Antimicrobial susceptibility of Neisseria gonorrhoeae isolates and treatment of gonorrhoea patients in Ternopil and Dnipropetrovsk regions of Ukraine, 2013-2018

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SUMMARY


Antimicrobial resistance (AMR) in Neisseria gonorrhoeae is a major public health concern globally. However, recent gonococcal AMR data from Eastern Europe are extremely limited and no AMR data for strains spreading in Ukraine has ever been internationally published. We investigated the AMR of N. gonorrhoeae isolates in two regions of Ukraine (Ternopil 2013-2018, Dnipropetrovsk 2013-2014), and, where information was available, the treatment administered to the corresponding gonorrhoea patients. Determination of minimum inhibitory concentration (MIC) of eight antimicrobials was performed using Etest and resistance breakpoints from the EUCAST were applied. Overall, 9.3% of the examined 150 isolates were resistant to ciprofloxacin, 6.0% to tetracycline, 2.0% to azithromycin, and 0.7% to benzylpenicillin. No isolates were resistant to ceftriaxone, cefixime, spectinomycin, or gentamicin. However, one (0.7%) isolate showed a MIC value of 0.125 mg/L for both ceftriaxone and cefixime, i.e. bordering resistance. Eighty-eight (67.2%) of 131 patients were administered dual therapy (ceftriaxone 1 g plus doxycycline/clarithromycin/azithromycin/ofloxacin) and 22 (16.8%) ceftriaxone 1 g monotherapy. Worryingly, 21 (16.0%) patients received monotherapy with clarithromycin/doxycycline/azithromycin/ofloxacin/benzylpenicillin. In conclusion, the antimicrobial susceptibility of gonococcal strains spreading in Ternopil and Dnipropetrovsk, Ukraine during 2013–2018 was high. Low levels of resistance to ciprofloxacin, tetracycline, azithromycin, and benzylpenicillin were found, but no resistance to the internationally recommended ceftriaxone, cefixime, or spectinomycin. Ceftriaxone 1 g should remain as...
empiric first-line treatment, in dual therapy with azithromycin or doxycycline or in monotherapy. Continued and expanded gonococcal AMR surveillance in Ukraine is essential to monitor the susceptibility to particularly extended-spectrum cephalosporins, azithromycin and doxycycline.

Key words: *Neisseria gonorrhoeae*, Gonorrhoea, Antimicrobial resistance (AMR), ceftriaxone, azithromycin, Ukraine

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Gonorrhoea is the second most prevalent bacterial sexually transmitted infection (STI) globally (1,2). In Ukraine, 4779 gonorrhoea cases (11.3 per 100,000 population) was reported in 2017. However, the reported incidence in Ukraine is highly underestimated due to the suboptimal screening of asymptomatic individuals, limited testing of particularly extragenital specimens, rare use of sensitive laboratory diagnostics, and incomplete case reporting and epidemiological surveillance.

Worryingly, *Neisseria gonorrhoeae* has developed antimicrobial resistance (AMR) to all drugs introduced for gonorrhoea treatment (3,4). The extended-spectrum cephalosporin (ESC) ceftriaxone is the only remaining option for empiric first-line monotherapy in most countries. However, decreased susceptibility or resistance has emerged in many settings globally (4-24) and failures to cure pharyngeal gonorrhoea with ceftriaxone monotherapy were confirmed in several countries (10,25-30). Consequently, WHO global gonorrhoea

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treatment guidelines and guidelines in Europe, Australia, USA, and Canada recommend dual antimicrobial therapies, mainly ceftriaxone 250-500 mg×1 intramuscularly (IM) plus azithromycin 1-2 g×1 orally (3,7,31-33). It is a grave concern that the first global failure to cure pharyngeal gonorrhoea with dual therapy was reported in 2016 (34), international transmission of a ceftriaxone-resistant strain in 2015-2018 has been verified (16-22), and in 2018 the first gonococcal isolates with ceftriaxone resistance and high-level azithromycin resistance were reported from England (23) and Australia (24). Enhanced gonococcal AMR surveillance is essential, to identify emerging AMR, monitor AMR trends, and to ensure effective patient management by timely refinements of treatment guidelines.

The European Gonococcal Antimicrobial Surveillance Program (Euro-GASP) is monitoring the AMR in the European Union/European Economic Area (EU/EEA) countries (8,9,35-37). However, in the non-EU/EEA countries of the WHO European Region (38), gonococcal AMR surveillance is extremely limited, and mainly sporadically available in Russia and Belarus (39-44). In Ukraine, no AMR data for gonococcal strains spreading in Ukraine has ever been internationally published. The gonorrhoea treatment in Ukraine is mainly guided by two national orders of the Ukrainian Ministry of Health, which were updated in 2004 and 2009, respectively (45,46). For treatment of uncomplicated gonorrhoea, these guidelines recommend particularly ceftriaxone 1 g×1 IM, but also include cefotaxime 1 g×1, spectinomycin 2-4 g×1, and several different macrolides (azithromycin, but also erythromycin, clarithromycin, josamycin, roxithromycin, spiramycin), fluoroquinolones, tetracyclines, penicillins, and aminoglycosides (45,46).
This study investigated i) the AMR of gonococcal isolates in Ternopil (2013-2018) and Dnipropetrovsk (2013-2014) regions of Ukraine to eight previously or currently recommended therapeutic antimicrobials, ii) the antimicrobial treatment administered to the corresponding gonorrhoea patients, and iii) provide quality-assured AMR data for informing the national gonorrhoea treatment guidelines in Ukraine (45,46), which have not been updated since 2009.

MATERIAL AND METHODS

Study population
Consecutive patients with urogenital complaints attending the Ternopil and Dnipropetrovsk regional clinical dermatovenerological dispensaries, Ukraine, July 2013-August 2018 were included. Cervical specimens from females and urethral specimens from males were collected, and written informed consent was obtained from all patients. Clinical and epidemiological data, including gender, age, symptoms, sexual orientation, and treatment administered, were collected. Exclusion criteria included being not sexually active or not providing informed consent. All patients were aimed to be managed in accordance with the national orders of the Ukrainian Ministry of Health (45,46).

*Neisseria gonorrhoeae* culture
All swabs were inoculated on the selective Chocolate agar™+PolyViteX VCAT3 media (bioMérieux, Marcy-l’Étoile, France), followed by an incubation for 24-48 hours at 36°C in a humid candle jar. Isolates were confirmed as *N. gonorrhoeae* by identification of Gram-negative diplococci in microscopy, rapid oxidase reaction, rapid sugar utilisation test (Neisseria-test; PLIVA-Lachema Diagnostika s.r.o., Brno, Czech Republic), and matrix-
assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS; Microflex LT, Bruker Daltonik, Bremen, Germany), and subsequently preserved at -72°C.

Antimicrobial susceptibility testing
The minimum inhibitory concentrations (MICs, mg/L) of eight antimicrobials were determined by Etest (bioMérieux, Marcy-l'Étoile, France) on Difco GC Medium Base agar (Becton, Dickinson and Company, Sparks, USA) supplemented with 1% Isovitalex (Becton, Dickinson and Company, Sparks, USA) and 1% Hemoglobin (Becton, Dickinson and Company, Sparks, USA), with agar plates incubated for 18-20 hours at 36±1°C in a humid 5% CO₂-enriched atmosphere. Results were interpreted using whole MIC dilutions and, where available, breakpoints for susceptibility (S) and resistance (R) according to the European Committee on Antimicrobial Susceptibility Testing breakpoints (EUCAST) (47). For gentamicin, previously published interpretative criteria were used (48). ß-lactamase production was identified using a Nitrocefin test (Oxoid, Basingstoke, England). The 2016 WHO N. gonorrhoeae reference strains (49-51) were used for quality controls.

Ethical approval
The study was approved by the Bioethic Commission of I. Horbachevsky Ternopil State Medical University, in accordance with the excerpt from Minutes No. 51 of the Bioethic Commission of I. Horbachevsky Ternopil State Medical University, dated 14.01.2019.
RESULTS

Neisseria gonorrhoeae isolates and gonorrhoea patient characteristics

Gonococcal isolates (n=150) from 150 patients were cultured in 2013 (n=14), 2014 (n=40), 2015 (n=33), 2016 (n=25), 2017 (n=13), and 2018 (n=10) in Ternopil region and in 2013-2014 (n=15) in Dnipropetrovsk region.

The 150 isolates were cultured from 130 (86.7%) males and 20 (13.3%) females. The mean age for the females was 28.0 years (median age: 26.0 years; range: 17-80 years) and for the males 29.2 years (median age: 28.0 years; range: 16-60 years). The age distribution was similar during 2013-2018. Clinical data was available from 132 (88%) patients, and of these, 94.7% (107/113) of males had an urethral discharge and 89.5% (17/19) of females had a vaginal discharge. The sexual orientation was reported for 133 (88.7%) patients, of which 96.2% (n=128) reported to be heterosexuals and 3.8% (n=5) reported to be men-who-have-sex-with-men (MSM).

Antimicrobial susceptibility of N. gonorrhoeae isolates (n=150) from Ukraine, 2013-2018

Table 1.

Overall, 9.3% (0%) of isolates were resistant (intermediately susceptible) to ciprofloxacin (MIC range: <0.002->32 mg/L), 6.0% (8%) to tetracycline (0.125-32 mg/L), 2.0% (14%) to azithromycin (0.032-1 mg/L), and 0.7% (29.3%) to benzylpenicillin (0.003->32 mg/L). No isolates were resistant to ceftriaxone (<0.002-0.125 mg/L), cefixime (<0.016-0.125 mg/L), spectinomycin (4-24 mg/L), or gentamicin (2-8 mg/L). Notably, one (0.7%) isolate showed MIC=0.125 mg/L of both ceftriaxone and cefixime, which is bordering resistance (51) (Table 1). This isolate was additionally resistant to ciprofloxacin and intermediately susceptible to azithromycin and benzylpenicillin. Only one (0.7%) β-lactamase producing isolate was
detected. The number of AMR isolates was low and no major trends over time could be elucidated.

The MIC distributions for ceftriaxone and azithromycin, used in the internationally recommended dual gonorrhoea therapy (3,7,31-33), were examined in detail. Briefly, 66% of isolates showed a ceftriaxone MIC of \( \leq 0.002 \) mg/L, but a defined population representing 32% of isolates had higher MICs of ceftriaxone (MIC=0.004-0.032 mg/L). Most (84%) isolates belonged to a susceptible wild type MIC distribution for azithromycin, but 16% of isolates showed an intermediate susceptibility or resistance.

**Antimicrobial treatment of gonorrhoea patients (n=131) in Ukraine**

Treatment data was available from 131 (87.3%) patients and 14 antimicrobial treatment regimens were administered (Table 2).

**Table 2**

The most frequently given treatment was ceftriaxone 1 g plus doxycycline 100 mg twice daily for five days (33.6% of cases), followed by ceftriaxone 1 g plus clarithromycin 500 mg twice daily for five days (22.9%), and ceftriaxone 1 g monotherapy (16.8%). Dual therapy with ceftriaxone 1 g plus azithromycin 1.5 g, i.e., given over 3-6 days, was administered to 6.1% of patients. For sporadic patients (2.3%), combinations of ceftriaxone 1 g plus two additional antimicrobials were administered. Nevertheless, 16.0% of patients were not treated with any ceftriaxone and instead monotherapies with clarithromycin, doxycycline, benzylpenicillin, azithromycin, and ofloxacin (Table 2).
DISCUSSION

This study describes the first internationally reported gonococcal AMR data for isolates cultured in Ukraine, i.e., 2013-2018 in Ternopil and Dnipropetrovsk, quality-assured according to WHO standards (49-52). The AMR levels were surprisingly low compared to the neighbouring EU/EEA countries (8,9,53), Belarus (40) and Russia (41-44), and many countries globally (6). It would be valuable to compare the relatively susceptible Ukrainian gonococcal population with the populations internationally, i.e. using whole-genome sequencing which is utilized in the EU/EEA surveillance (54).

The prevalence of resistance to the previously recommended gonorrhoea antimicrobials ciprofloxacin and tetracycline was 9.3% and 6.0%, respectively, in Ukraine. None of these antimicrobials should be recommended for empirical first-line gonorrhoea monotherapy in Ukraine, which is in line with most settings globally (6,8,9,39-44). Worryingly, doxycycline and ofloxacin were used for monotherapy of 4.6% and 0.8%, respectively, of the current patients and tetracyclines or fluoroquinolones need to be excluded from the empiric treatment recommended in the Ukrainian treatment guidelines (45,46). Interestingly, β-lactamase producing gonococcal strains appear exceedingly rare (1/150 isolates) in Ukraine, as in the neighbouring East-European countries Belarus and Russia (39-43). This indicates that no β-lactamase producing strains have emerged and no imported such strains have managed to establish any domestic transmission in Ukraine. No isolates showed chromosomal benzylpenicillin resistance either, but 29.3% of isolates displayed an intermediate susceptibility, which questions the use of benzylpenicillin in any empiric gonorrhoea therapy.
Regarding currently internationally recommended gonorrhoea therapeutics, azithromycin resistance (2%) was also rare compared to in most EU/EEA countries (8,9), and the neighbouring East-European countries Belarus and Russia (40,43,44), and all resistant Ukrainian isolates (n=3) had a low azithromycin MIC (1 mg/L), which make the internationally recommended ceftriaxone plus azithromycin dual therapy (3,7,31-33), used in 6.1% of cases, highly effective. Also the most frequently administered treatment, i.e. ceftriaxone plus doxycycline dual therapy used for 33.6% of patients, should be highly effective and additionally this regimen effectively eradicate concomitant Chlamydia trachomatis infections (55). All isolates were susceptible to ceftriaxone, cefixime, spectinomycin, and gentamicin, and in general the MICs of these antimicrobials were low. Nevertheless, one (0.7%) isolate was bordering resistance to ceftriaxone and cefixime. This stresses the importance of strengthened and expanded gonococcal AMR surveillance in Ukraine. The reasons for the low gonococcal AMR levels in Ukraine are unknown. However, it might be explained by the long-term use of ceftriaxone 1 g (in monotherapy or dual therapy) as the recommended empirical first-line treatment of uncomplicated gonorrhoea (since 2004). Furthermore, cefixime or other less potent oral cephalosporins have never been recommended or frequently used in Ukraine which has most likely limited the selection pressure for ESC resistance, and no gonococcal strain with ESC resistance might have been imported from other countries and/or managed to establish domestic transmissions in Ukraine.

Worryingly, many additional antimicrobials are recommended in the Ukrainian gonorrhoea treatment guidelines (45,46) and unfortunately these antimicrobials remain to be used. These recommended antimicrobials include several additional cephalosporins, macrolides, fluoroquinolones, penicillins, tetracyclines, aminoglycosides, and trimethoprim.
in combination with sulfamethoxazole and, obviously, most of these antimicrobials should be excluded from the Ukrainian gonorrhoea treatment guidelines (45,46). Furthermore, in Ukraine antimicrobials are easily available “over-the-counter” in the pharmacies without any prescriptions, which is crucial to abandon to mitigate self-medication.

The limitations of this study included the moderate number of isolates from only two regions of Ukraine, no extragenital specimens examined, no data regarding treatment outcome, and no typing of the isolates. Accordingly, the quality-assured surveillance of gonococcal AMR, and treatment failures, needs to be strengthened and expanded in Ukraine. Increasing the number of representative isolates collected annually and including additional regions, pharyngeal and rectal specimens, and improved patient epidemiological data are urgently needed, to provide a strong evidence base for regular refinements of the Ukrainian gonorrhoea treatment guidelines (45,46). For expansion of the gonococcal AMR surveillance, training of clinicians and laboratory workers (e.g., in sample collection, sample transportation, and gonococcal culture and AMR testing), implementation of quality-assured gonococcal culture in many Ukrainian laboratories, and political and financial commitment are imperative.

In conclusion, we describe the first internationally reported gonococcal AMR data for isolates cultured in Ukraine, i.e., in Ternopil and Dnipropetrovsk regions in 2013-2018. This study also included Ukraine in the WHO Global GASP (6). The antimicrobial susceptibility was surprisingly high and only relatively low levels of resistance to ciprofloxacin, tetracycline, azithromycin, and benzylpenicillin were found. No resistance to the currently recommended first-line ceftriaxone (or cefixime), spectinomycin and gentamicin was identified. Accordingly, ceftriaxone 1 g intramuscularly can remain as first-line empiric
treatment (in monotherapy or dual therapy with azithromycin or doxycycline). Continued, strengthened and expanded quality-assured surveillance of gonococcal AMR and ideally also gonorrhoea treatment failures in Ukraine is essential to monitor AMR emergence and trends of the susceptibility to particularly ESCs (ceftriaxone and cefixime), azithromycin and doxycycline, and to provide evidence-based AMR data for timely and regular refinements of the gonorrhoea treatment guidelines in Ukraine (45,46), which is essential to ensure effective management and control of gonorrhoea in Ukraine.

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We are especially grateful to Oleksandr I. Khara for extensive and invaluable support in 2013-2017. We would also like to thank the following collaborators for clinical and technical assistance: Luidmyla P. Gayova, Volodymyr V. Romaniv, Valentyna Y. Grytsuik, Ihor Kohut, Oksana Hlushok, Vitaliy Hlushok, Ruslan Semenyna, Natalia Obal, Yaroslav Krushelnytskyi, Lilia Ivachova, Inna Shumeiko, Natalia Yarosevych, and Irina Makogon.

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Table 1. Antimicrobial susceptibility in *Neisseria gonorrhoeae* isolates (n=150) from Ternopil and Dnipro regions, Ukraine, 2013-2018

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Number of isolates (%; 95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0 (0%; 0-2.4%)</td>
</tr>
<tr>
<td>Cefixime</td>
<td>0 (0%; 0-2.4%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>3 (2.0%; 0.4-5.7%)</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>0 (0%; 0-2.4%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>14 (9.3%; 5.2-15.1%)</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>1 (0.7%; 0.02-3.7%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>9 (6.0%; 2.8-14.1%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 (0%; 0-2.4%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The clinical breakpoints (susceptible, resistant) were as follows: ceftriaxone and cefixime (MIC≤0.125 mg/L, MIC>0.125 mg/L), azithromycin (MIC≤0.25 mg/L, MIC>0.5 mg/L), ciprofloxacin (MIC≤0.032 mg/L, MIC>0.064 mg/L), spectinomycin (MIC≤64 mg/L, MIC>64 mg/L), benzylpenicillin (MIC≤0.064 mg/L, MIC>1.0 mg/L), tetracycline (MIC≤0.5 mg/L, MIC>1.0 mg/L), and gentamicin (MIC≤4 mg/L, MIC>16 mg/L) (47,48). EUCAST current definitions: S, Susceptible-standard dosing regimen; I, Susceptible-increased exposure; and R, Resistant.

<sup>b</sup>NA, not applicable.
Table 2. Antimicrobial treatment of gonorrhoea patients (n=131) diagnosed in Ternopil, Ukraine, 2013-2018

<table>
<thead>
<tr>
<th>Prescribed antimicrobials</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone monotherapy</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone 1 g intramuscularly (IM)</td>
<td>22 (16.8%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM plus doxycycline 100 mg orally twice daily for 5 days</td>
<td>44 (33.6%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM plus clarithromycin 500 mg orally twice daily for 5 days</td>
<td>30 (22.9%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM plus azithromycin 500 mg orally once daily for 3 days</td>
<td>8 (6.1%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM plus ofloxacin 200 mg orally twice daily for 5 days</td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM, doxycycline 100 mg orally twice daily for 5 days, clarithromycin 500 mg orally twice daily for 5 days</td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM plus azithromycin 250 mg orally once daily for 6 days</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Ceftriaxone 1 g IM, azithromycin 500 mg orally once daily for 3 days followed by clarithromycin 500 mg orally twice daily for 5 days</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Other antimicrobial monotherapies</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin 500 mg orally twice daily for 5 days</td>
<td>11 (8.4%)</td>
</tr>
<tr>
<td>Doxycycline 100 mg orally twice daily for 5 days</td>
<td>6 (4.6%)</td>
</tr>
<tr>
<td>Azithromycin 500 mg orally once daily for 3 days</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Ofloxacin 200 mg orally twice daily for 5 days</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Benzylpenicillin 1 MLN units IM eight times daily for 4 days</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Benzylpenicillin 1 MLN units IM eight times daily for 5 days</td>
<td>1 (0.8%)</td>
</tr>
</tbody>
</table>

MLN, Millions.