Genome-based epidemiology and antimicrobial resistance determinants of Neisseria gonorrhoeae isolates with decreased susceptibility and resistance to extended-spectrum cephalosporins in Argentina in 2011–16

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Objectives: Our aim was to describe the molecular epidemiology and antimicrobial resistance determinants of isolates of Neisseria gonorrhoeae with decreased susceptibility and resistance to extended-spectrum cephalosporins (ESCs) in Argentina in 2011–16.

Methods: Gonococcal isolates (n = 158) with decreased susceptibility and resistance to ESCs collected in 2011–16 across Argentina were subjected to WGS and antimicrobial susceptibility testing for six antimicrobials.

Results: In total, 50% of the isolates were resistant to cefixime, 1.9% were resistant to ceftriaxone, 37.3% were resistant to azithromycin and 63.9% of the isolates showed an MDR phenotype. Resistance and decreased susceptibility to ESCs was mainly associated with isolates possessing the mosaic penA-34.001, in combination with an mtrR promoter A deletion, and PorB1b amino acid substitutions G120K/A121N. Phylogenetic analysis revealed two main clades of circulating strains, which were associated with the N. gonorrhoeae multiantigen sequence typing (NG-MAST) ST1407 and closely related STs, and characterized by a high prevalence rate, wide geographic distribution and temporal persistence.

Conclusions: N. gonorrhoeae isolates with decreased susceptibility and resistance to ESCs in Argentina have emerged and rapidly spread mainly due to two clonal expansions after importation of one or two strains, which are associated with the international MDR NG-MAST ST1407 clone. The identification of the geographical dissemination and characteristics of these predominant clones may help to focus action plans and public health policies to control the spread of ESC resistance in Argentina. Dual antimicrobial therapy (ceftriaxone plus azithromycin) for gonorrhoea needs to be considered in Argentina.

Introduction

Neisseria gonorrhoeae has developed resistance to all antimicrobials used for gonorrhoea treatment, and the levels of resistance to traditional gonorrhoea antimicrobials, including penicillins, tetracyclines, fluoroquinolones and earlier-generation macrolides, are high worldwide.1–4 Extended-spectrum cephalosporins (ESCs), i.e. cefixime and ceftriaxone, are the last remaining options for empirical first-line monotherapy of gonorrhoea. In recent decades, decreased susceptibility to ESCs has been described worldwide, and resistance to cefixime has also emerged in many countries.1–5 The initial emergence of ESC resistance, including the first cases of high-level resistance to ceftriaxone published in 2011–12,6–8 resulted in large public health concerns globally, and dual antimicrobial therapy (mainly 250–500mg of ceftriaxone plus 1–2g of azithromycin) was introduced as the recommended first-line empirical treatment of uncomplicated gonorrhoea in many
countries. Worryingly, the first global case of failure to treat pharyngeal gonorrhoea with recommended dual therapy was reported in 2016 in England. Moreover, during recent years, a ceftriaxone–resistant strain initially described in Japan in 2015 (FC428) has spread internationally and been subsequently reported in Australia, Canada, Denmark, France and Ireland. It is a grave concern that the first gonococcal strain with ceftriaxone resistance and high-level azithromycin resistance was identified in England in early 2018, followed by two similar cases in Australia. This serious development threatens the effectiveness of the recommended dual antimicrobial treatment (ceftriaxone plus azithromycin).

In Argentina, the national guidelines recommend empirical first-line monotherapy with ceftriaxone (125–250mg) or cefixime (400mg) for uncomplicated gonorrhoea. Worryingly, ESC decreased susceptibility and resistance has emerged in N. gonorrhoeae during the last 5 years in Argentina.

Molecular epidemiological studies have been used to describe gonococcal isolates with decreased susceptibility and resistance to ESCs in many settings worldwide. These studies have mainly used N. gonorrhoeae multiantigen sequence typing (NG-MAST) and identified gonococcal clones that are important for driving transmission of MDR gonococci within national and international sexual networks. NG-MAST ST1407 and MLST ST1901 strains have been spreading internationally and have been associated with MDR and resistance to ESCs, and showed capacity to develop high-level ceftriaxone resistance.

WGS provides a measure of the distribution, stability and expansion of circulating gonococcal clones, which can inform implementation of measures for prevention, treatment and control of gonorrhoea. Compared with NG-MAST and MLST, WGS provides a substantially more accurate and ideal resolution, and detection of antimicrobial resistance (AMR) determinants and accordingly prediction of AMR. WGS can also identify transmission events and reveal geographical and temporal spread of N. gonorrhoeae. However, WGS has not been previously used to elucidate the gonococcal population spreading in Argentina.

Our aims were to describe the genome-based epidemiology and molecular AMR determinants in N. gonorrhoeae isolates with decreased susceptibility and resistance to ESCs across Argentina in 2011–16.

Materials and methods

N. gonorrhoeae isolates

We examined 158 gonococcal isolates from male and female gonorrhoea patients (one isolate per patient) attending sexually transmitted infection (STI) clinics across Argentina. These isolates were collected through the Gonococcal Antimicrobial Susceptibility Surveillance Programme–Argentina (GASSP-AR) from 2011 to 2016 and selected based on their decreased susceptibility or resistance to ceftriaxone (MICs > 0.06mg/L) and/or cefixime (MICs > 0.125mg/L). The investigated isolates (n = 158) were selected from 3478 isolates collected across all the seven health regions of Argentina: Central Region (n = 839), Ciudad Autónoma de Buenos Aires (CABA) (n = 825), South Region (n = 545), Buenos Aires Province (n = 462), North-East Region (n = 671), Central West Region (n = 75) and North-West Region (n = 61). All gonococcal isolates were species confirmed by culture on selective agar medium, Gram-stained microscopy, oxidase test, superoxol test, carbohydrate utilization test and MALDI-TOF MS (Microflex LT, Bruker Daltonik, Bremen, Germany). Isolates were preserved at – 80°C. All examined gonococcal isolates were cultured and preserved as part of the routine diagnostics. Isolates were sent to the National Reference Laboratory without patient identification data, but minimal information including age, gender and sexual orientation was requested. Ethical approval was not required.

Antimicrobial susceptibility testing

N. gonorrhoeae isolates were subcultured onto Difco GC medium agar base (Becton Dickinson, Franklin Lakes, NJ, USA) supplemented with 1% Britalex enrichment supplement (Britania Lab, Buenos Aires, Argentina) for 18–24h at 35°C in a humid 5% CO2-enriched atmosphere prior to antimicrobial susceptibility testing. The MICs of ceftriaxone, cefixime, azithromycin, benzylpenicillin, tetracycline and ciprofloxacin (Richet laboratory, Buenos Aires, Argentina) were determined using an agar dilution method. The MICs were interpreted as susceptibility, intermediate susceptibility and resistance using the breakpoints stated by EUCAST. Cefinase disc test (Becton Dickinson) determined β-lactamase production. For quality control, the gonococcal reference strain ATCC 49226 and the 2008 WHO gonococcal reference strains were used.

MDR N. gonorrhoeae was defined based on a modified version of the definition published by Tapsall et al. i.e. MDR isolates were resistant to one or more of the antimicrobials in category 1 (antimicrobials currently generally recommended for gonorrhoea treatment: ceftriaxone, cefixime and azithromycin), plus resistant to two or more of the antimicrobials listed in category 2 (antimicrobials now less frequently used for gonorrhoea treatment: ciprofloxacin, benzylpenicillin and tetracycline).

WGS

Genomic DNA was extracted by using the Qiagen DNA blood mini kit (QIAGEN). WGS was performed on all isolates using the Nextera XT DNA library preparation kit and the Illumina MiSeq Platform (Illumina, San Diego, CA, USA). A customized version of CLC Genomic Workbench software v.9.5.5 (CLC bio, Qiagen) was used for downstream analysis. AMR determinants including penA, mtrR, penB (parB1b mutations), porA, gyrA, parC and rpsL were identified based on the de novo assembly. The frequency of macrolide resistance mutations (A2059G, C2611T) in the 23S rRNA gene was identified using the integrated mapping and quality-based variant detection within the CLC Genomic Workbench. The MLST and NG-MAST alleles were determined using an agar dilution method. The MLST and NG-MAST (http://www.ng-mast.net/) and NG-STAR (https://ngstar.canada.ca/) databases were used to assign allele numbers and STs. Closely related NG-MAST STs were grouped using previously described genogroup definitions.

Reads for all isolates were mapped against the chromosome of the WHO-F18,16,18,19 reference strain using SMALT (v 0.7.6) with GATK indel realignment. SNP calling was performed using bcftools (v 0.1.19) within SAMtools (v0.1.19). A Maximum Likelihood (ML) tree was inferred from the whole-genome alignment using RAxML v8 under a generalized time-reversible model of evolution with a Gamma correction for among-site rate variation with four rate categories. Recombinant regions were identified and removed using Gubbins, and a total of 10273 SNPs remained for the phylogenetic analysis.

Results

The 158 gonococcal isolates were collected from males (87.3%), from females (9.5%) and from cases with gender not reported (3.2%). Patient age was reported for 139 isolates. The mean age (range) of the males and females was 27.4 (16–70) years and 18.7 (3–29) years, respectively. Patient sexual orientation data were available for <10% of isolates; where available, 16.6% of isolates...
were from MSM. The anatomical site of infection was: urogenital (84.8%), pharyngeal (1.3%), anorectal (0.6%), other (1.3%) and site not reported (12.0%).

The isolates were identified across all the seven health regions in Argentina: 82 (51.9%) in the Central Region, 26 (16.5%) in CABA, 20 (12.7%) in the South Region, 14 (8.9%) in Buenos Aires Province, 7 (4.4%) in the North-East Region, 5 (3.2%) in the Central West Region and 4 (2.5%) in the North-West Region. However, 78 (49.4%) of the 158 isolates were cultured in Córdoba Province (in the Central Region), which is the second most populated province in Argentina (Figure 1).

**Antimicrobial susceptibility**

Among 3478 gonococcal isolates obtained across Argentina in 2011–16, 60.3% (2097/3478) were resistant to ciprofloxacin, 45.6% (1586/3478) to benzylpenicillin and 35.3% (1228/3478) to tetracycline. The susceptibility to azithromycin did not differ greatly by year, with <5% of the isolates annually showing resistance.

The antimicrobial susceptibility of all selected gonococcal isolates (n = 158) is summarized in Table 1. Briefly, 98.7% (156/158) of isolates were resistant to ciprofloxacin, 94.9% (150/158) to tetracycline, 89.9% (142/158) to benzylpenicillin, 50.0% (79/158) to cefixime, 37.3% (59/158) to azithromycin and 1.9% (3/158) to ceftriaxone (identified in 2014 in Rio Negro (n=1) and in 2015 in Córdoba (n=1) and Buenos Aires (n=1); MIC = 0.25–0.5 mg/L). β-Lactamase production was detected in 1.9% (3/158) of isolates. The number of cefixime-resistant isolates increased from 2/351 (0.6%) isolates in 2011 to a peak of 32/728 (4.4%) isolates in 2015, after which the number decreased to 10/709 (1.4%) isolates in 2016. Two (66.7%) of the three ceftriaxone-resistant isolates and 27 (34.2%) of the 79 cefixime-resistant isolates were also resistant.
to azithromycin. In total, 63.9% (101/158) of the isolates showed an MDR phenotype and 1 (0.6%) of the isolates was resistant to all antimicrobials examined.

**Molecular AMR determinants**

The presence of main mutations associated with resistance to β-lactams (in the penA, mtrR, porB1b and penA genes), ciprofloxacin (in gyrA and parC), tetracycline (in rpsJ) and azithromycin (in the 23S rRNA gene) is shown in Table 2. Briefly, 90.5% (n = 143) of the isolates contained a mosaic penA gene, which encodes a mosaic PBP2 that is the main lethal target for β-lactam antimicrobials. The most common mosaic penA allele was penA-34.001 (n = 136), followed by penA-10.002 (n = 3), penA-10.001 (n = 2), penA-34.014 (n = 1) and penA-52.002 (n = 1). The remaining 15 isolates (9.5%) had non-mosaic penA alleles, comprising penA-13.001 (n = 5), penA-5.002 (n = 3), penA-9.001 (n = 2), penA-12.002 (n = 2), penA-19.001 (n = 1), penA-19.003 (n = 1) and penA-44.001 (n = 1). Mutations in the promoter region and/or coding sequence of the mtrR gene, which cause overexpression of the MtrCDE efflux pump and decreased susceptibility to many antimicrobials, were observed in all isolates. A single nucleotide (A) deletion in the 13bp inverted repeat of the mtrR promoter was observed in 155 (98.1%) of the isolates. Five (3.2%) of the isolates contained additionally the MtrR G45D amino acid mutation. However, three (1.9%) isolates only contained the A39T amino acid mutation in MtrR. Non-synonymous substitutions at amino acids G120 and A121 in the porin PorB1b, associated with decreased intake of many antimicrobials and resistance to β-lactam antimicrobials, were observed in 151 (95.6%) and 152 (96.2%) of the isolates, respectively. The substitutions G120K/A121N were observed in 87.3% (138/158) of the isolates, followed by G120K/A121D in 7.6% (12/158). One (0.6%) isolate had G120K/A121G and one (0.6%) only A121S. The L421P mutation in PBP1 (penA1 penicillin resistance determinant) was observed in all isolates.

Isolates resistant to ceftriaxone and/or cefixime (n = 80) were observed in all seven health regions: Central Region, n = 41; CABA, n = 15; South Region, n = 13; Buenos Aires Province, n = 6; North-East Region, n = 3; Central West Region, n = 1; and North-West Region, n = 1. Sixty-eight (85%) and five (6.3%) of these isolates possessed mosaic penA-34.001 and mosaic penA-10.001/10.002, respectively, both associated with decreased susceptibility and resistance to ESCs. Four (5) and five (6.3%) isolates possessed mosaic penA alleles 5.002 (n = 2), 19.001/19.003 (n = 2), 9.001 (n = 1), 13.001 (n = 1) and 52.002 (n = 1). All of these five penA alleles, except 52.002, are non-mosaic alleles. However, three of these non-mosaic penA alleles code for PBP2 with specific amino acid substitutions (penA-5.002, G542S; penA-9.001, P551L; and penA-13.001, A501V and P551S) associated with decreased susceptibility or resistance to ESCs. Additional AMR determinants for ESCs were found in most of the resistant isolates, e.g. mtrR (A deletion in promoter: 96.3%) and penB (G120K and A121N/G/D, 95.0%) (Table 3).

None of the isolates with azithromycin resistance contained the C2611T and A2059G (Escherichia coli numbering) target mutations in the 23S RNA gene. However, all of them had the characteristic single nucleotide deletion (A) in the mtrR promoter. No mtrR120 SNP, which generates a novel promoter for the mtrCDE operon47 was found. Fluoroquinolone resistance has been attributed to SNPs in the QRDRs of the gyrA and parC genes. All ciprofloxacin-resistant isolates (n = 156) had double GyrA amino acid substitutions in positions 91 and 95 (S91F/D95G) and G542S; penA-156) had double GyrA amino acid substitutions in positions 91 and 95 (S91F/D95G) and G542S; parC substitutions (D86N, S87R/N, S88P or E91G). The most common amino acid substitutions were GyrA S91F/D95G (n = 151, 95.6%) and ParC S87R (n = 147, 93.0%). The VS7M amino acid substitution in the ribosomal protein S10 (rpsJ gene), which is involved in chromosomal resistance to tetracyclines, was found in all isolates.

### Molecular epidemiology and phylogenomic analysis

Using NG-MAST, 54 different STs were observed among the 158 N. gonorrhoeae isolates. ST1407 predominated (n = 71), followed by ST3378 (n = 7), ST2318 (n = 4), ST3149 (n = 4), ST3343 (n = 4), ST925 (n = 3), ST2212 (n = 3), ST3294 (n = 3) and ST16027 (n = 3), and 34 STs were represented by single isolates (Table 2). Two NG-MAST genogroups comprising three or more isolates, G1407 (n = 131) and G625 (n = 3), were identified, which encompassed 134 (84.8%) of the isolates. MLST identified 13 different STs, and the most frequent STs were ST9101 (n = 103), ST9365 (n = 27), ST13592 (n = 8), ST13593 (n = 5) and ST7827 (n = 4). NG-STAR showed 18 different types, with type 90 (n = 134) predominated, followed by type 38 (n = 3), type 937 (n = 3) and type 1008 (n = 3). The WGS phylogeny revealed that the majority of the ESC resistance was due to clonal expansions of two strains resulting in two main clades, i.e. clade 1 (C1) and clade 2 (C2) (Figure 2). In total, 134 (84.8%) isolates were grouped into C1 (n = 101) and C2 (n = 43) clades, i.e. clade 1 (C1) and clade 2 (C2). The WGS phylogeny revealed that the majority of the ESC resistance was due to clonal expansions of two strains resulting in two main clades, i.e. clade 1 (C1) and clade 2 (C2) (Figure 2). In total, 134 (84.8%) isolates were grouped into C1 (n = 101) and C2 (n = 43) clades.

### Table 1. Antimicrobial susceptibility of 158 N. gonorrhoeae isolates with decreased susceptibility or resistance to ceftriaxone and/or cefixime cultured across Argentina in 2011-16

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC$_{50}$ (mg/L)</th>
<th>MIC$_{90}$ (mg/L)</th>
<th>MIC range (mg/L)</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>16</td>
<td>16</td>
<td>0.06–32</td>
<td>98.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4</td>
<td>4</td>
<td>0.25–32</td>
<td>94.9</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>2</td>
<td>4</td>
<td>0.5–16</td>
<td>89.9</td>
</tr>
<tr>
<td>Cefixime</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125–0.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.5</td>
<td>1</td>
<td>0.03–4</td>
<td>37.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.06</td>
<td>0.125</td>
<td>0.06–0.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

MIC$_{50}$, MIC of an antimicrobial inhibiting 50% of isolates; MIC$_{90}$, MIC of an antimicrobial inhibiting 90% of isolates.
Table 2. Health region of isolation, molecular AMR determinants and NG-MAST of 158 N. gonorrhoeae isolates with decreased susceptibility or resistance to ESCs cultured across Argentina from 2011 to 2016

<table>
<thead>
<tr>
<th>Health region of isolation</th>
<th>penA allele (n)</th>
<th>MtrR&lt;sup&gt;a&lt;/sup&gt; (n)</th>
<th>PorB&lt;sup&gt;b&lt;/sup&gt; (n)</th>
<th>PBP1&lt;sup&gt;c&lt;/sup&gt; (n)</th>
<th>Tetracycline S10&lt;sup&gt;d&lt;/sup&gt; (n)</th>
<th>Ciprofloxacin GyrA (n)</th>
<th>Ciprofloxacin ParC (n)</th>
<th>Azithromycin 23S rRNA (2059/2611) (n)</th>
<th>NG-MAST ST (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central, CABA, Buenos Aires, North-East, South, Central West, North-West</td>
<td>mosaic</td>
<td>34 (137)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt;</td>
<td>(136), MtrR A39T (1)</td>
<td>G120K; A121N (136), L421P (137)</td>
<td>V57M (137)</td>
<td>S91F; D95G (136), S91F; D95A (1)</td>
<td>S87R (136), S87N (1)</td>
<td>WT/WT (137)</td>
</tr>
<tr>
<td>North-East, Buenos Aires, CABA</td>
<td>10 (5)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt; (4), A&lt;sup&gt;−&lt;/sup&gt;; MtrR G45D (1)</td>
<td>G120K; A121D (5)</td>
<td>L421P (5)</td>
<td>V57M (5)</td>
<td>S91F; D95G (4), S91F; D95N (1)</td>
<td>S87R (4), S87N (1)</td>
<td>WT/WT (5)</td>
<td>ST1407 (71), ST3378 (7), ST3149 (4), ST3431 (4), ST2212 (3), ST3294 (3), ST16027 (3), ST3158 (2), ST3709 (2), ST4459 (2), ST4936 (2), ST6314 (2), ST13123 (2), ST15461 (2), ST16028 (2), ST16036 (2), single STs (24)</td>
</tr>
<tr>
<td>Central, Buenos Aires, CABA</td>
<td>52 (1)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt; (1), MtrR G45D (1)</td>
<td>G120K; A121N (1)</td>
<td>L421P (1)</td>
<td>V57M (1)</td>
<td>S91F; D95G (4), S91F; D95A (1)</td>
<td>S86N (4), S87R (3)</td>
<td>WT/WT (5)</td>
<td>ST925 (3), ST1424 (1), ST2958 (1), ST8921 (1)</td>
</tr>
<tr>
<td>Buenos Aires, CABA, non-mosaic</td>
<td>5 (3)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt; (3)</td>
<td>G120K; A121D (4), A121S (1)</td>
<td>L421P (3)</td>
<td>V57M (3)</td>
<td>S91F; D95G (3)</td>
<td>S87R (3)</td>
<td>WT/WT (3)</td>
<td>ST8509 (2), ST16032 (1)</td>
</tr>
<tr>
<td>Central, Buenos Aires, North-East</td>
<td>19 (2)</td>
<td>MtrR A39T (2)</td>
<td>NA (2)</td>
<td>L421P (2)</td>
<td>V57M (2)</td>
<td>S91F; D95A (2)</td>
<td>WT (2)</td>
<td>WT/WT (2)</td>
<td>ST625 (2)</td>
</tr>
<tr>
<td>South, CABA</td>
<td>12 (2)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt; (2)</td>
<td>G120K; A121D (2)</td>
<td>L421P (2)</td>
<td>V57M (2)</td>
<td>S91F; D95G (2)</td>
<td>S87R (2)</td>
<td>WT/WT (2)</td>
<td>ST225 (1), ST437 (1)</td>
</tr>
<tr>
<td>North-East, South</td>
<td>9 (2)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt; (2)</td>
<td>G120K; A121N (1), G120K; A121D (1)</td>
<td>L421P (2)</td>
<td>V57M (2)</td>
<td>S91F; D95G (1)</td>
<td>E91G (1)</td>
<td>WT/WT (2)</td>
<td>ST3620 (1), ST13064 (1)</td>
</tr>
<tr>
<td>North-East</td>
<td>44 (1)</td>
<td>A&lt;sup&gt;−&lt;/sup&gt; (1)</td>
<td>NA (1)</td>
<td>L421P (1)</td>
<td>V57M (1)</td>
<td>S91F; D95G (1)</td>
<td>E91G (1)</td>
<td>WT/WT (1)</td>
<td>ST16034 (1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>A<sup>−</sup> indicates an adenine deletion in the 13 bp inverted repeat sequence of the mtrR promoter.

<sup>b</sup>NA indicates the presence of a porB1a allele.

<sup>c</sup>Ribosomal protein S10 is encoded by the rpsJ gene.
Table 3. Molecular profiles of 80 N. gonorrhoeae isolates resistant to ceftriaxone and/or cefixime

<table>
<thead>
<tr>
<th>No. of isolates with the following MIC (mg/L)</th>
<th>Resistance determinants</th>
<th>penA</th>
<th>MtrR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PorB&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PBP1</th>
<th>NG-MAST genogroup (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ceftriaxone</td>
<td>cefixime</td>
<td>0.06</td>
<td>0.125</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>56</td>
<td>65</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mosaic penA allele.
<sup>b</sup>Non-mosaic penA allele.
<sup>c</sup>A– indicates an adenine deletion in the promoter region.
<sup>d</sup>NA indicates the presence of a porB1a allele.

(n = 33). C1 mainly comprised isolates belonging to MLST ST1901 (91.1%), NG-STAR ST90 (98%) and NG-MAST G1407 (96%). The isolates in C1 displayed a high level of resistance to cefixime (48.5%) and azithromycin (48.5%); there was one (1.0%) ceftriaxone-resistant isolate (MIC = 2mg/L). The rate of C1 isolates increased significantly from 2011 to 2016 (P < 0.05), from 1.3% (2/158) in 2011 to 11.4% (18/158) in 2016, with a peak of 24.7% (39/158) in 2015. Isolates in C1 were initially identified in CABA and the Chaco Province, respectively. C2 isolates showed a high level of resistance to cefixime (48.5%) and 24.2% resistance to azithromycin, but no isolate was resistant to ceftriaxone. All isolates possess mosaic penA–34 (96.3% of isolates belonged to NG-MAST G1407).

The isolates with decreased susceptibility and resistance to ESCs showed a high level of resistance to other antimicrobials, with most of them (63.9%) displaying an MDR profile. These results show that gonococcal MDR phenotypes have continued to spread in Argentina since they were first detected in 2011. In Argentina, the national guidelines recommend empirical first-line monotherapy with ceftriaxone (125–250mg) or cefixime (400mg) for uncomplicated gonorrhoea. Azithromycin (1g) or doxycycline (100mg twice daily for 7 days) are recommended for treatment of Chlamydia trachomatis or Mycoplasma genitalium infections. Furthermore, Argentinian guidelines recommend antimicrobial treatment if an STI is suspected but not confirmed. This practice can result in a further induction or selection of resistance in N. gonorrhoeae as well as M. genitalium. In many other countries, dual antimicrobial therapy with ceftriaxone plus azithromycin is recommended as first-line empirical treatment as a strategy to stem the development and/or spread of gonococcal AMR. Similar dual antimicrobial therapy needs to be considered in Argentina for all gonorrhoea cases to mitigate the selection of MDR isolates.

The decreased susceptibility and resistance to ESCs in Argentina was mainly associated with mosaic penA–34.001, which is commonly found in NG-MAST ST1407 and genetically related (sub)clones. Also F89, the second clinical strain characterized as XDR, belonged to ST1407. This strain displayed high-level resistance to cefixime (MIC = 2mg/L) and cefixime (MIC = 4mg/L) due to an additional SNP coding for an A501P amino acid substitution in its mosaic penA–34.001 (resulting in mosaic penA–42.001). Emergence of azithromycin resistance is also of

Discussion

WGS SNP analysis showed that the emergence and rapid spread of N. gonorrhoeae isolates with decreased susceptibility and resistance to ESCs across Argentina in 2011–16 were mainly due to two clonal expansions resulting in two N. gonorrhoeae clades possessing mosaic penA–34 (96.3% of isolates belonging to NG-MAST G1407).

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concern, as it is recommended in combination with ceftriaxone in dual antimicrobial therapy for gonorrhoea. Specific SNPs in the 23S rRNA gene result in high-level (A2059G: MIC/C21 256mg/L) or intermediate-level (C2611T: MIC/C21 4mg/L) azithromycin resistance.29 In our study, the azithromycin-resistant isolates had MICs ranging from 1 to 4mg/L, but the A2059G and C2611T mutations were not observed in any isolate. However, mutations in the mtrR gene or its promoter region, which increase azithromycin MICs by causing an overexpression of the MtrCDE pump efflux, were observed in all azithromycin-resistant isolates.4,47 Another worrisome finding in our study was that azithromycin resistance was mainly associated with isolates harbouring mosaic penA-34 and, accordingly, a higher level of decreased susceptibility or resistance to ESCs. Continued evolution and widespread transmission of these isolates might challenge the effectiveness of current therapeutic options, i.e. both ESC monotherapy and ceftriaxone plus azithromycin dual therapy. The isolates with decreased susceptibility or resistance to ESCs additionally showed a high percentage of ciprofloxacin resistance, and cumulative mutations were observed in the QRDRs of gyrA and parC as previously reported.26

In the current study, the SNP-based phylogenomics grouped the isolates into two main clades and revealed that NG-MAST G1407 was predominant among N. gonorrhoeae isolates with decreased susceptibility or resistance to ESCs across Argentina in 2011–16. G1407 has been associated with decreased susceptibility and resistance to ESCs and has also been shown to be responsible for treatment failures with ESCs and azithromycin in many countries.4,23,26,48 This clone, and subsequently evolved subclones, is thought to have originated in Japan and later spread worldwide, with the first report of NG-MAST ST1407 (MLST ST1901) in Kanagawa, Japan in 2003.49 In the present study, the Argentinian NG-MAST ST1407 isolates showed a high level of genomic similarity to the predominant ST1407 clone reported already in the late 2000s in other settings such as the USA,20 Canada33 and Europe.26

Figure 2. Phylogenomic analysis of 158 N. gonorrhoeae isolates with decreased susceptibility or resistance to ESCs cultured from 2011 to 2016 across Argentina. The main phylogenetic clades were designated as C1 and C2. The first three columns at the termini of the phylogenetic tree represent date of collection, location and patient gender for each isolate. Immediately following the first columns are three columns that represent the AMR phenotype profile. The following four columns represent AMR determinants. Grey represents penA-34, adenosine deletion in the mtrR promoter and G120K/A121N amino acid substitutions in PorB. Green represents the absence of mutations in the 23S rRNA. The last three columns describe the major NG-MAST genogroup and NG-STAR and MLST STs.
indicating that importation and subsequent spread across Argentina have occurred. The two clades found in Argentina were characterized by a high prevalence rate, but also by a temporal persistence, suggesting an outbreak that persisted across the period of study and was caused by two independent but temporally simultaneous introductions (in CABA and Córdoba) and subsequent clonal expansions. This also indicates that these strains have a high biological fitness and/or, due to the rapid spread, have been introduced into high-risk, frequently transmitting sexual networks. Nevertheless, due to the high similarity of the isolates in C1 and C2, it cannot be excluded that initially only one strain was imported and has since evolved into the two clades, for example during spread in different populations. Analysis of a larger number of isolates would be valuable in order to clarify the complete phylogeny of gonococcal isolates in Argentina, including the relationships between C1 and C2, in detail. Unfortunately, limited data regarding sexual orientation of patients were available, but C1 (93.1%) was more associated with male patients (including MSM) than C2 (78.8%). However, our results show that isolates in both clades are spreading within both the MSM population and the heterosexual population in Argentina. Increased awareness of the dissemination of ESC-resistant and MDR gonococcal strains is crucial. Accordingly, there is a need to strengthen the AMR surveillance system in order to also detect treatment failures, identify communities at high risk and trace sexual contacts, in addition to molecular studies for better understanding of the AMR mechanisms and molecular epidemiology of N. gonorrhoeae to maximize the effectiveness of currently available antimicrobials for gonorrhoea treatment.

In conclusion, N. gonorrhoeae isolates with decreased susceptibility and resistance to ESCs in Argentina have emerged and rapidly spread due to two clonal expansions, which are both associated with the international MDR NG-MAST ST1407 clone. This study indicates that dual antimicrobial therapy (ceftriaxone plus azithromycin) needs to be considered in Argentina. Appropriate antimicrobials for gonorrhoea treatment.

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Transparency declarations
None to declare.

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