10$) and variants R714G and K823E in the *mtrD* gene); ceftriaxone and cefixime SIR status and *penA* mosaic alleles ($n > 10$) and variants (A501T/V and P551L/S); ciprofloxacin SIR status and *gyrA* S91F, D95G/L, and *parC* S87I/N/R variants; tetracycline SIR status, *tetM* allele (American or Dutch) and *rpsJ* V57M variant; benzylpenicillin SIR status, TEM allele, and variants in *ponA* (L421P) and *porB1b* (G120K and A121N). This figure can be further explored in: https://microreact.org/project/vr-gonococcus-genomic-epidemiology.

lineages without mosaics (Table S14), despite most of them being recent lineages less than 20 years since the most recent common ancestor, suggesting antibiotic-driven selective sweeps.

Discussion

The landscape of AMR gonococcal infections in the Valencian Region is consistent with the resistance patterns observed in Spain [21,22] and the rest of Europe [5,14,18,19], The rate of azithromycin resistance in this region has mimicked the global increasing trend, with an overall prevalence of 17.7% resistant isolates, slightly higher than that previously reported by a multicentric study (15.1%) [21]. Phenotypic resistance to azithromycin was principally linked to genetic mutations in the different copies of the 23S rRNA gene, and to the presence of mosaic structures in the *mtrR* promoter and/or the *mtrD* gene. Particularly, *mtr* mosaic 2 (*mtrR* promoter and *mtrD* mosaic 2) was the most frequent and significantly associated with higher MICs [13], such as other mosaic and semi-mosaic variants in the *mtrD* gene [14] (variants 13, 34, 35, 39 and the newly identified variant 88). Of those, all except semi-mosaic 13 co-occurred with *mtrR* promoter mosaic 2 (Table S2). These findings underscore the key role of efflux pump alterations in conferring azithromycin resistance [13].

The downward trend in the median MIC values for ceftriaxone and cefixime is likely linked to the global decline of the mosaic *penA*-carrying NG-STAR CC90 lineage [14]. The rate of ciprofloxacin resistance in the Valencian Region showed an increasing trend and was higher than previously reported [14,21,22]. The detection of ParC D86N in 7.3% of the isolates, a variant also associated with reduced susceptibility to gepotidacin [15,16] could threaten novel treatment options for gonorrhoea in the region. Tetracycline and benzylpenicillin resistance remained relatively stable throughout the study.

The increase of mosaic *mtr* alleles in recent years in the region is consistent with trends reported in other EU/EEA countries [14,18], highlighting the role of recombination events in the evolution of *N. gonorrhoeae* resistance [23]. The major circulating

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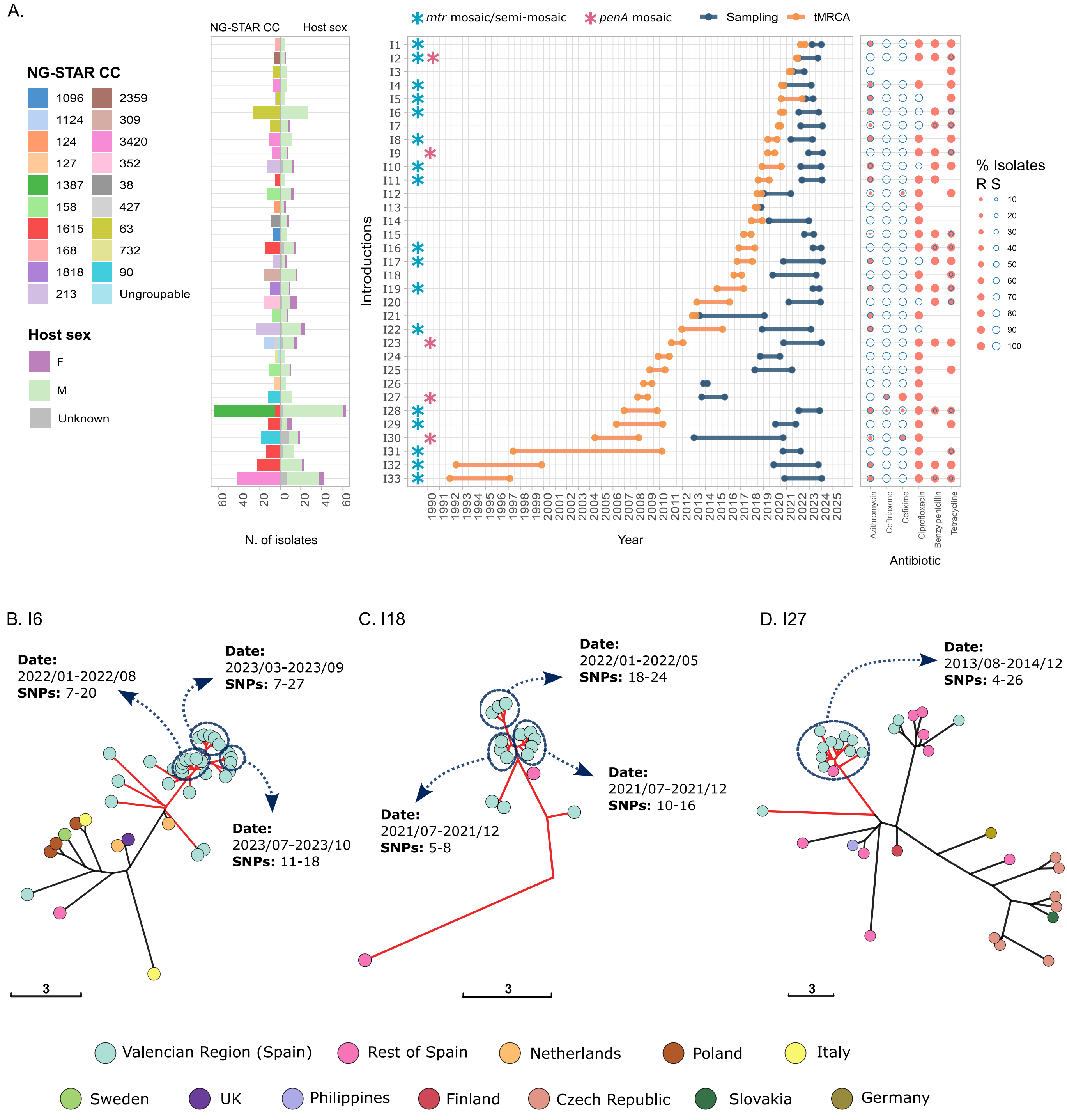

Fig. 5. Overview of the *Neisseria gonorrhoeae* lineages introduced into the Valencian Region. (a) Number of isolates belonging to each *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) Clonal Complex (CC) and the distribution by sex for each introduced lineage. Sampling dates are represented by blue lines and the estimated date of the introductions (time of the most recent common ancestor, tMRCA) by orange lines. Lineages carrying *penA* (pink) and *mtr* mosaics/semi-mosaics (teal) are marked with stars. The rightmost graph represents the proportion of phenotypically antimicrobial resistant and susceptible isolates reported for the isolates in each introduced lineage. (b), (c), and (d) Minimum spanning trees linking the isolates in introductions I6, I18, and I27, respectively, where the colour of the nodes indicates the origin of each sample: the Valencian Region, rest of Spain, or other countries (see legend). Introductions I6 and I27 are shown in context, with red branches indicating the introduced lineage. The single nucleotide polymorphism (SNP) range and date range of collection for the potential transmissions are also displayed.

lineages in the Valencian Region, NG-STAR CC1615 and CC63, which were among the most prevalent in Europe in 2020 [14], played a key role in the local dissemination of AMR gonorrhoea. CC1615 and CC63 were linked to the presence of *mtr* mosaics, with CC1615 showing a significant association with azithromycin and ciprofloxacin resistance. Previous studies reported that the COVID-19 pandemic had an impact on the diversity of gonococcal isolates, leading to a temporary decrease in the diversity of circulating lineages [24,25]. However, a decrease in diversity was not detected around 2020–2021 in the Valencian Region (Fig. 3(b)).

Our results identified multiple lineage introductions into the region that led to local sustained transmissions since the 1990s (Fig. 5). This is consistent with historical evidence of a notable increase in national and international tourism into the Valencian Region from the 1990s–2000s [26]. These introduced lineages carried significantly more isolates with *mtr* mosaics and phenotypic resistance to azithromycin than isolates causing sporadic or nonsustained transmissions. Lineages carrying a mosaic *mtr* exhibited significantly lower substitution rates than those lacking these mosaic variants, reflecting the evolutionary impact of azithromycin usage on recently-emerged *N. gonorrhoeae* lineages, where antibiotic-driven selection pressure reduced genetic diversity through selective sweeps. Importantly, the majority of strains isolated in the Valencian Region (74.0%) were intermixed with isolates from Spain and the rest of Europe, indicating constant bidirectional transmission between the Valencian Region likely driven by international mobility. The sex distribution observed among sustained introductions, with lineages exclusively detected in males, suggested a transmission dynamic likely shaped by specific sexual networks, such as men who have sex with men. However, transmission networks in lower-risk groups and, especially women, may be underrepresented in this study. This is because these groups may show higher proportions of asymptomatic infections that did not seek medical assistance and from which cultured isolates were not obtained. Further studies based on high-throughput sequencing from direct samples will be key to unravel the real picture. Other limitations in the study include differences in the number of isolates available per year, especially over the first part of the study period, as well as differences in the geographical distribution of isolates, although no intra-region conclusions are drawn from the study.

In conclusion, this study provides a comprehensive insight into the dynamics and evolution of AMR *N. gonorrhoeae* lineages at the local level, demonstrating that both persistent introductions and sustained local transmissions are central to the spread of AMR strains. Results from this work reinforce that genome-based enhanced surveillance systems can strengthen public health responses tailored to local sexual networks and transmission patterns. This, together with targeted public health interventions and timely outbreak responses, will be key to mitigate the dissemination of AMR gonococcal lineages at both the local and wider scale.

CRediT authorship contribution statement

Leonor Sánchez-Busó: Conceptualization, Methodology, Coordination, Laboratory investigation, Statistical analysis, Analyzation, Data interpretation, Data access, Publication. Andrea Sánchez-Serrano: Methodology, Laboratory investigation, Statistical analysis, Analyzation, Data interpretation, Data access, Publication. Fernando González-Candelas: Support, Data curation. Carlos Francés-Cuesta: Support, Data curation. Lidia Ruiz-Roldán: Support. Nuria Jiménez-Hernández: Performed all the Illumina library preparation and high-throughput sequencing. Javier Colomina-Rodríguez: Data collection, Investigation, Data transfer. Devi Carolina Salas-Olortegui: Data collection, Investigation, Data transfer. Maria Jesús Castaño-Aroca: Data collection, Investigation, Data transfer. José Miguel Sahuquillo-Arce: Data collection, Investigation, Data transfer. Araceli Molina de Diego: Data collection, Investigation, Data transfer. José Luis López-Hontangas: Data collection, Investigation, Data transfer. José Luis Ramos Martí: Data collection, Investigation, Data transfer. Olalla Martínez-Macias: Data collection, Investigation, Data transfer. Nieves Orta Mira: Data collection, Investigation, Data transfer. Inmaculada Vidal Català: Data collection, Investigation, Data transfer. Victoria Dominguez-Márquez: Data collection, Investigation, Data transfer. Silvia Madrid Camacho: Data collection, Investigation, Data transfer. José Miguel Nogueira-Coíto: Data collection, Investigation, Data transfer. Cori Gázquez Gómez: Data collection, Investigation, Data transfer. Victoria Ortiz De La Tabla Ducasse: Data collection, Investigation, Data transfer. Oihana Sabalza-Baztán: Data collection, Investigation, Data transfer. María Gil-Fortuño: Data collection, Investigation, Data transfer. Óscar Pérez Olaso: Data collection, Investigation, Data transfer. Noelia Hernández Pérez: Data collection, Investigation, Data transfer. Mercedes Roig Cardells: Data collection, Investigation, Data transfer. All the authors read, commented on, and approved the final manuscript.

Transparency declaration

Potential conflict of interest

The authors declare no competing interests.

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Data availability

Individual data associated with the isolates and patients is included in Online Supplement 2 and in Microreact (https://microreact.org/project/vr-gonococcus-genomic-epidemiology). The genome data derived from this study is available at the European Nucleotide Archive public repository (PRJEB83795). Contextual genome data is already available in public repositories. Experimental and analytical protocols can be made available from the corresponding author after publication on reasonable request. Online Supplement files are available from Zenodo: https://doi.org/10.5281/zenodo.17519415.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.12.026.

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