

GONOCOCCI – PATHOGENS OF GROWING IMPORTANCE. PART 1. CURRENT DATA ON DIAGNOSTICS, GENOTYPING AND THERAPY.

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Abstract: *Neisseria gonorrhoeae* is an aetiological agent of gonorrhoea, which is a sexually transmitted disease (STD), a public health challenge worldwide. Gonorrhoea is undoubtedly a serious health risk, especially for women, due to its often asymptomatic course and the possibility of upper genital tract complications such as pelvic inflammatory disease (PID), which can result in infertility. The data show that the diagnostic method for *N. gonorrhoeae* should be specific, simple, sensitive, rapid and inexpensive. Currently, phenotypic identification methods have been dominated by NAAT methods, which allow detection and identification of gonococcus directly in the clinical specimen. However, up today molecular methods do not allow full determination of drug susceptibility.

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Keywords: AMR, diagnosis, gonorrhoea, molecular typing, STD

1. Introduction

Neisseria gonorrhoeae is a Gram-negative diplococcus called gonococcus. It belongs to the genus *Neisseria* and the family *Neisseriaceae*. *N. gonorrhoeae* is an obligate human pathogen, the aetiological agent of gonorrhoea. Another human pathogenic species of this family is *N. meningitidis*. In contrast, eight commensal species are part of the human microbiome (*N. cinerea*, *N. elongata*, *N. flavescens*, *N. lactamica*, *N. mucosa*, *N. polysaccharaea*, *N. sicca*, *N. subflava*), colonizing mainly the mucous membranes of the upper respiratory tract, genitourinary tract, and anal area. Commensal *Neisseria* species can be opportunistic pathogens for humans (Humbert and Christodoulides 2019).

Gonorrhoea is a sexually transmitted disease that spreads through direct contact with secretions. Infection can also occur through vertical transmission from infected mother to child during childbirth. Gonorrhoea is an acute or chronic disease that develops mainly in

the columnar and transitional epithelium of the lower genitourinary tract. It less commonly affects the anus, pharynx, conjunctiva, or cornea. The squamous epithelium is less susceptible to infection (Bignell *et al.* 2013).

In Poland, gonococcal infection has been subject to mandatory reporting since 1948. This time, the recorded incidence was about 180 cases per 100,000 people. Polish data on gonorrhoea epidemiology come from annual reports published by the National Institute of Public Health – National Institute of Hygiene (NIZP PZH, www.pzh.gov.pl). Since 2014, data on gonococci isolated in Poland are presented in ECDC reports of the Euro-GASP program (ECDC 2022). Since the 1980s, there has been a decline in reported incidence, from 87.6 per 100,000 in 1981 to 9.7 in 1992 and less than 2 per 100,000 in 2000 (Młynarczyk-Bonikowska *et al.* 2014). In 2014, only 458 cases were reported in Poland, with an incidence of 1.19 per 100,000, and in 2021, only 287 cases of gonorrhoea were reported, with an incidence of 0.75 per 100,000. After the Covid-19 pandemic

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in 2022, 630 cases of gonorrhoea were reported, with an incidence of 1.67 per 100,000. In 2023, there was twice an increase in cases – 1372, indicating 3.64 per 100,000. But still, the number of registered gonorrhoea in Poland is low compared to the European average. However, the Polish data may be underestimated significantly (NIZP PZH – PIB 2022, ECDC 2022). Globally, the number of cases is increasing. According to WHO, more than 82.4 million cases of gonorrhoea were reported in 2020, which ranks it as the second most common bacterial sexually transmitted disease after *Chlamydia trachomatis* infection (WHO 2021). According to epidemiological reports from the E.U. area, incidence has increased from 6.6 per 100,000 in 2009 to 31.6 per 100,000 in 2019 (ECDC 2023). In the U.S., an even higher incidence rate was found in 2020: 206.5 per 100,000, and there was a further increase of 4.6% in 2021. This may be due to better epidemiological surveillance (Rowley *et al.* 2019; WHO 2021, ECDC 2023).

The clinical picture of *N. gonorrhoeae* infection in men and women differs fundamentally. The first symptoms of infection usually appear within a week after sexual intercourse (usually between 3–7 days). In men, acute urethritis develops that is difficult to overlook, with the presence of a mucopurulent discharge (> 80% of cases), as well as dysuria (> 50%). The asymptomatic infections in men are rare (< 10%). In untreated patients, a complication of prolonged infection can be testicular inflammation, epididymitis, and prostatitis (Bignell *et al.* 2013). In women, *N. gonorrhoeae* infection mainly affects the cervical canal or urethra, causing gonococcal cervicitis or urethritis, respectively. The disease can present as a change in the type of vaginal discharge (about 50%), lower abdominal pain (< 20%), or dysuria (10–15%). In women, the symptoms of infection are much milder than in men. In more than 50% of cases, the infection in women is asymptomatic, resulting in a lack of recognition and treatment and the development of different complications. The main sequel of gonorrhoea infection in women worth to be listed is Bartholin's gland inflammation, pelvic inflammatory disease (PID) involving the endometrium, fallopian tubes and ovaries, perianal abscesses, ovarian abscess, tubal factor infertility (TFI), ectopic pregnancy, Fitz-Hugh-Curtis syndrome (FHCS), perihepatitis. An unrecognized infection also facilitates the transmission of the pathogen to sexual partners. An ascending gonococcal infection involving the upper urogenital tract develops in 10–20% of cases. Consequently, it can be threatened with disseminated gonococcal infection (DGI) and gonococcal arthritis in both sexes (Mroczkowski 1998; Bignell *et al.* 2013; Workowski *et al.* 2021). Gonococcal infection may be the reason for infertility globally (Chemaitelly *et al.* 2021).

Noteworthy is the significant difference in the number of strains isolated from women and men, possibly due to differences in the clinical picture of this infection in both sexes and its epidemiology. According to ECDC's 2020 reports (ECDC 2022), between 2014 and 2020, among all strains analyzed for resistance, isolates from women ranged from 14.7% to 18.2%. In 2019, the reporting of gonorrhoea cases in men and women in Europe was 48 per 100,000 population and 16 per 100,000 population, respectively, resulting the male-to-female ratio of gonorrhoea reported cases 3 to 1. In Poland, the male-to-female ratio of reported gonorrhoea was almost ten times higher, as high as 29 to 1 (ECDC 2023). It is noted that gonococcal strains isolated from men come from two groups of patients – heterosexual men and men who have sex with men (Xiridou *et al.* 2015; Sánchez-Busó *et al.* 2022). As mentioned above, there has been an increase in gonococcal infections between 2010 and 2019 among both men and women, but the highest increase has been in the group of men who have sex with men (ECDC 2023). The observed significant differences in the detection of gonococcal infections in men and women in Poland compared to European data may, therefore, be the result of an underestimation of the prevalence of Polish infections among heterosexual partners and the more frequent performance of diagnostic tests in men who have sex with men due to increased awareness of such risks in these individuals.

In both sexes, the picture of gonococcal infection is different. The often asymptomatic or nonspecific course of infection in the vagina and cervix can be easily overlooked by female patients. Gonococcal infections are more easily detected, diagnosed, and treated correctly in men who report to specialists with severe symptoms of infection. The question arises as to what other factors may be associated with the lower rates of infection recorded in women. The issue requires further research based on epidemiological and clinical patient data.

2. Diagnosis of gonococcal infection

The diagnosis of gonorrhoea is based on the detection of gonococci or their genetic material in swabs taken from the genitourinary tract, anus, pharynx, and conjunctival sac, using culture or molecular methods based on nucleic acid amplification NAATs (*Nucleic Acid Amplification Tests*) (Fig. 1). The gold standard for diagnosis of gonococcal infection for many years was a culture of *N. gonorrhoeae*, which has now been replaced mainly by molecular methods (CDC 2014). However, it should be noted that at the current stage of diagnostic development, only classical microbiological culture methods allow the complete determination of drug susceptibility of clinical strains of *N. gonorrhoeae*.

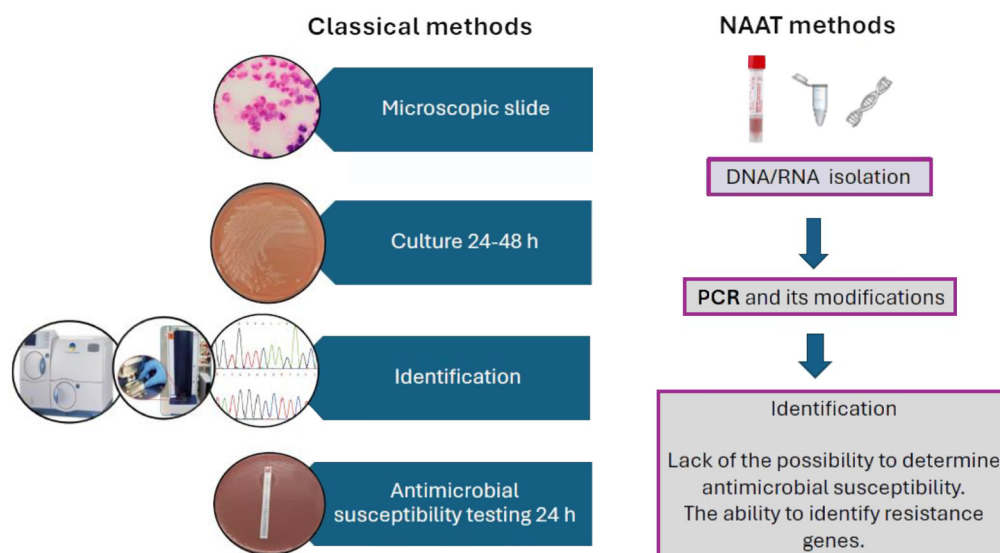


Fig. 1. Methods used in the diagnosis of *N. gonorrhoeae*.

2.1. Microscopy

Microscopically, *N. gonorrhoeae* is a Gram-negative diplococcus with a diameter of 0.6 to 1 μm , arranged in characteristic pairs resembling coffee beans. It does not have a capsule and does not produce spores.

Direct Gram-stained microscopic preparation should always be performed for purulent or mucopurulent urethral discharge in men, a symptom of gonococcal urethritis. Observing numerous polymorphonuclear leucocytes (PMNs) and Gram-negative gonococci within or closely associated with some PNMs in the microscopic image is diagnostically significant and allows us to identify gonococcal infection in clinically symptomatic men. In this case, if the preparation is appropriately performed and evaluated by an experienced microbiologist, the microscopic examination has high sensitivity (90–95%) and specificity (95–100%) (Janda and Gaydos 2007). However, direct microscopic examination has shown low sensitivity (<55%) in diagnosing asymptomatic men. It is also the case for the diagnosis of cervicitis (sensitivity < 50%) and rectal inflammation (sensitivity < 40%). Hence, direct examination of Gram-stained preparation of smears from the vagina, cervical canal, pharynx, or rectum has no diagnostic significance (Bignell *et al.* 2013). Methylene blue (M.B.) stain may also establish the intracellular location of gonococci in leucocytes (PMNs) of male urethral exudate accompanying gonococcal urethritis (Workowski 2021).

2.2. Culture methods

N. gonorrhoeae is an aerobic bacterium with high nutritional requirements, is oxidase- and catalase-positive and oxidizes only glucose to acid. It requires an energy source of glucose, pyruvate or lactate and

the presence of cysteine to grow on culture media. Because of changes in metabolic pathways, some isolates show special growth requirements for amino acids, purines, and pyrimidines (Ng and Martin 2005; Quillin *et al.* 2018).

The culture of gonococci requires special nutrient-rich growth media and selective media that inhibit the growth of other microorganisms on human mucous membranes. The pathogen grows at a temperature of 35–37°C in an atmosphere with up to 5–10% CO_2 and increased humidity. Gonococci outside the host are sensitive to environmental factors. They die within hours at temperatures above 40°C. The bacteria are sensitive to drying, light and low concentrations of disinfectants. Collected clinical materials should be inoculated into culture media as soon as possible. Culture media with added animal protein are used, primarily chocolate agar, modified Thayer-Martin (MTM) medium, Roiron medium, Martin-Lewis (ML) medium, GC-Lect agar and New York City (NYC) agar. MTM, ML, and GC-Lect media based on chocolate agar are supplemented with growth factors (e.g., IsoVitalex, PolyVitex, and Kellogg supplement). NYC medium is a transparent medium based on peptone, corn starch with yeast extract, horse plasma and lyophilized horse erythrocytes. Roiron medium contains, among others, enzymatic casein hydrolysate, horse plasma and glucose. Antibiotics and antimycotics are added to selective media to inhibit the growth of commensal microorganisms present in clinical materials. Vancomycin and colimycin inhibit Gram-positive and Gram-negative microorganisms, respectively, trimethoprim *Proteus* species, while nystatin, amphotericin B or anisomycin have antifungal effects (Janda and Gaydos 2007).

For urogenital tract specimens, cultures for *N. gonorrhoeae* are usually conducted in parallel on Columbia

agar with the addition of 5% sheep blood, chocolate agar without any selection factors, and selective medium with the selection factors mentioned above. An example of a selective medium is chocolate agar with polyvitex with the addition of vancomycin, colimycin, amphotericin and trimethoprim (Chocolate + PVS + VCAT). Basal media without the addition of antibiotics are necessary to ensure the growth of those few *N. gonorrhoeae* strains that show sensitivity to antibiotics present in selective media. On chocolate agar, gonococci grow after a 24- to 48-hour culture as small, round, smooth colonies of greyish-beige colour or clear and shiny, 0.5 to 2 mm in diameter. Their morphology depends on the number of *in vitro* passages. Fine, elevated colonies are associated with the production of pili.

Further morphotypes appear during passages on solid media, variants of larger diameter, flat colonies, often with irregular edges. Under the influence of phase changes of Opa surface proteins, dull and iridescent variants appear. Due to the different types of colonies, cultures on chocolate agar can give the impression of a mixed culture (Janda and Gaydos 2007).

2.3. Species identification

After a 24–48 hour incubation, the characteristic small grey-beige or transparent colonies of *N. gonorrhoeae* grown on chocolate agar or selective medium require isolation to identify the species level and determine drug susceptibility. Identification by classical microbiological methods is based on the evaluation of colony morphology on chocolate medium, finding of Gram-negative diplococci in a Gram-stained slide, confirmation of the ability to produce oxidase, catalase, and the results of biochemical tests, considering carbohydrate degradation and enzymatic activity. The bacterium oxidizes glucose and does not metabolize maltose, sucrose, fructose or lactose. It shows proline aminopeptidase (PIP) activity, while it does not have β -galactosidase nor γ -glutamylaminopeptidase activity, unlike *N. lactamica* and *N. meningitidis*. The specific enzymatic activity is used to identify *N. gonorrhoeae* in manual enzyme chromogenic assays such as API NH – bioMérieux, Gonochek II – TCS Biosciences, *Neisseria* PET – BioConnections (Alexander *et al.* 2005; Meyer and Buder 2020). The automated system VITEK 2 (bioMérieux) also uses biochemical traits for species identification of *N. gonorrhoeae*. A modern identification method by which gonococci can be reliably identified is MALDI-TOF (*Matrix-Assisted Laser Desorption/Ionization – Time Of Flight*) mass spectrometry (Ilin *et al.* 2009; Cassagne *et al.* 2011; Carannante *et al.* 2015; van Belkum *et al.* 2013; Morrel *et al.* 2018), which analyses the protein profile, mainly of the microbe's ribosomal proteins. 16S rRNA gene sequencing can also be used

for species identification of *N. gonorrhoeae*. A fragment of the *rplF* gene (413 pz), encoding the L6 50S ribosomal protein, is a suitable genetic target for differentiating this species (Bennett *et al.* 2014).

2.3.1. VITEK 2

VITEK 2 Compact (bioMérieux, France) is an automated system commonly used by clinical laboratories for routine microbiological diagnosis. Using disposable NH ID cards with 30 different substrates for biochemical and enzymatic reactions, identification of *N. gonorrhoeae* is possible. NH ID cards provide reliable identification results for six clinically relevant species of the *Neisseria* genus: *N. gonorrhoeae*, *N. meningitidis*, *N. cinerea*, *N. elongata*, *N. lactamica* and *N. sicca*. The panel of biochemical reactions assays the distribution of sugars and the activity of 7 peptidases: arginine, lysine, leucine, tyrosine, L-proline, phenylalanine and alanine-phenylalanine-proline. The assay requires multiplying the strain and making a suspension of bacteria with a density equivalent to 2.7–3.3 of the McFarland scale from fresh, preferably from an 18–24 hour culture on chocolate medium. The identification process takes about 6 hours. Results are generated through the system's database, with specific confidence levels and percentage probability ranges reflecting the compliance level with species-typical responses. Ranges $\geq 96\%$, 93–95%, 89–92%, and 85–88% correspond to species identification at excellent, very good, good, and acceptable levels. The “low distinction” results when 2 or 3 species show an identical numerical pattern. In such cases, the percentage probability of species identification is subjected to the manual selection of one of the options. Inconclusive results, when a given numerical pattern corresponds to more than three taxonomic groups or is atypical, require repetition after verifying the purity of the sample. Biochemical methods have been widely used for years in identifying *N. gonorrhoeae* (Alexander *et al.* 2005; Meyer and Buder 2020). It was important for microbiological diagnostics that in 2000, mutants with a deletion in the *pip* gene appeared. These strains did not express proline aminopeptidase (PIP), an enzyme the presence of which is a differentiating feature in biochemical tests. The spread of gonococci not producing PIP was reported in England, Denmark, Australia, and New Zealand after 2000. Such strains showed high genetic relationships (Unemo *et al.* 2007).

2.3.2. MALDI-TOF

Matrix-Assisted Laser Desorption/Ionization – Time Of Flight (MALDI-TOF) mass spectrometry for analyzing the protein profile, mainly of the microbial ribosomal proteins, is increasingly used in routine microbial

diagnostics (Ilina *et al.* 2009; Cassagne *et al.* 2011; van Belkum *et al.* 2013; Carannante *et al.* 2015; Morrel *et al.* 2018). This system uses laser energy to desorb biomolecules that co-crystallize with the matrix and ionize them. The matrix plays a key role in this process. Positively charged ions are accelerated in the electric field of the device's through-tube at a speed proportional to their mass and reach the detector at different time intervals. According to the *Time Of Flight* (TOF) measured in nanoseconds, the signal value obtained, converted to the ion's molecular mass ratio to its electric charge (m/z), creates a specific pattern of spectral peaks.

The value of the identification index can take point values from 0 to 3, which are logarithms of the spectra's consistency values. Scores above 2, consistent with "A" that excludes a mixed bacterial population, indicate high confidence in species identification. A score of 2,300 to 3,000 is a reliable identification of microorganism to the species level, a score of 2,000–2,299 is a reliable identification to the genus level and a highly probable species identification result, values of 1,700–1,999 indicate a probable identification result to the genus level, and below 1,699 indicate an unreliable identification result. The usefulness of mass spectrometry in the identification of *N. gonorrhoeae* has been described in papers by other authors (Schweitzer *et al.* 2016; Ilina *et al.* 2009; Carannante *et al.* 2015; Buchanan *et al.* 2016; Morrel *et al.* 2018). Buchanan *et al.* (2016) conducted a retrospective analysis of the identification results of 1,090 gonococci by two methods: manual, biochemical using API NH assays (bioMerieux) and spectrometric using the SepsiTyper® system (Bruker) or VITEK® MS (bioMerieux), estimated the positive predictive value for the MALDI TOF method at 99.3%. Morel *et al.*

noted the need for cautious interpretation of gonococcal identification results for strains isolated from non-genital infections due to the presence of commensal species of the genus *Neisseria* (Morrel *et al.* 2018). Strains that do not produce PIP and the arylamidases TyrA and APPA and do not oxidase glucose were incorrectly identified by the VITEK 2 biochemical method but correctly by the MALDI TOF method (Bruker) (Plakhova *et al.* 2020; Nosov *et al.* 2022).

2.4. Molecular methods

According to the Centers for Disease Control and Prevention (CDC), NAAT methods are currently recommended for diagnosing and screening gonococcal infections among men and women (Table I). They have high sensitivity and specificity and the added advantage of simultaneously detecting other sexually transmitted diseases (*Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*). Examples of NAATs that detect *N. gonorrhoeae* approved by the Food and Drug Administration (FDA) for clinical use are shown in Table I (CDC 2014; Low and Unemo 2016). Recommendations for transporting protected swabs for gonorrhoea diagnosis by molecular methods are less restrictive compared to culture methods. Any delay in inoculation of materials into appropriate microbiological media can adversely affect the result of *N. gonorrhoeae* culture.

In contrast, this is not the case with NAAT methods. Longer sample storage time does not impair the method sensitivity. The very high sensitivity of molecular methods, associated with the ability to multiply

Table I
FDA-approved molecular diagnostic tests for the clinical diagnosis of gonorrhea, based on CDC Recommendations (2014)

NAAT test detected pathogens	Producer	Method	Molecular targets <i>N. gonorrhoeae</i>
Aptima NG assay <i>N. gonorrhoeae</i>	(Hologic/Gen-Probe, SanDiego, California)	TMA	Specific region of 16S rRNA, different than in the Aptima Combo 2 assay
Aptima Combo 2 assay <i>C. trachomatis</i> , <i>N. gonorrhoeae</i>	(Hologic/Gen-Probe, SanDiego, California)	TMA	Specific region of 16S rRNA
Abbott Real Time 2000 CT/NG <i>C. trachomatis</i> , <i>N. gonorrhoeae</i>	Abbott Molecular Inc. Des Plaines, Illinois	qPCR	48 bp sequence within the <i>Opa</i> gene
Cobas CT/NG <i>C. trachomatis</i> , <i>N. gonorrhoeae</i>	Roche Molecular Diagnostics, Branchburg, New Jersey	PCR	two sequences within the fragment DR 9A and DR 9B (DR – <i>direct repeat</i>)
BD ProbeTec ET CT/GC Amplified DNA assay <i>C. trachomatis</i> , <i>N. gonorrhoeae</i>	Becton Dickinson, Sparks, Maryland	SDA	Chromosomal pilin gene-inverting protein homolog
BD ProbeTec NG Q <i>N. gonorrhoeae</i>	Becton Dickinson, Sparks, Maryland	SDA	Chromosomal pilin gene-inverting protein homolog
Xpert CT/NG Assay <i>C. trachomatis</i> , <i>N. gonorrhoeae</i>	Cepheid, Sunnyvale, California	qPCR	two specific chromosomal DNA sequence

TMA – Transcription-mediated amplification, SDA – Strand displacement amplification, qPCR – Real-Time Polymerase chain reaction

even single copies of target DNA or RNA, allows diagnostic use of samples with low bacterial loads, such as the initial urine stream or vaginal swabs. On the other hand, due to the high sensitivity, there is the possibility of false-positive results, such as in cases of cross-reactions with genetic material from other species of the genus *Neisseria* or patients just after treatment, due to the persistence of the pathogen's DNA in the body. With molecular techniques, it is essential to strictly control the course of laboratory procedures to ensure the reliability of test results.

Most importantly, all safeguards should be used to prevent contamination of the laboratory environment with pathogen amplicons, the presence of which can be a source of false positives. Control of the efficiency of isolation, amplification and detection process of the pathogen's DNA is ensured by using so-called positive controls, and each time, the inclusion of negative control, i.e. sterile water, verifies the possibility of contamination of, for example, reagents or the environment. To date, the development and good standardization of molecular methods have improved the diagnosis of gonococcal infections but have not enabled routine determination of drug susceptibility.

2.5. The importance of drug susceptibility assessment

Antimicrobial resistance (AMR) of *N. gonorrhoeae* has a multifactorial basis, both chromosomal and plasmid-mediated (Unemo *et al.* 2016; Unemo and Shafer 2014). The drug susceptibility profiles of gonococci and plasmid and chromosomal resistance prevalence vary by geographic region and change over time. The increasing antimicrobial resistance of the pathogen is a global problem and requires constant monitoring (Tapsall *et al.* 2009; Town *et al.* 2020). With the introduction of sulfonamides and then penicillin for the treatment of gonorrhoea in the 1930s, *N. gonorrhoeae* has acquired resistance to successive groups of antibiotics formerly used in therapeutic regimens: sulfonamides, penicillin, tetracyclines, fluoroquinolones (Ohnishi *et al.* 2011; Unemo and Shafer 2014; Grad *et al.* 2016). Third-generation cephalosporins, along with azithromycin or tetracycline, appeared in treatment recommendations, but the use of these antibiotics may soon prove less effective or even ineffective (Grad *et al.* 2016; Martin *et al.* 2016; Martin *et al.* 2019). Strains with a wild-type phenotype regarding drug susceptibility, which do not show resistance or reduced sensitivity to antibiotics, have become increasingly rare. Multidrug-resistant strains emerge worldwide, such as those with Multi Drug Resistance (MDR) or Extensively Drug-Resistant (XDR) phenotypes. *N. gonorrhoeae* strains with the MDR phenotype are defined as resistant or with reduced sensitivity to one of the antibiotics recommended for current empiric

therapy (i.e., cefixime, ceftriaxone or azithromycin) with resistance to two other therapeutic groups (e.g., fluoroquinolones or tetracyclines). In contrast, strains with extensive drug resistance, XDR *N. gonorrhoeae*, are understood to be resistant or with reduced sensitivity to both recommended drugs, with concurrent resistance to two other antibiotic groups (Tapsall *et al.* 2009). In 2011, the first case of treatment failure of gonorrhoeal pharyngitis caused by a strain with the XDR phenotype was described in Japan (Ohnishi *et al.* 2011). Europe's first ceftriaxone-resistant gonococcal strains were isolated in France in 2010 (Unemo *et al.* 2012) and Spain in 2011 (Carnicer-Pont *et al.* 2012). They showed phenotypic and genotypic similarity (NG-MAST 1407, MLST ST1901). In 2018, XDR strains emerged in the U.K. and Australia, resistant to ceftriaxone and with high azithromycin resistance (≥ 256 mg/l) (Eyre *et al.* 2017; Whiley *et al.* 2018; Jennison *et al.* 2019). Due to the increasing resistance of gonococci, the WHO presented the "Global Action Plan to Control the Spread and Prevent Antibiotic Resistance of *N. gonorrhoeae*" (WHO 2012), and in 2017, *N. gonorrhoeae* was included in the list of bacterial pathogens for which there is a particular need for new drugs (WHO 2021). The drug susceptibility of gonococci is monitored mainly through widely conducted national and international surveillance programs: WHO-Global GASP (*Gonococcal Antimicrobial Surveillance Programme*) (Unemo *et al.* 2019), Euro GASP (Cole *et al.* 2013), US-GISP (*Gonococcal Isolate Surveillance Project*) (Grad *et al.* 2016), Canadian GASP (Martin *et al.* 2019), Australian AGSP (*Australian Gonococcal Surveillance Programme*) (Lahra *et al.* 2015), UK GRASP (*Gonococcal Resistance to Antimicrobials Surveillance Programme*) (Unemo *et al.* 2020).

2.5.1. Antimicrobial susceptibility determination methods

Drug susceptibility testing of *N. gonorrhoeae* isolates, according to current recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as well as Clinical and Laboratory Standards Institute (CLSI), requires the determination of values of minimum inhibitory concentrations – MIC (mg/l). Two determination methods are recommended: the WHO's preferred method of serial antibiotic dilutions in agar (Unemo *et al.* 2013) or gradient strips. The antibiotic gradient strip method combines the diffusion method with the serial dilution method, requiring the use of media recommended by the strip manufacturer (Unemo *et al.* 2013; Liu *et al.* 2015; Papp *et al.* 2018). The range of MIC limits applicable in U.S. and European recommendations differs slightly (Tab. II). A reference strain of *N. gonorrhoeae* ATCC 49226 is recommended for quality control of

Table II.
MIC breakpoint range for *N. gonorrhoeae* according
to EUCAST recommendations v 12.0 2023.01.01 and CLSI 2020

Antibiotic (abbreviation)	MIC limit values (mg/l)			
	EUCAST v 12.0 2023		CLSI 2020	
	≤ W	> O	≤ W	> O
Penicillin (P)	0,06	1,0	0,06	2,0
Cefixime (CFM)	0,125	0,125	0,25	–
Ceftriaxone (CRO)	0,125	0,125	0,25	–
Cefotaxime (CTX)	0,125	0,125	0,5	–
Azithromycin (AZM)*	1,0 (ECOFF)		1,0	–
Ciprofloxacin (CIP)	0,03	0,06	0,06	1,0
Tetracycline (T)	0,5	0,5	0,25	2,0
Spectinomycin (SPT)	64,0	64,0	32,0	128,0

Azithromycin* – According to EUCAST v.12.0 recommendations for azithromycin, it is possible to provide MIC values (mg/l) without a sensitive/resistant interpretation. The ECOFF value (epidemiological cut-off value) is 1.0 mg/l, in the case of MIC value ≤ 1 mg/l, azithromycin can be used in combination with another active antibiotic. MIC value > 1 mg/l indicates the presence of acquired resistance mechanisms.

the procedure. Determination of MIC values for penicillin-resistant strains, according to EUCAST recommendations, should be extended by testing the ability to produce penicillinase using the cephinase assay. Nitrocefin (a chromogenic cephalosporin) paper discs allow the detection of penicillinase activity in live *N. gonorrhoeae* isolates, thanks to the disc's colour change from yellow to red. The colour reaction results from the hydrolysis of the amide bond in the β-lactam ring of cephalosporin by bacterial β-lactamase.

Routinely used in microbiological diagnostics to determine drug susceptibility, the disk-diffusion method is not recommended for gonococci but can be used due to limited resources. Interpretation of drug susceptibility results based on zones of inhibition around antibiotic discs is presented by CLSI (<https://clsi.org>) (Singh *et al.* 2012, Mal *et al.* 2016).

2.6. Genotypic analysis methods

Over the past 30 years, several methods have been developed to analyze the genetic diversity of *N. gonorrhoeae* strains. They aim to identify polymorphisms at a single locus, several loci, or even the entire genome. The variability of gonococcal strains was assessed based on patterns of electrophoretically separated strands of amplified DNA, which allowed the demonstration of, among others, different plasmid profiles (PPA – *Plasmid Profile Analysis*), restriction fragment length polymorphism (RFLP/PFGE pulse electrophoresis method), ribotyping and Opa protein typing (Unemo and Dillon 2011). The use of sequencing techniques allowed

the differentiation of gonococci initially for the *porB* gene (Bash *et al.* 2005; Liao *et al.* 2009), and then, after expanding the scope, gave the possibility to type several loci. Nowadays the most popular molecular typing schemes are MLST (Maiden *et al.* 1998), NG-MAST (Martin *et al.* 2004), NG-STAR (Demczuk *et al.* 2017). The whole genome sequencing method (WGS) is now playing an increasingly important role in epidemiological and phylogenetic studies, as well as in monitoring antibiotic resistance of gonococci. This method certainly facilitates surveillance of circulating *N. gonorrhoeae* strains in an epidemiological context (Eyre *et al.* 2018; Harris *et al.* 2018; Sánchez-Busó *et al.* 2019; Sánchez-Busó *et al.* 2022). The Multi-Locus Sequence Typing (MLST) method is based on the sequencing of 7 housekeeping genes: *abcZ* (putative ABC transporter), adenylate kinase (*adk*), *aroE* (shikimate dehydrogenase), *fumC* (fumarate hydratase), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), *pgm* (phosphoglucomutase) (Maiden 2008). Sequence type relatedness analysis allowed us to define MLST CC clonal complexes grouping related clonal lines. With the same scheme being used to type other species in the human genus *Neisseriaceae*, the pathogenic *N. meningitidis* and the commensal *N. lactamica*, the MLST database is a valuable source of data for genetic analyses within the genus and broad studies in an evolutionary context over the long term (Maiden 2008; Harrison *et al.* 2020). As of June 2022, the PubMLST database contained 16700 ST types for the genus *Neisseria*. The PubMLST website is maintained by the Department of Zoology at Oxford University (Jolley *et al.* 2018). *N. gonorrhoeae* Multi-Antigen Sequence Typing (NG-MAST) method is based on the sequencing of two highly variable loci within the *porB* (490 bp) and *tbpB* (390 bp) genes, which encode two *N. gonorrhoeae* membrane proteins: porin B (PorB) and transferrin binding protein (tbpB), respectively. The combination of both loci allows the determination of the Sequence Type (ST). NG-MAST STs are grouped into geno-groups for phylogenetic and epidemiological analysis (Chisholm *et al.* 2013). The NG-MAST method is widely used worldwide. The website <https://pubmlst.org> contains the current database for typing with this method. As of May 2023, 12735 *porB* and 3147 *tbpB* alleles and 21490 ST profiles of *N. gonorrhoeae* have been published on the site (Jolley *et al.* 2018). *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) is the typing scheme that considers seven chromosomal determinants of *N. gonorrhoeae* resistance to β-lactam antibiotics, macrolides, fluoroquinolones and tetracyclines. The method requires sequencing of the following loci: *penA*, *mtrR*, *porB*, *ponA*, *gyrA*, *parC* and 23S *rRNA* and is consistent with classical Sanger sequencing and WGS (Demczuk *et al.* 2017). The unique sequence of

each gene is assigned an allele number, while the combination of alleles of seven genes allows to determine the sequence type of NG-STAR. Sequences can be analyzed and compared through an online application in a publicly available database (<https://ngstar.canada.ca/>) maintained by the Public Health Agency of Canada and the National Microbiology Laboratory. As of May 2023, 5259 ST types of *N. gonorrhoeae* have been published on the NG-STAR database website. NG-STAR typing allows analysis of strain relatedness, tracking of clonal lineage development and monitoring of antibiotic resistance (Golparian *et al.* 2021).

2.7. Rapid diagnostic tests

Today's challenge is to develop a quicker, more straightforward, and more sensitive diagnostic method to detect *N. gonorrhoeae* infection (Bignell *et al.* 2013; Li *et al.* 2019). Both standard methods for identifying *N. gonorrhoeae* – NAAT or culturing of the pathogen prove expensive and require specialized laboratory equipment, making its use in low- and middle-income countries much limited or impossible.

In 2004, the WHO drew attention to the urgent need for rapid diagnostic tests for bacterial sexually transmitted diseases. The ASSURED criteria were established to determine the characteristics that such a diagnostic test should have. First and foremost, it is to be affordable, sensitive and specific, avoiding false results. In addition, it is to be user-friendly, i.e., simple to perform, with a minimum number of steps and requiring no advanced training. Moreover, the test must be fast and durable to survive various transportation conditions. It should also require no additional equipment (Land *et al.* 2019).

Rapid gonococcal diagnostic tests are emerging on the market. Such tests allow bypassing the multi-step and lengthy identification of the pathogen. Most often, they consist of a carrier such as a cassette, frequently containing all the reagents necessary for analysis, in which the collected sample is placed directly and inserted into the appropriate analyzer. These tests allow rapid identification of the pathogen for up to 30 minutes. However, they have limitations. First, not every test will prove suitable for every type of sample.

One rapid diagnostic test is Xpert CT/NG, which uses the GeneXpert platform (Cepheid). It is a real-time *in vitro* PCR performed directly from the collected sample. The FDA approves the test for use in urine, vaginal, cervical, rectal, and pharynx samples. It shows a sensitivity and specificity between 86–99% (Herbst De Cortina *et al.* 2016; Doernberg *et al.* 2019). Another rapid test is binx health io CT/NG (binx health, INC), a PCR-based qualitative test consisting of a stationary device and easy-to-use coffers. The waiting time for the result is about 30 minutes. As of 2019, the FDA has

authorized the use of this test in vaginal swab samples. In vaginal samples, its high sensitivity and specificity of 96–97.7% was stated (Van Der Pol and Gaydos 2021; Gaydos *et al.* 2013). The Truelab Preal Time micro PCR system (Molbio Diagnostics Pvt Ltd) is a rapid semi-quantitative PCR-based test. The entire process involves sample collection and an automated extraction system. Then, the nucleic acid is transferred to an analyzer chip using a fluorophore-capturing optical sensor for determination. Analysis results are available after about an hour. The test is not FDA-certified (Nair *et al.* 2016). The STI Array test (Randox Biosciences) requires the Vivalytic Analyzer (Bosch Healthcare Solutions), a device that enables quantitative PCR reaction, to perform the assay. It comes with a coffer containing all the necessary reagents for the analysis (Adamson *et al.* 2020). A different type of assay is loop-mediated isothermal amplification (LAMP). It is a reliable, low-cost, sensitive, rapid technique for amplifying nucleic acids. It has been widely used to identify pathogens, including SARS-CoV-2, *Mycobacterium tuberculosis* and *Bruceella* (Shete *et al.* 2019). DNA polymerase in the LAMP method can effectively amplify target genes at 58–69°C (Notomi *et al.* 2000). The *N. gonorrhoeae* – LAMP-PNB assay combines isothermal amplification with a polymer nanoparticle-based biosensor. The entire identification process takes about 60 minutes (Wong *et al.* 2018; Chen *et al.* 2021). The documented development of rapid diagnostic methods for *N. gonorrhoeae* infection is insufficient. There is still a need for specific, sensitive assays that are simple and inexpensive.

2.8. Therapy of uncomplicated gonorrhoea

According to current European recommendations for treating uncomplicated gonorrhoea in adults, ceftriaxone is administered intramuscularly in a single dose of 1 g (Unemo *et al.* 2021). In CDC recommendations for anogenital and pharyngeal infections, the dose of ceftriaxone depends on the patient's body weight (500 mg for individuals <150 kg and 1 g for individuals >150 kg). If chlamydial infection has not been excluded, chlamydia should be treated with doxycycline 100 mg orally twice daily for seven days, except for pregnant patients for whom a single dose of azithromycin 1 g is recommended. An alternative treatment for uncomplicated gonococcal infections of the urethra or anus is intramuscular gentamicin in a single dose of 240 mg with azithromycin (2 g) or a single oral dose of cefixime (800 mg). In cases of gonococcal throat infections with an allergy to ceftriaxone, CDC recommends consultation with an infectious disease specialist to choose alternative therapy. The treatment regimen is the same for HIV-positive patients. Abstaining from sexual activity is necessary during treatment and for

seven days afterwards. According to recommendations, all patients diagnosed with gonorrhoea should also be tested for other STIs, including chlamydia, syphilis, and HIV. Recent sex partners of diagnosed persons should be referred for testing and presumptive treatment (Workowski *et al.* 2021).

3. Conclusion

Gonococcal infection, a sexually transmitted disease caused by *N. gonorrhoeae*, is a public health challenge worldwide. Initially, the primary method of identifying this pathogen was using microscopy. Microscopy is a sensitive enough diagnostic method only in men with clinical urethritis symptoms. Unfortunately, in the case of swabs taken from the vagina, cervical canal, pharyngeal or rectum, the microscopic slide has no diagnostic significance. Currently, culture and phenotypic identification methods have been dominated by NAAT methods, which allow the detection and identification of the species directly in the clinical specimen. However, today's molecular methods do not allow for the complete determination of drug susceptibility. This makes empirical therapy predominate over targeted therapy in the treatment of gonorrhoea. The reason for deviating from classical culture methods in diagnosing gonorrhoea is the biology of the pathogen, its high growth requirements, and the sensitivity of gonococci to the conditions of sample transport and incubation of clinical materials.

The increasing antimicrobial resistance of *N. gonorrhoeae* is a global problem and requires constant monitoring. Although determining MIC values by serial dilutions or using antibiotic-gradient strips is costly and time-consuming, taking a minimum of 3 days, it is still irreplaceable despite attempts to construct an assay detecting gonococcal resistance at the molecular level. Difficulties in developing a suitable molecular method for antimicrobial susceptibility determination of *N. gonorrhoeae* relate to the nature of this pathogen, its variability, and precisely the multifactorial, complex genetic basis of resistance. An additional problem is that clinical specimens such as swabs taken from the genitourinary tract, pharynx or rectum are microbe-rich material, which creates the possibility of cross-reactions in molecular tests.

A steadily increasing number of gonococcal infections are being reported globally, also in low- and middle-income regions. The problem in poor areas is often the lack of proper diagnosis of gonorrhoea due to the shortage of medical equipment and even electricity. Hence, considering the difficulties of developing countries, a specific, sensitive, rapid, and inexpensive method of gonorrhoea diagnosis is highly needed.

Gonorrhoea is undoubtedly a severe health risk, especially for women, due to its often asymptomatic course and the possibility of upper genital tract complications such as PID, which can result in infertility. Gonorrhoea infection prevalence in infertile populations was several folds higher than that for the general population globally, according to the meta-analysis by Chemaitelly *et al.* (2021). Prophylaxis using immunization is unavailable, as attempts to develop an effective vaccine have failed. Screening is not routinely performed in the population. Because of these facts, the epidemiology of *N. gonorrhoeae* infection and drug resistance should be monitored continuously, as infection surveillance and antibiotic therapy are the only means of controlling gonorrhoea in the current situation. It is necessary to use the approved, sensitive diagnostic methods and rational treatment of gonococcal infections based on recommendations and reliable knowledge of medical microbiology and pharmacology.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

References:

1. Adamson P.C., Loeffelholz M.J., Klausner J.D.: Point-of-care testing for sexually transmitted infections: A review of recent developments. *Arch. Pathol. Lab. Med.* **144**, 1344–1351 (2020)
2. Alexander S., Ison C.: Evaluation of commercial kits for the identification of *Neisseria gonorrhoeae*. *J. Med. Microbiol.* **54**, 827–831 (2005)
3. Bash M.C., Zhu P., Gulati S., McKnew D., Rice P.A., Lynn F.: Por Variable-region typing by DNA probe hybridization is broadly applicable to epidemiologic studies of *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* **43**, 1522–1530 (2005)
4. Bennett J.S., Watkins E.R., Jolley K.A., Harrison O.B., Maiden M.C.: (2014). Identifying *Neisseria* species by use of the 50S ribosomal protein L6 (rplF) gene *J. Clin. Microbiol.* **52**, 1375–1381 (2014)
5. Bignell C. & Unemo M.: European STI Guidelines Editorial Board. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. *Int. J. STD AIDS.* **24**, 85–92 (2013)
6. Buchanan R., Ball D., Dolphin H., Dave J.: Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry for the identification of *Neisseria gonorrhoeae*. *Clin. Microbiol. Infect.* **22**, 815.e5–815.e7 (2016)
7. Carannante A. & Stefanelli P. *et al.*: Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for identification and clustering of *Neisseria gonorrhoeae*. *BMC Microbiol.* **15**, 142 (2015)

8. Carnicer-Pont D., Smithson A., Fina-Homar E., Bastida M.T.: Gonococcus antimicrobial resistance surveillance working group. First cases of *Neisseria gonorrhoeae* resistant to ceftriaxone in Catalonia, Spain, May 2011. *Enferm. Infecc. Microbiol. Clin.* **30**, 218–219 (2012)
9. Cassagne C., Ranque S., Normand A.C., Fourquet P., Thiebault S., Planard C., Hendrickx M., Piarroux R.: Mould routine identification in the clinical laboratory by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *PLoS One*. **6**, e28425 (2011)
10. CDC Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*-2014. *MMWR Recomm. Rep.* **63**, 1–19 (2014)
11. Chemaitelly H., Majed A., Abu-Hijleh F., et al.: Global epidemiology of *Neisseria gonorrhoeae* in infertile populations: systematic review, meta-analysis and metaregression. *Sex Transm Infect.* **97**(2), 157–169 (2021)
12. Chen X., Zhou Q., Wu X., Wang S., Liu R., Dong S., Yuan W.: Visual and rapid diagnosis of *Neisseria gonorrhoeae* using loop-mediated isothermal amplification combined with a polymer nanoparticle-based biosensor in clinical application. *Front. Mol. Biosci.* **8**, 702134 (2021)
13. Chisholm S.A., Unemo M., Quaye N., Johansson E., Cole M.J., Ison C.A., Van de Laar M.J.: Molecular epidemiological typing within the European Gonococcal Antimicrobial Resistance Surveillance Programme reveals predominance of a multidrug-resistant clone. *Euro Surveill.* **18**, 20358 (2013)
14. Demczuk W. & Martin I. et al.: *Neisseria gonorrhoeae* sequence typing for antimicrobial resistance, a novel antimicrobial resistance multilocus typing scheme for tracking global dissemination of *N. gonorrhoeae* strains. *J. Clin. Microbiol.* **55**, 1454–1468 (2017)
15. Doernberg S.B. & Klausner J.D. et al.: Simultaneous evaluation of diagnostic assays for pharyngeal and rectal *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using a master protocol. *Clin. Infect. Dis.* **71**, 2314–2322 (2019)
16. ECDC: European Centre for Disease Prevention and Control. Gonorrhoea. In: ECDC. Annual epidemiological report for 2019. *Stockholm: ECDC* (2023)
17. ECDC: Gonococcal antimicrobial susceptibility surveillance in the Europe Union/European Economic Area. Summary of results 2020. *Stockholm: ECDC* (2022)
18. Eyre D.W. & Paul J. et al.: WGS to predict antibiotic MICs for *Neisseria gonorrhoeae*. *J. Antimicrob. Chemother.* **72**, 1937–1947 (2017)
19. Eyre D.W. & Andersson M.I. et al.: Gonorrhoea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. *Euro Surveill.* **23**, 1800323 (2018)
20. Gaydos C.A. & Hook E.W. et al.: Performance of the Cepheid CT/NG Xpert Rapid PCR Test for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* **51**, 1666–72 (2013)
21. Golparian D., Sánchez-Busó L., Cole M., Unemo M.: *Neisseria gonorrhoeae* Sequence typing for antimicrobial resistance (NG-STAR) clonal complexes are consistent with genomic phylogeny and provide simple nomenclature, rapid visualization and antimicrobial resistance (AMR) lineage predictions. *J. Antimicrob. Chemother.* **76**, 940–944 (2021)
22. Grad Y.H., Harris S.R., Kirkcaldy R.D., Green A.G., Marks D.S., Bentley S.D., Trees D., Lipsitch M.: Genomic epidemiology of gonococcal resistance to extended-spectrum cephalosporins, macrolides, and fluoroquinolones in the United States, 2000–2013. *J. Infect. Dis.* **214**, 1579–87 (2016)
23. Harris S.R. & Unemo M. et al.: Public health surveillance of multidrug-resistant clones of *Neisseria gonorrhoeae* in Europe: a genomic survey. *Lancet Infect. Dis.* **18**, 758–768 (2018)
24. Harrison O.B., Cehovin A., Skett J., Jolley K.A., Massari P., Genco C.A., Tang C.M., Maiden M.C.J.: *Neisseria gonorrhoeae* population genomics: use of the gonococcal core genome to improve surveillance of antimicrobial resistance. *J. Infect. Dis.* **222**, 1816–1825 (2020)
25. Herbst De Cortina S., Bristow C.C., Joseph Davey D., Klausner J.D.: A systematic review of point of care testing for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*. *Infect. Dis. Obstet. Gynecol.* **2016**, 4386127 (2016)
26. Humbert M.V. & Christodoulides M.: Atypical, yet not infrequent, infections with *Neisseria* species. *Pathogens (Basel, Switzerland)*, **9**, 10 (2019)
27. Ilina E.N. & Govorun V.M. et al.: Direct bacterial profiling by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry for identification of pathogenic *Neisseria*. *J. Mol. Diagn.* **11**, 75–86 (2009)
28. Janda W.M. & Gaydos C.A.: *Neisseria*, manual of clinical microbiology. *ASM Press*, **12**, 52 (2007)
29. Jennison A.V., Whitley D., Lahra M.M., Graham R.M., Cole M.J., Hughes G., Fifer H., Andersson M., Edwards A., Eyre D.: Genetic relatedness of ceftriaxone-resistant and high-level azithromycin resistant *Neisseria gonorrhoeae* cases, United Kingdom and Australia, February to April 2018. *Euro Surveill.* **24**, 1900118 (2018)
30. Jolley K.A., Bray J.E., Maiden M.C.J.: Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* **3**, 124 (2018)
31. Lahra M.M., Enriquez R.P., National *Neisseria* network: Australian gonococcal surveillance programme. *Commun. Dis. Intell. Q Rep.* **39**, 628–630 (2015)
32. Land K.J., Boeras D.I., Chen X.S., Ramsay A.R., Peeling R.W.: REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol.* **4**, 46–54 (2019)
33. Li S., Liu Y., Wang Y., Chen H., Liu C., Wang Y.: Lateral flow biosensor combined with loop-mediated isothermal amplification for simple, rapid, sensitive, and reliable detection of *Brucella* spp. **12**, 2343–2353 (2019)
34. Liao M., Helgeson S., Gu W.M., Yang Y., Jolly A.M., Dillon J.A.: Comparison of *Neisseria gonorrhoeae* multiantigen sequence typing and porB sequence analysis for identification of clusters of *N. gonorrhoeae* isolates. *J Clin Microbiol.* **47**, 489–491 (2009)
35. Liu G., Tang C.M., Exley R.M.: Non-pathogenic *Neisseria*: members of an abundant, multi-habitat, diverse genus. *Microbiology*, **161**, 1297–1312 (2015)
36. Low N., Unemo M.: Molecular tests for the detection of antimicrobial resistant *Neisseria gonorrhoeae*: when, where, and how to use? *Curr. Opin. Infect. Dis.* **29**, 45–51 (2016)
37. Maiden M.C. & Spratt B.G. et al.: Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc. Natl. Acad. Sci. USA*, **95**, 3140–3145 (1998)
38. Maiden M.C.: Population genomics: diversity and virulence in the *Neisseria*. *Curr. Opin. Microbiol.* **11**, 467–471 (2008)
39. Mal P.B., Jabeen K., Farooqi J., Unemo M., Khan E.: Antimicrobial susceptibility testing of *Neisseria gonorrhoeae* isolates in Pakistan by E-test compared to calibrated dichotomous sensitivity and clinical laboratory standards institute disc diffusion techniques. *BMC Microbiol.* **16**, 236 (2016)

40. Martin I. Mulvey M.R. *et al.*: Multidrug-resistant and extensively drug-resistant *Neisseria gonorrhoeae* in Canada, 2012–2016. *Can. Commun. Dis. Rep.* **45**, 45–53 (2019)
41. Martin I. & Mulvey M.R. *et al.*: Decline in decreased cephalosporin susceptibility and increase in azithromycin resistance in *Neisseria gonorrhoeae*, Canada. *Emerg Infect Dis.* **22**, 65–67 (2016)
42. Martin I.M., Ison C.A., Aanensen D.M., Fenton K.A., Spratt B.G.: Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. *J. Infect. Dis.* **189**, 1497–1505 (2004)
43. Meyer T. & Buder S.: The Laboratory diagnosis of *Neisseria gonorrhoeae*: current Testing and future demands. *Pathogens*, **9**, 91 (2020)
44. Młynarczyk-Bonikowska B., Serwin A.B., Golparian D., Walter de Walthoffen S., Majewski S., Koper M., Malejczyk M., Domeika M., Unemo M.: Antimicrobial susceptibility/resistance and genetic characteristics of *Neisseria gonorrhoeae* isolates from Poland, 2010–2012. *BMC Infect. Dis.* **14**, 65 (2014)
45. Morrel F., Jacquier H., Desroches M., Fihman V., Kumanski S., Cambau E., Decousser J.W., Berçot B.: Use of Andromas and Bruker MALDI-TOF MS in the identification of *Neisseria*. *Eur. J. Clin. Microbiol. Infect. Dis.* **37**, 2273–2277 (2018)
46. Mroczkowski T.F.: Rzeżączka. Choroby przenoszone drogą płciową. Wyd. PZWL (1998)
47. Nair C.B., Manjula J., Subramani P.A., Nagendrappa P.B., Manoj M.N., Malpani S., Pullela P.K., Subbarao P.V., Ramamoorthy S., Ghosh S.K.: Differential diagnosis of malaria on Truelab Uno[®], a Portable, Real-Time, MicroPCR Device for Point-Of-Care Applications. *PLoS One*. **11**, e0146961 (2016)
48. Ng L.K., Martin I.E.: The laboratory diagnosis of *Neisseria gonorrhoeae*. *Can. J. Infect. Dis. Med. Microbiol.* **16**, 15–25 (2005)
49. NIZP http://wwwold.pzh.gov.pl/oldpage/epimeld/index_p.html
50. Nosov N., Kubanov A., Solomka V., Deryabin D.: Biochemical Atypia in Russian *Neisseria gonorrhoeae* Clinical Isolates Belonging to the G807 NG-MAST Genogroup/ST1594 MLST. *Microorganisms*, **10**, 2271 (2022)
51. Notomi T., Okayama H., Masubuchi H., Yonekawa T., Watanabe K., Amino, N.: Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **28**, 63 (2000)
52. Ohnishi M., Golparian D., Shimuta K., Saika T., Hoshina S., Iwasaku K., Nakayama S., Kitawaki J., Unemo M.: Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. *Antimicrob. Agents Chemother.* **55**, 3538–3545 (2011)
53. Papp J.R., Rowlinson M.C., O'Connor N.P., Wholehan J., Razeq J.H., Glennen A.: Ware D., Iwen P.C., Lee L.V., Hagan C.: Accuracy and reproducibility of the Etest to detect drug-resistant *Neisseria gonorrhoeae* to contemporary treatment. *J Med Microbiol.* **67**, 68–73 (2018)
54. Plakhova X.I., Petrova N.P., Nikonorov A.A., Kubanov A.A.: Biochemical atypia in the modern russian strains of *Neisseria gonorrhoeae*. *Klin. Lab. Diagn.* **65**, 507–511 (2020)
55. Quillin S.J., Seifert H.S.: *Neisseria gonorrhoeae* host adaptation and pathogenesis. *Nat. Rev. Microbiol.* **16**, 226–240 (2018)
56. Rowley J. & Taylor M.M. *et al.*: *Chlamydia*, gonorrhea, trichomoniasis and syphilis: global prevalence and incidence estimates. *Bull World Health Organ.* **97**, 548–62 (2019)
57. Sánchez-Busó L., Cole M.J., Spiteri G.: Europe-wide expansion and eradication of multidrug-resistant *Neisseria gonorrhoeae* lineages: a genomic surveillance study. *Lancet Microbe.* **3**, e452–e463 (2022)
58. Sánchez-Busó L., Golparian D., Corander J., Grad Y.H., Ohnishi M., Flemming R., Parkhill J., Bentley S.D., Unemo M., Harris S.R.: The impact of antimicrobials on gonococcal evolution. *Nat Microbiol.* **4**, 1941–1950 (2019)
59. Schweitzer V.A., van Dam A.P., Hananta I.P., Schuurman R., Kusters J.G., Rentenaar R.J.: Identification of *Neisseria gonorrhoeae* by the Bruker biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system is improved by a database extension. *J Clin Microbiol.* **54**, 1130–1132 (2016)
60. Shete P.B., Farr K., Strnad L., Gray C.M., Cattamanchi A.: Diagnostic Accuracy of TB-LAMP for Pulmonary Tuberculosis: a Systematic Review and Meta-Analysis. *BMC Infect. Dis.* **19**, 268 (2019)
61. Singh V., Bala M., Kakran M., Ramesh V.: Comparative assessment of CDS, CLSI disc diffusion and Etest techniques for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*: a 6-year study. *BMJ Open.* **2**, e000969 (2012)
62. Tapsall J.W., Ndowa F., Lewis D.A., Unemo M.: Meeting the public health challenge of multidrug- and extensively drug-resistant *Neisseria gonorrhoeae*. *Expert Rev. Anti. Infect. Ther.* **7**, 821–834 (2009)
63. Town K., Harris S., Sánchez-Busó L., Cole M.J., Pitt R., Fifer H., Mohammed H., Field N., Hughes G.: Genomic and phenotypic variability in *Neisseria gonorrhoeae* antimicrobial susceptibility, England. *Emerg. Infect. Dis.* **26**, 505–515 (2020)
64. Unemo M., Del Rio C., Shafer W.M.: Antimicrobial resistance expressed by *Neisseria gonorrhoeae*: A major global public health problem in the 21st Century. *Microbiol. Spectr.* **4**, 3 (2016)
65. Unemo M. & Dillon J.A.: Review and international recommendation of methods for typing *Neisseria gonorrhoeae* isolates and their implications for improved knowledge of gonococcal epidemiology, treatment, and biology. *Clin Microbiol Rev.* **24**, 447 (2011)
66. Unemo M., Ison C.A., Cole M., Spiteri G., van de Laar M., Khotenashvili L.: Gonorrhea and gonococcal antimicrobial resistance surveillance networks in the WHO European Region, including the independent countries of the former Soviet Union. *Sex Transm. Infect.* **89**, 42–46 (2013)
67. Unemo M., Lahra M.M., Cole M., Galarza P., Ndowa F., Martin I., Dillon J.R., Ramon-Pardo P., Bolan G., Wi T.: World Health Organization global gonococcal antimicrobial surveillance program (WHO GASP): Review of new data and evidence to inform international collaborative actions and research efforts. *Sex Health.* **16**, 412–25 (2019)
68. Unemo M., Palmer H.M., Blackmore T., Herrera G., Fredlund H., Limnios A., Nguyen N., Tapsall J.: Global transmission of prolyliminopeptidase-negative *Neisseria gonorrhoeae* strains: implications for changes in diagnostic strategies. *Sex Transm. Infect.* **83**, 47–51 (2007)
69. Unemo M., Ross J., Serwin A.B., Gomberg M., Cusini M., Jensen J.S.: 2020 European guideline for the diagnosis and treatment of gonorrhea in adults. *Int. J. STD AIDS.* **95**, 64 (2020)
70. Unemo M. & Shafer W.M.: Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: Past, evolution, and future. *Clin. Microbiol. Rev.* **27**, 587–613 (2014)
71. Unemo M., Golparian D., Nicholas R., Ohnishi M., Gallay A., Sednaoui P.: High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob. agents chem.* **56**, 1273–1280 (2012)
72. Unemo M., Ross J., Serwin A.B., Gomberg M., Cusini M., Jensen J.S.: Background review for the '2020 European guideline for the diagnosis and treatment of gonorrhea in adults. *Int J STD AIDS.* **32**(2), 108–126 (2021)
73. van Belkum A., Durand G., Peyret M., Chatellier S., Zambardi G., Schrenzel J., Shortridge D., Engelhardt A., Dunne W.M.: Rapid clinical bacteriology and its future impact. *Ann. Lab. Med.* **33**, 14–27 (2013)

74. Van Der Pol B. & Gaydos C.A.: A profile of the binx health io® molecular point-of-care test for chlamydia and gonorrhea in women and men. *Expert Rev. Mol. Diagn.* **21**, 861–868 (2021)
75. Whiley D.M., Jennison A., Pearson J., Lahra M.M.: Genetic characterization of *Neisseria gonorrhoeae* resistant to both ceftriaxone and azithromycin. *Lancet Infect. Dis.* **18**, 717–718. (2018)
76. WHO. Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*. WHO (2012)
77. WHO Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. Accountability for the global health sector strategies 2016–2021: actions for impact. Geneva: WHO (2021)
78. Wong Y.P., Othman S., Lau Y.L., Radu S., Chee, H.Y.: Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of microorganisms. *J. Appl. Microbiol.* **124**, 626–643 (2018)
79. Workowski K.A., Bachmann L.H., Chan P.A. *et al.*: Sexually transmitted infections treatment guidelines, 2021. *MMWR Recomm Rep.* **70**, 1–187 (2021)
80. Xiridou M., Soetens L.C., Koedijk F.D., Van Der Sande M.A., Wallinga J.: Public health measures to control the spread of antimicrobial resistance in *Neisseria gonorrhoeae* in men who have sex with men. *Epidemiol. Infect.* **143**, 1575–1584 (2015)