MOLECULAR SURVEILLANCE OF GONOCOCCAL CIPROFLOXACIN SUSCEPTIBILITY/ RESISTANCE IN BULGARIA, 2022-2023

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ABSTRACT

Background: The emergence and spread of antimicrobial resistance in *Neisseria gonorrhoeae* is a significant public health issue, with Euro-GASP conducting surveillance across the EU-EEA. The advent of molecular diagnostics for *N. gonorrhoeae* may possibly limit the surveillance data because it is dependent on gonococcal culture for phenotypic susceptibility testing. Ideally, molecular diagnostics should combine identification and resistance detection but the complexity of gonococcal molecular genetics of resistance is a major barrier for test development. Currently, resistance prediction in *N. gonorrhoeae* is accurate only for fluoroquinolones and there are commercial kits available on the market that allow antimicrobial resistance surveillance and individualized treatment.

The study examined Bulgaria's gonococcal fluoroquinolone resistance rate for 2022-2023 by molecular methods and compared it with previous years and EU-EEA trends.

Methods: The commercial ResistancePlus® GC assay was used to predict ciprofloxacin susceptibility/ resistance in 66 *Neisseria gonorrhoeae*-positive DNA samples from patients, diagnosed in 2022-2023.

Results: The identified fluoroquinolone resistance rate in 2022-2023 was 68.2%. The majority of the cases were males in the age group 20-29 (50.8%), the most common mode of transmission was MSM (77%) and 17% of the cases with known HIV status were positive.

Conclusion: This study found a higher than before fluoroquinolone resistance rate (68.2%) in Bulgaria, following the trend in Europe. In the EU-EEA, ciprofloxacin resistance increased to 65.9% in 2022. Molecular testing for predicting susceptibility/ resistance is suitable for effective antimicrobial resistance surveillance and individualized treatment decisions.

Keywords:

Bulgaria, gonorrhea, *Neisseria gonorrhoeae,* ciprofloxacin, antimicrobial resistance

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INTRODUCTION

Gonorrhoea is a sexually transmitted infection (STI) caused by *Neisseria gonorrhoeae*. Typical genital infections present as urethritis among men and cervicitis among women, but a broad spectrum of clinical presentations and complications can occur[1]. The oropharynx, anorectum and conjunctiva are sites of extragenital infection. The most common complications include epididymitis and pelvic inflammatory disease (PID), and although the frequency of disseminated gonococcal infection (DGI) decreased over the last decades[1], discrete outbreaks of DGI have recently been documented[2]. Many infected women remain asymptomatic or have only minor symptoms, resulting in delayed diagnosis, complications and uninterrupted transmission[1].

The incidence of gonorrhoea has increased in many countries around the globe during the last decade. For example, in the European Union–European Economic Area (EU–EEA) notification rates per 100 000 population increased continuously between 2014 and 2019 (from 5.9 cases in 2014 to 10.4 cases in 2019). After a decrease in 2020 to 9.5 cases, notification rates increased again in 2021 to 11.7 cases and in 2022 to 17.9 cases per 100 000 population[3]. The year 2022 marks the highest number of gonorrhoea cases in the EU-EEA over the last decade and the majority

of countries (25/28) observed substantial increases in 2022 in comparison to the previous year[4].

In Bulgaria, in contrast to the general trend in the EU-EAA, the notification rates of gonorrhoea per 100 000 population over the last ten years have been steadily decreasing (from 2.3 cases in 2014 to 0.04 in 2021[4,5]). Although our country has still one of the lowest notification rates (less than one case per 100 000 population), in 2022, a significant increase of more than 50 % was observed in comparison to the previous year[4]. In 2023, the notification rates doubled once again[6].

Current recommendations for the treatment of gonorrhea are published in the "*2020 European guideline for the diagnosis and treatment of gonorrhoea in adults*" from the IUSTI, and include parenteral therapy with a third-generation cephalosporin and accompanying treatment with azithromycin for additional coverage[7].

Alternative treatment options are limited, given the widespread gonococcal resistance to numerous antibiotic classes. For example, owing to the worldwide increase of *N. gonorrhoeae* strains resistant to fluoroquinolones, these agents now have limited clinical usefulness. Nonetheless, fluoroquinolones are highly effective against infection caused by susceptible gonococci, with cure rates of at least 98% for all anatomic sites[8]. Although their empirical use should not be recommended, these drugs could have an important role in patients with contraindications to cephalosporins, in whom no other option is feasible. In such cases, a culture isolate should be obtained so that susceptibility testing can be performed. Indeed, the above strategy for permissive fluoroquinolone use has been adopted by the British guidelines[9].

Gonococcal antimicrobial resistance remains a major problem and surveillance is necessary to address this threat. Across the EU-EEA surveillance is conducted by the European Gonococcal Antimicrobial Surveillance Program (Euro-GASP). Euro-GASP provides important data at the European level but is dependent on gonococcal culture for susceptibility testing. The use of nucleic acid amplification tests (NAATs) as routine diagnostics is increasing in many countries as well as in Bulgaria. When compared to cultures, NAATs offer several advantages including greater sensitivity, higher throughput, self-collected samples, and potential identification of more than one pathogen in one sample[10,11]. Ideally, NAATs would combine identification and resistance detection.

However, the complexity of *N. gonorrhoeae* resistance determination presents a major barrier for test development. For example, resistance to third-generation cephalosporins is predicted by four genes (*penA, penB, mtrR* and *ponA*), with the main determinant being the mosaic *penA* alleles[12]. Predicting azithromycin resistance necessitates the detection of *mtrD/mtrR* promoter mosaic 2 or semi-mosaic *mtrD* and not only 23S rRNA target mutations, which were previously the main cause of azithromycin resistance[13].

Currently, the prediction of resistance based on genetic characterization in *N. gonorrhoeae* is accurate only for fluoroquinolones, as the absence of mutations in serine codon 91 of the *gyrA* gene predicts susceptibility. A commercial test that combines pathogen identification with quinolone resistance determination is now available in Australia and Europe (SpeeDx ResistancePlus GC)[14]. The utilization of those tests in clinical settings allows the implementation of the resistance-guided therapy for gonococcal treatment with fluoroquinolones, which has already demonstrated success in some studies[15,16]. The results suggest that in the right patient population, fluoroquinolones could be very useful for treatment and could slow down antimicrobial resistance generation by reducing exposure to cephalosporins[15,16].

In our previous study from 2018-2021[17], a high prevalence of fluoroquinolone resistance in Bulgaria (59%) was detected by validated assays targeting resistance mutations in the *gyrA* and *parC* genes. This study aimed to make a comprehensive analysis of the rates of gonococcal fluoroquinolone resistance and epidemiological data of the cases in Bulgaria for 2022-2023 and to compare it with the results of previous years and the general trends in EU-EEA.

METHODS AND MATERIALS

Study population

From January 2022 to December 2023, a total of 1179 individuals (median age 30; 73.6% males and 26.4% females) attending the Center for Sexual Health

"CheckPointSofia" for voluntary and confidential HIV testing were referred to the National Center of Infectious and Parasitic Diseases (NCIPD, Sofia, Bulgaria) for gonorrhea testing based on symptoms and high-risk sexual behavior.

The routine testing was performed with Real-Time PCR (Neisseria gonorrhoeae Real-TM assay, Sacace Biotechnologies srl, Como, Italy). Following the diagnostic testing, DNA samples were stored frozen at −80 °C at NCIPD for further analysis.

During the study period, 66 *N. gonorrhoeae*-positive DNA samples (one sample per gonorrhea patient/episode) were obtained and confirmed with culture (n = 34) or with the *porA/opa* assay[18] (n = 32). When no isolate was available, confirmation by repeat testing with a PCR targeting another genetic sequence was imperative due to the suboptimal specificity of commercial assays, with the *porA/opa* assay being highly suitable for that purpose (clinical sensitivity and specificity of 100% and 99.3%, respectively). Phenotypic susceptibility testing of the corresponding *N. gonorrhoeae* isolates was performed by determining the minimal inhibitory concentrations (MIC), using gradient strips (Liofilchem srl, Italy) and interpreting the results according to EUCAST. The *N. gonorrhoeae*-positive DNA samples included genital (n = 33), pharyngeal (n = 10), ano-rectal (n = 21), and eye swabs (n = 2).

Detection of ciprofloxacin susceptibility/resistance markers

All stored DNA extracts from *N. gonorrhoeae*-positive samples were retrospectively analyzed by the commercial CE-IVD/IVDR certified ResistancePlus® GC assay (SpeeDx Pty Ltd, Sydney, Australia). The kit is intended to simultaneously detect the bacterium *N. gonorrhoeae* and the *gyrA* S91 (wild type) or *gyrA* S91F (mutant) markers that are associated with susceptibility or resistance to the fluoroquinolone antibiotic, ciprofloxacin. The assay was performed, according to the manufacturer's instructions on LightCycler® 480 Instrument II (LC480 II, Roche). All data were analyzed and reported using the ResistancePlus® GC (LC480) v1.0 analysis software.

Ethics and informed consent:

Written informed consent was obtained from all patients for epidemiological data collection and microbiological sample testing as required by the National Law and Ethics Committee at the National Center of Infectious and Parasitic Diseases.

RESULTS

***Neisseria gonorrhoeae*-positive cases**

The prevalence of *N. gonorrhoeae* among the referred individuals was 5.6% (95% CI 4.2% to 6.9%). The median age of the patients with confirmed gonorrhoea was 29 (age range 19–49). The male-to-female ratio was 21:1. The largest proportion of

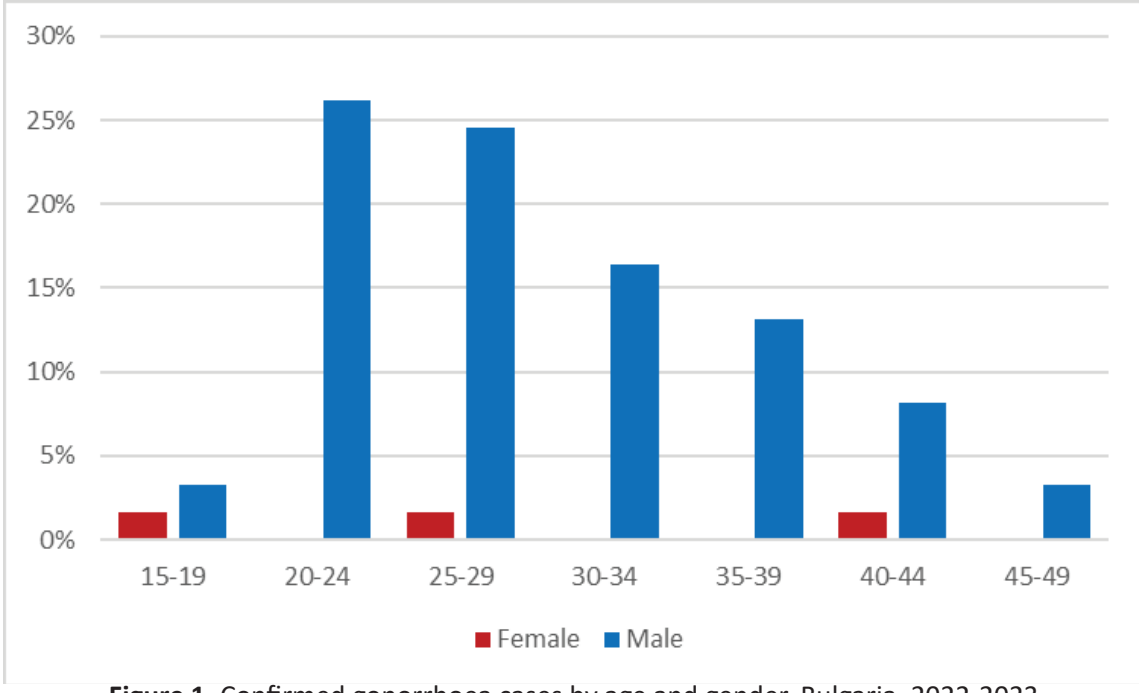

Figure 1. Confirmed gonorrhoea cases by age and gender, Bulgaria, 2022-2023

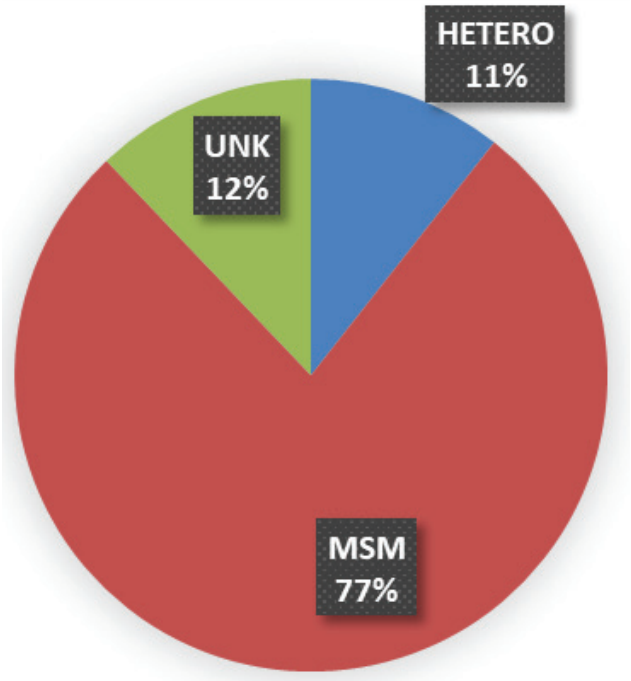

Figure 2. Confirmed gonorrhoea cases by transmission category, Bulgaria, 2022-2023

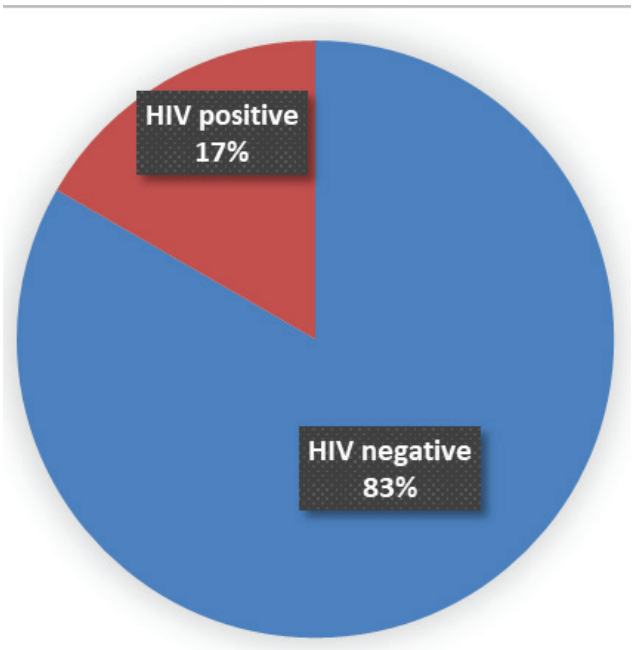

Figure 3. Confirmed gonorrhoea cases with known HIV status (n=60), Bulgaria, 2022-2023

cases was among males in the age group 20–24 years (26.2% of cases), followed by males in the age group 25–29 years (24.6%), (Figure 1).

Among the confirmed gonorrhoea cases, 77% were reported as men who have sex with men (MSM), 11% were reported as heterosexuals (57% males and 43% females), and 12% had no information on the mode of transmission (Figure 2).

Data on the HIV status of gonorrhoea cases reported in 2022 and 2023 were provided for 60 cases (91%). Among cases with known HIV status, 17% were HIV-positive (Figure 3).

Phenotypic susceptibility testing

According to the performed phenotypic susceptibility testing, of the available 34 *N. gonorrhoeae* isolates, 14 were susceptible and 20 were resistant to ciprofloxacin.

ResistancePlus® GC assay for ciprofloxacin resistance/susceptibility prediction

Detection of *N. gonorrhoeae*. The ResistancePlus® GC assay correctly identified all 66 DNA samples as *Neisseria gonorrhoeae*-positive.

Detection of *gyrA* S91 (wild type) and *gyrA* S91F (mutant). The *gyrA* S91 wild type was identified in 20 (30.3%) of the *N. gonorrhoeae*-positive samples, *gyrA* S91F was detected in 45 (68.2%) of the samples, and indeterminate *gyrA* results were obtained for one (1.5%) sample.

For the 34 *N. gonorrhoeae*-positive samples paired with *N. gonorrhoeae* isolates, ResistancePlus® GC assay detected GyrA S91F in all 20 samples resistant to ciprofloxacin according to the phenotypic susceptibility testing; and GyrA wild type was established in the other 14 samples. Accordingly, both the sensitivity and specificity of the assay for phenotypic prediction of the antimicrobial resistance was 100%.

The detected *gyrA* S91F (ciprofloxacin resistance) rate was 58.8% in the DNA samples paired with culture and 78.1% in the samples without confirmed culture (Table 1).

DISCUSSION

In this study, the prevalence of *N. gonorrhoeae* infections among individuals attending the Center for Sexual Health "CheckPointSofia" for voluntary and confidential HIV testing was estimated and gonococcal fluoroquinolone resistance rates were

Table 1. Investigation of *N. gonorrhoeae*-positive DNA samples (n=66) by ResistancePlus® GC assay for ciprofloxacin resistance/susceptibility prediction, Bulgaria, 2022-2023

	ResistancePlus® GC assay			
Samples (n)	**N. gonorrhoeae (*opa* + *porA*)**	***gyrA* S91 (wild type)**	***gyrA* S91F (mutant)**	**Intermediate**
N. gonorrhoeae-positive samples (66)	66	20 (30.0%)	45 (68.2%)	1 (1.5%)
• *Samples with paired culture (34)*	34	14 (41.2%)	20 (58.8%)	-
• *Samples without confirmed culture (32)*	32	6 (18.8%)	25 (78.1%)	1 (3.1%)

investigated by detection of *gyrA* S91 wild type or *gyrA* S91F mutant (ciprofloxacin susceptible or ciprofloxacin resistant, respectively). The prevalence of *N. gonorrhoeae* infection was 5.6% (95% CI 4.2% to 6.9%) and the detected fluoroquinolone resistance rate was 68.2%. Ciprofloxacin used for the treatment of gonococcal infections has the advantages of providing effective oral treatment of both urogenital and extragenital infections, limited side effects, and reduced selective pressure for emergence and spread of resistance to dual therapy with ceftriaxone and azithromycin[19]. Nevertheless, its current use in Bulgaria should be considered only after confirmed susceptibility.

Data on gonococcal antimicrobial resistance from the Balkan Peninsula countries is available only from Bulgaria and Greece. The detected fluoroquinolone resistance rate in Bulgaria was higher compared to neighboring Greece (66%)[3] and it had substantially increased in comparison to the previous Bulgarian study in 2021 (59%)[17]. The increase in Bulgaria is following the overall trend in Europe, where the proportion of isolates showing resistance to fluoroquinolones noticeably increased during the last several years: 65.9% in 2022 compared to that observed in 2020 (57.7%)[20].

Regarding the obtained epidemiological data, it was found that the average age of patients with gonococcal infection is decreasing in comparison to previous observations (from an average of 32 years in 2019[21] to 29 years in the current study), with almost one-third of patients identified to be less than 25 years old (31.2%). It is well known that young adults bear the highest burden of STIs . This fact may be attributed to several factors, i.e. riskier sexual behaviors, lack of awareness, social stigma, and/or limited access to sexual health services[22]. Promoting sexual education, regular testing, and safe practices are crucial in addressing this trend. In the present study, the male-to-female ratio was very high (21:1), and only several cases of gonorrhea in women were identified. The comprehensive data regarding the male-to-female ratio for Bulgaria for the last ten years are close to the EU-EEA averages (ranging from 3.5 in 2014 to 4.7 in 2020). From 2021 on, a predominance of male gonococcal patients started, exceeding a ratio of 10:1, and even - lack of confirmed cases in women[3]. Although our neighboring countries (such as Greece, Serbia and Romania) also report high male-to-female ratios (26.7:1, 12.2:1 and 10.5:1, respectively[3]) the fact is concerning, since women with asymptomatic gonorrhea may not seek healthcare services. While gonorrhea often may be asymptomatic in women, it can still lead to serious complications if left untreated[1]. Encouraging regular testing and awareness about the risks of untreated infections is crucial and should be considered in local guidelines and programs. Obtaining epidemiological data regarding transmission and HIV status for gonorrhoea patients can be challenging due to stigma and privacy concerns by patients and limited resources for surveillance and data collection by health professionals. Efforts to improve data collection should involve collaboration between healthcare

providers, laboratories, and public health agencies. In the present study, data on the mode of transmission were collected in 88% of cases, and HIV status was established in 91%. Transmission data show that in Bulgaria, the largest burden of gonococcal infection goes to MSM (77% of all cases), and epidemiological interventions to reduce incidence in this group would have an impact on enhancing the control and prevention of gonococcal infection. Of all cases with known HIV status, 17% were positive, which is above the average proportion in EU-EEA (12%[4]). The prevalence of HIV co-infection among people with gonorrhoea is an important concern. Studies have shown that the discharge of HIV in body secretions of people co-infected with gonorrhoea is significantly higher , increasing the risk of HIV transmission by 3 to 5 times[23,24]. While specific percentages may differ across populations, understanding this co-infection is crucial for effective prevention and treatment strategies.

The significant increase in gonococcal notification rates in the last two years in Bulgaria, although on a smaller scale in terms of absolute number of cases, gives grounds for public health concern. Gonorrhoea has been recognized as a significant public health problem internationally and prevention, diagnosis, and therapy strategies are used to reduce the burden of the disease[25]. Ongoing also is the improvement of antimicrobial stewardship through the implementation and use of better surveillance systems to detect antimicrobial resistance in *N. gonorrhoeae* and to inform locally appropriate treatment[17]. Sensitive and specific molecular assays for the prediction of *N. gonorrhoeae* antimicrobial resistance are needed, both to inform personalized treatment and for antimicrobial resistance surveillance, which is further highlighted by the WHO global action plan[26].

Compared to cultural diagnostics and phenotypic susceptibility testing, molecular assays such as the ResistancePlus® GC assay have many advantages, including superior sensitivity and high specificity, shorter turnaround time, automation, high throughput and potential for use as rapid tests[27]. However, a limitation of the molecular assays for the detection of resistance determinants is that they cannot detect new determinants of antimicrobial resistance. They cannot provide a complete profile of antimicrobial resistance with minimum inhibitory concentrations of the antimicrobials, either[11]. In addition, it can be very labor-intensive to detect more than one resistance determinant.

In the current study, the commercially available ResistancePlus® GC assay showed high sensitivity and specificity for the detection of *N. gonorrhoeae*, as compared with the confirmatory *porA/opa* assay[18]. The assay showed also an excellent ability to detect and distinguish *gyrA* S91 wild type and *gyrA* S91F and to predict ciprofloxacin resistance/ susceptibility compared with phenotypic testing. The results support the intended use of the assay for both detecting the ciprofloxacin resistance/susceptibility and confirming positive results of commercial PCR assays for gonorrhoea diagnostics, when no isolate is available. Furthermore, it can be effectively used for antimicrobial resistance surveillance and individualized treatment with ciprofloxacin, which is easily accessible and administered as an oral regimen[28]. Unlike our previous study where both *gyrA* and *parC* genes were investigated, in the present study only *gyrA* was targeted because it has proven to be the most effective target for the detection of ciprofloxacin resistance, i.e. mutations in *parC* are never found without concurrent mutations in *gyrA*[17,27].

However, one sample (1.5%) remained indeterminate for *gyrA*, which is likely due to low *N. gonorrhoeae* load in the clinical DNA sample and/or inhibition and cross-reactions with other Neisseria species[29] In particular, extragenital sites (such as the case) are challenging for molecular resistance prediction, because these sites frequently harbor non-gonococcal Neisseria species as commensals and many identical or very similar DNA sequences from other commensal species, including resistance determinants[30].

Finally, a statistically significant difference (p=0.049) was found in the resistance rates in DNA samples paired with culture (58.8%) and DNA samples without confirmed culture (78.1%), making the comprehensive fluoroquinolone resistance rate higher than conventionally reported (68.2%). This is of utmost importance because conventional gonococcal antimicrobial resistance surveillance is done only by phenotypic testing. Given the fact

that in EU/EEA (Bulgaria included) about half of the confirmed gonorrhoea cases are not cultured and no phenotypic testing is performed, it should be acknowledged that conventional surveillance is non-comprehensive and antimicrobial resistance could be much more prevalent than reported. This underlines the importance of a national laboratory network able perform culture studies, alongside with molecular studies in order to obtain comprehensive data about the spread of gonococcal antimicrobial resistance in our country.

CONCLUSION

The fluoroquinolone resistance rate found in this study (68.2%) was significantly higher than that observed a few years ago (59%). The increased prevalence of fluoroquinolone resistance in Bulgaria follows the general trend in Europe during the recent years. In EU-EEA the proportion of isolates showing resistance to ciprofloxacin substantially increased: 65.9% in 2022 as compared to 62.8% and 57.7% in 2021 and 2020 respectively. The molecular testing for predicting ciprofloxacin susceptibility/ resistance in gonococcal infections is very suitable for supporting effective gonococcal antimicrobial resistance surveillance, and simultaneously with diagnostics - individualized treatment decisions, thus reducing exposure to unnecessary empiric therapy and slowing the spread of resistance.

REFERENCES

1. Bennett, J. E., Dolin, R. & Blaser, M. J. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases E-Book: 2-Volume Set. (Elsevier health sciences, 2019).
2. Tang, E. C. et al. Characterizing the Rise of Disseminated Gonococcal Infections in California, July 2020-July 2021. Clin. Infect. Dis. 76, 194-200 (2023). https://doi.org/10.1093/cid/ciac805
3. European Centre for Disease Prevention and Control. Surveillance atlas of infectious diseases. https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases (2024).
4. European Centre for Disease Prevention and Control. Gonorrhoea. In: ECDC. Annual epidemiological report for 2022. Stockholm: ECDC. (2024).
5. European Centre for Disease Prevention and Control. Gonorrhoea. In: ECDC Annual Epidemiological Report for 2017. Stockholm: ECDC. 1-5 (2017).
6. National Center of Public Health and Analyses & MoH. Annual analysis of infectious diseases in Bulgaria. https://ncpha.government.bg/index/3031-godishni-danni.html (2024).
7. Unemo, M. et al. 2020 European guideline for the diagnosis and treatment of gonorrhoea in adults. Int. J. STD AIDS (2020). https://doi.org/10.1177/0956462420949126
8. Unemo, M., Golparian, D. & Eyre, D. W. Antimicrobial resistance in Neisseria gonorrhoeae and treatment of gonorrhea. Neisseria gonorrhoeae methods Protoc. 37-58 (2019). https://doi.org/10.1007/978-1-4939-9496-0_3
9. Fifer, H., Saunders, J., Soni, S., Sadiq, S. T. & FitzGerald, M. 2018 UK national guideline for the management of infection with Neisseria gonorrhoeae. Int. J. STD AIDS 31, 4-15 (2020). https://doi.org/10.1177/0956462419886775
10. Workowski, K. A. Sexually transmitted infections treatment guidelines, 2021. MMWR. Recomm. Reports 70, (2021). https://doi.org/10.15585/mmwr.rr7004a1
11. Meyer, T. & Buder, S. The Laboratory Diagnosis of Neisseria gonorrhoeae: Current Testing and Future Demands. Pathog. (Basel, Switzerland) 9, (2020). https://doi.org/10.3390/pathogens9020091
12. Golparian, D. & Unemo, M. Antimicrobial resistance prediction in Neisseria gonorrhoeae: current status and future prospects. Expert Rev. Mol. Diagn. 22, 29-48 (2022). https://doi.org/10.1080/14737159.2022.2015329
13. Golparian, D. et al. Antimicrobial-resistant Neisseria gonorrhoeae in Europe in 2020 compared with in 2013 and 2018: a retrospective genomic surveillance study. The Lancet Microbe 10, 1-11 (2024).
14. Hadad, R. et al. Evaluation of the SpeeDx ResistancePlus® GC and SpeeDx GC 23S 2611 (beta) molecular assays for prediction of antimicrobial resistance/susceptibility to ciprofloxacin and azithromycin in Neisseria gonorrhoeae. J. Antimicrob. Chemother. 76, 84-90 (2021). https://doi.org/10.1093/jac/dkaa381
15. Allan-Blitz, L. T., Adamson, P. C. & Klausner, J. D. Resistance-Guided Therapy for Neisseria gonorrhoeae. Clin. Infect. Dis. 75, 1655-1660 (2022). https://doi.org/10.1093/cid/ciac371
16. Klausner, J. D. et al. Resistance-Guided Treatment of Gonorrhea: A Prospective Clinical Study. Clin. Infect. Dis. 73, 298-303 (2021). https://doi.org/10.1093/cid/ciaa596
17. Philipova, I., Levterova, V., Simeonovski, I. & Kantardjiev, T. High rate of fluoroquinolone resistant Neisseria gonorrhoeae detected by molecular surveillance of antimicrobial resistance determinants in Bulgaria. Biotechnol. Biotechnol. Equip. 36, 942-948 (2022). https://doi.org/10.1080/13102818.2022.2146532
18. Goire, N., Nissen, M. D., LeCornec, G. M., Sloots, T. P. & Whiley, D. M. A duplex Neisseria gonorrhoeae real-time polymerase chain reaction assay targeting the gonococcal porA pseudogene and multicopy opa genes. Diagn. Microbiol. Infect. Dis. 61, 6-12 (2008). https://doi.org/10.1016/j.diagmicrobio.2007.12.007
19. Melendez, J. H. et al. Can ciprofloxacin be used for precision treatment of gonorrhea in public STD clinics? Assessment of ciprofloxacin susceptibility and an opportunity for point-of-care testing. Pathogens 8, 189 (2019). https://doi.org/10.3390/pathogens8040189
20. European Centre for Disease Prevention and Control. Gonococcal antimicrobial susceptibility surveillance in the European Union/European Economic Area - Summary of results for 2022. (2024).
21. Philipova, I. et al. PREVALENCE OF NEISSERIA GONORRHOEAE AND MYCOPLASMA GENITALIUM IN BULGARIAN POPULATION FROM SOFIA MUNICIPALITY OF REPRODUCTIVE AGE, 2018-2019. Probl. Infect. Parasit. Dis. 49, 20-26 (2021). https://doi.org/10.58395/pipd.v49i2.56
22. Tao, X., Ghanem, K. G., Page, K. R., Gilliams, E. & Tuddenham, S. Risk factors predictive of sexually transmitted infection diagnosis in young compared to older patients attending sexually transmitted diseases clinics. Int. J. STD AIDS 31, 142-149 (2020). https://doi.org/10.1177/0956462419886772
23. Kalichman, S. C., Pellowski, J. & Turner, C. Prevalence of sexually transmitted co-infections in people living with HIV/

AIDS: systematic review with implications for using HIV treatments for prevention. Sex. Transm. Infect. 87, 183 LP - 190 (2011). https://doi.org/10.1136/sti.2010.047514

24. Lami, F. et al. Incidence and spontaneous clearance of gonorrhea and chlamydia infections among men who have sex with men : a prospective cohort study in. 1-9 (2024) doi:10.3389/fpubh.2024.1348686. https://doi.org/10.3389/fpubh.2024.1348686
25. World Health Organization. Global health sector strategies on, respectively, HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030. (2022).
26. World Health Organization. Global action plan to control the spread and impact of antimicrobial resistance in Neisseria gonorrhoeae. https://apps.who.int/iris/%0Ahandle/10665/44863.
27. Jensen, J. S. & Unemo, M. Antimicrobial treatment and resistance in sexually transmitted bacterial infections. Nat. Rev. Microbiol. (2024) doi:10.1038/s41579-024-01023-3. https://doi.org/10.1038/s41579-024-01023-3
28. Hadad, R., Cole, M. J., Ebeyan, S., Jacobsson, S. & Tan, L. Y. Evaluation of the SpeeDx ResistancePlus. J. Antimicrob. Chemother. 2611, 84-90 (2021). https://doi.org/10.1093/jac/dkaa381
29. Allan-Blitz, L.-T. et al. Improved determination of Neisseria gonorrhoeae gyrase A genotype results in clinical specimens. J. Antimicrob. Chemother. 74, 2913-2915 (2019). https://doi.org/10.1093/jac/dkz292
30. Golparian, D. & Unemo, M. Antimicrobial resistance prediction in Neisseria gonorrhoeae: current status and future prospects. Expert Rev. Mol. Diagn. 22, 29-48 (2022). https://doi.org/10.1080/14737159.2022.2015329