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GONORRHOEA IN 21ST CENTURY – INTERNATIONAL AND POLISH SITUATION

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ABSTRACT

Gonorrhoea, according to the latest World Health Organization (WHO) estimates in 2008, is the most frequent bacterial sexually transmitted infection globally, accounting for 106.1 million new cases among adults. Of those cases, 3.4 (3.2%) million were in the WHO European Region. In the European Union and European Economic Area, the incidence of reported cases was 12.6 per 100,000 inhabitants in 2011. The highest incidences were noted in the United Kingdom (37.1), Latvia (24.4) and Ireland (18.6). However, in Poland from 2000 to 2011 the reported incidence declined and was only 0.8-0.9 per 100,000 inhabitants in 2011, that might indicate a suboptimal diagnostics and incomplete case reporting and epidemiological surveillance. A study surveying the diagnostics for gonorrhoea and the case reporting system, including the local and national epidemiological surveillance, in Poland is recommended. The high resistance in *Neisseria gonorrhoeae* to nearly all antimicrobials introduced for treatment of gonorrhoea is an exceedingly serious problem globally. A few years ago the first extensively-drug resistant *N. gonorrhoeae* strains with high-level resistance to ceftriaxone, the last remaining option for first-line empirical monotherapy, were reported. Due to this emergent situation, in 2012 the WHO and the European Centre for Disease Prevention and Control (ECDC) launched a global action plan and regional response plan, respectively, to combat the spread of multidrug resistant *N. gonorrhoeae*. Additionally, an updated European guideline on the diagnosis and treatment of gonorrhoea, recommending treatment with ceftriaxone together with azithromycin, was published in 2012. Worryingly, no antimicrobial susceptibility data for *N. gonorrhoeae* strains circulating in Poland have been internationally reported in several decades. It is imperative to implement some regular and quality assured antimicrobial susceptibility surveillance for *N. gonorrhoeae* in Poland and the official Polish treatment guidelines (from 1970s) recommending penicillin G as first-line treatment for gonorrhoea need to be promptly revised.

Key words: *gonorrhoea, epidemiology, Neisseria gonorrhoeae, antimicrobial resistance, diagnostics*

EPIDEMIOLOGY OF GONORRHOEA

In the latest report of the World Health Organization (WHO) on the global incidence and prevalence of four curable sexually transmitted infections (STIs) in 2008, the estimated number of new *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Treponema pallidum* infections was 276.4, 106.1, 105.7 and 10.6 million, respectively. Accordingly, the total estimated number of these four infections was 498.9 million, which represents an increase with 11.3% compared to the WHO estimates in 2005, and the highest increase (21.0%) was noticed for gonorrhoea. In 2008, the highest incidence of gonorrhoea was estimated in the WHO Western Pacific Region (42.0 million cases), WHO South-East Asia Region (25.4 million) and WHO Africa Region (21.1 million). In the WHO European Region (53 countries), 3.4 million cases of gonorrhoea was estimated (1).

In the European Union (EU) and European Economic Area (EEA) (30 countries in 2011), 39 179 new cases of gonorrhoea were reported from 28 countries (gonorrhoea was not mandatorily reported in Germany and Lichtenstein). The mean incidence (cases per 100,000 inhabitants) was 12.6. The highest incidences were noted in the United Kingdom (UK) (37.1; representing 59% of all cases reported in the EU/EEA), Latvia (24.4) and Ireland (18.6), and the lowest incidences in Slovenia (1.2), Portugal (1.1), Poland (0.8-0.9) and Luxemburg (0.4) (2-4).

From 2007 to 2011, in total an increase in gonorrhoea incidence (by 19%) was noticed in the EU/EEA, however, in several countries the relative increase in reported gonorrhoea cases was over 50%, e.g., in Finland, Greece, Ireland, Luxemburg, Portugal and Slovakia. Persons aged 25-34 years constituted 30-33% of all patients in 2000-2011. Persons below 25 years of age constituted 43% in 2011. The incidence of gonorrhoea was 21.2 and 7.6 cases per 100,000 inhabitants in males and females, respectively (2). This nearly 3-fold higher incidence in males compared to females in 2011 can partly be explained by the more frequently symptomatic infection in males, making it easier to diagnose, but also the relatively high incidence of infection in men who have sex with men (MSM).

Accordingly, in many countries MSM constitute, compared to several other subpopulations, a high-frequency transmitting group in regards to gonorrhoea. In the EU/EEA in 2011, MSM constituted 33% of all cases (45% of all cases in males). The highest MSM proportions were reported in Portugal (71%), the Netherlands (55%), and Norway (48%), but data regarding sexual orientation were only available from 19 countries. Eleven percent of all gonorrhoea patients were HIV-positive, but data was available from only ten countries (2).

In countries neighbouring Poland, in 2011 the incidences were below 10 (Slovakia - 3.6, Czech Republic - 6.7, Lithuania - 7.6), between 20 and 30 (Ukraine - 20.1, Kaliningrad – 27.5) and over 30 (in Belarus - 33.4) cases per 100,000 inhabitants (2,3). However, in Poland the reported incidence declined from 1.9 in 2000 to only 0.8-0.9 per 100,000 inhabitants in 2010 and 2011, with male-to-female ratio of the reported gonorrhoea cases of approximately 9:1 in 2011 (2-4; www.pzh.gov.pl).

ANTIMICROBIAL RESISTANCE IN *NEISSERIA GONORRHOEAE*

A rapid increase in antimicrobial resistance (AMR) in *N. gonorrhoeae* to all antimicrobials introduced for the treatment of gonorrhoea has been observed since many decades. Most commonly, gonococcal isolates resistant to a given antimicrobial have emerged only 10-20 years after its introduction into the treatment of gonorrhoea, and then the resistant strains have been spreading globally (5,6). Penicillin, introduced in 1943, became the treatment of first choice at the initial dose of 100,000 – 150,000 units. However, during the subsequent decades treatment failures forced to repeatedly increase the dose of penicillin administered. Finally, in 1976 two penicillinase-producing *N. gonorrhoeae* strains with high-level resistance to penicillin, originating in Africa and Asia, were reported in the United States of America (USA) and UK and in the mid-1980s also chromosomally-mediated high-level penicillin resistance was described, which initiated a change to other antimicrobials for the treatment of gonorrhoea (5,6). Gonococcal isolates with high-level resistance to tetracycline (mediated by the *tetM* gene carried on a conjugal plasmid) were reported in 1985, and in the USA all tetracyclines were withdrawn from the treatment guidelines only one year later (5,6). *N. gonorrhoeae* isolates resistant to fluoroquinolones (due to specific mutations in the *gyrA* and *parC* genes, encoding the enzymes DNA Gyrase and DNA topoisomerase IV, respectively) emerged in Asia, i.e. in Japan, already in the beginning of the 1990s (6). Those *N. gonorrhoeae* isolates resistant to ciprofloxacin and other fluoroquinolones were then subsequently spread worldwide and their transmission resulted in the withdrawal of all fluoroquinolones from treatment recommendations in many of the current EU/EEA countries in early- to mid-2000s and in the USA in 2007. After this, the third-generation cephalosporins - ceftriaxone (initially recommended at a dose of 125 mg×1 intramuscularly) and cefixime (400 mg×1 orally), were the only remaining effective options for first-line empirical monotherapy of gonorrhoea (5,6). Nevertheless, some cases of treatment failures of gonococcal urethritis with cefixime regimen (200 mg bid during three consecutive days) were reported already in the early 2000s in Japan. In the EU/EEA, confirmed cefixime treatment failures were subsequently reported from 2010 and onwards in Norway, UK, Austria and France (5). At present time, treatment failures have also been verified in South Africa (7) and Canada (8). Most or all of those cases appear to have been caused by the gonococcal multilocus sequence typing (MLST) (9) ST1901 clone, most probably originating from Japan, which currently is mostly represented by the *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) (9) ST1407 but also subsequently evolved genetically closely related subtypes (5). Cefixime was withdrawn from the recommended therapeutic regimens for gonorrhoea in Japan in 2006 and in 2012 in USA and Europe (10-12).

Still, resistance to ceftriaxone remains rare. Nevertheless, verified treatment failures of pharyngeal gonorrhoea with doses up to 1000 mg×1 ceftriaxone have been described in Australia, Europe and Japan (5,13). The minimum inhibitory concentration (MIC) of ceftriaxone for the gonococcal isolates causing those failures ranged from 0.032 mg/L to 4 mg/L. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), isolates showing MIC>0.125 mg/L of ceftriaxone is considered as resistant. In the cases when full molecular epidemiological typing was performed, the isolates resulting in those treatment failures belonged to the MLST ST1901 or the in Japan

previously frequently spread ST7363 (5,13). Clearly, a few gonococcal clones with specific *penA* mosaic alleles as the main resistance determinant and, in addition, *mtrR* and *penB* alterations that further increase the MIC of the third-generation cephalosporins, have caused basically all the currently verified treatment failures (5,13).

It is of grave concern that the first strain of extensively-drug resistant (XDR; defined in 14) *N. gonorrhoeae*, with high-level resistance to most antimicrobials including ceftriaxone (ceftriaxone MIC=2-4 mg/L) and resulting in a ceftriaxone treatment failure of pharyngeal gonorrhoea, was described in 2011 in Kyoto, Japan. Molecular epidemiological studies revealed that this strain, referred to as H041 (MLST ST7363/NG-MAST ST4220), was related to the *N. gonorrhoeae* strains resistant to cefixime and causing treatment failures with cefixime in the early 2000s in Japan (13). Subsequently, gonococcal XDR isolates were described also from Quimper, France (15) and Catalonia, Spain (16); displaying MIC=1-2 mg/L of ceftriaxone. The isolates from France and Spain (all from MSM) belonged to the gonococcal clone MLST ST1901/NG-MAST ST1407 and, in general, were phenotypically and genetically exceedingly similar, indicating that they belong to the same XDR strain (15,16). The NG-MAST ST1407 clone, with decreased susceptibility or resistance to cefixime and ability to develop ceftriaxone resistance, is widely spread in Europe and basically worldwide. Thus, at least two gonococcal clones (namely: MLST ST1901 and MLST ST7363), with decreased susceptibility or resistance to third-generation cephalosporins and resulting in treatment failures, spread globally (5).

Due to this worrying situation with resistance emerging to the last remaining option for empirical first-line antimicrobial monotherapy of gonorrhoea, it is essential to substantially enhance the quality-assured surveillance of gonococcal AMR as well as treatment failures globally. The WHO Global Antimicrobial Surveillance Programme (GASP) was established in early 1990s, and revisited and relaunched in 2009. In the EU/EEA, the European Centre for Disease Prevention and Control (ECDC) is coordinating the surveillance of sexually transmitted infections including the European GASP (Euro-GASP). Furthermore, independent national GASP programs exist in the UK (Gonococcal Resistance to Antimicrobials Surveillance Programme - GRASP), USA (US Gonococcal Isolate Surveillance Project - GISP), and several additional countries. Worryingly no antimicrobial susceptibility data for *N. gonorrhoeae* isolates from Poland, with exception of β-lactamase production, have been internationally reported since the 1970s (17-21). The latest study published in Polish literature (in 1992) demonstrated a high gonococcal susceptibility to spectinomycin (99.0%), ofloxacin (94.2%) and penicillin (82.7%) (22). Accordingly, it is imperative to implement some regular and quality assured antimicrobial susceptibility surveillance for *N. gonorrhoeae* in Poland.

In response to this serious situation regarding *N. gonorrhoeae* multi-drug resistance and possibly emergence of untreatable gonorrhoea, the WHO published the 'Global Action Plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*' in June 2012. This Action Plan aims to, for example, increase the awareness on correct use of antibiotics, particularly in key populations including MSM; improve the laboratory diagnostics; establish networks of laboratories to perform quality-assured culture and AMR testing; strengthen the surveillance of *N. gonorrhoeae* AMR and gonorrhoea treatment failures; improve the detection, verification and follow-up of verified treatment failures; and substantially intensify the research to identify or develop alternative therapeutic strategies and particularly compounds for treatment of gonorrhoea (23). Region-specific response plans, with similar aims, for the EU/EEA and the USA were also launched in 2012 by the ECDC (Stockholm, Sweden) and Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia, USA), respectively (24,25).

A thoroughly revised and updated European guideline on gonorrhoea diagnosis and treatment was also finalized and approved in 2012 (12). In this 2012 guideline, ceftriaxone 500 mg×1 intramuscularly together with azithromycin 2 g×1 orally is the recommended first-line treatment for uncomplicated gonorrhoea of urethra, cervix, rectum or pharynx, i.e. when the antimicrobial susceptibility of the gonococcal strain is unknown. This regimen will also effectively eradicate possible concomitant *C. trachomatis* infections. Penicillins or tetracyclines are not recommended for treatment of any uncomplicated gonorrhoea cases, and azithromycin and ciprofloxacin/ofloxacin should only be used if antimicrobial susceptibility testing has proven the gonococcal strain susceptible (12). It is essential to promptly revise the official Polish treatment guidelines, from 1974, which recommend penicillin G as the first-line treatment for gonorrhoea.

CONTEMPORARY LABORATORY DIAGNOSIS OF GONORRHOEA

Gonorrhoea is frequently asymptomatic, particularly in women, pharynx and rectum, and symptoms, if present, are commonly non-specific. Accordingly, appropriate laboratory procedures are needed for diagnosis, case-finding and test-of-cure. The laboratory diagnosis of gonorrhoea is established by detection of *N. gonorrhoeae* (or its genetic material) in genital or extra-genital secretions using at least one of the follow-

ing methods: microscopy of Gram or methylene blue stained smear, culture or molecular methods (nucleic acid amplification tests – NAATs). Ideally, assessment of the AMR of gonococcal isolates should be an integral part of the laboratory diagnosis of gonorrhoea.

Gonococci can be identified as intracellular diplococci in polymorphonuclear leukocytes in microscopy (magnification ×1000) of Gram or methylene blue stained smears. The method has advantages as low cost and almost immediate results but the performance characteristics highly depend on the experience of the microscopist. Moreover, it can provide a high sensitivity (95%) and specificity (97%) in the diagnosis of gonorrhoea in symptomatic men with urethral discharge (12,26,27). However, microscopy is not recommended as the sole method for the diagnosis of gonorrhoea in asymptomatic patients, or of cervical, pharyngeal or rectal gonococcal infections, as negative results do not exclude infection due to the low sensitivity of microscopy. For example, only 40-60% of culture-positive cervical specimens are positive in microscopy (12,26,27).

Culture, the old 'gold standard', offers high sensitivity (however, compared to NAATs lower sensitivity particularly for extragenital samples such as pharyngeal and rectal), up to 100% specificity (if appropriate species verifying assays are applied) and is the only method that enables AMR testing. Nevertheless, the method is relatively slow and, to obtain high sensitivity and specificity, it is crucial to strictly optimize the conditions for sample collection, transport, storage and the culture methodology, as gonococci are exceedingly sensitive to external environmental factors. Urethral, endocervical, rectal, pharyngeal or conjunctival swabs are incubated on nutritious selective culture media, with antimicrobials added to inhibit growth of other bacteria and fungi, for 24-48 hours in a temperature of 35-36°C and in an atmosphere enriched with 4-6% CO_2. Colonies of typical appearance should be presumptively identified as gonococci with microscopy after Gram staining and oxidase test (showing rapid positive reaction). Ideally and for definitive identification, also at least one species verifying assay should be used, i.e. a carbohydrate utilization assay (*N. gonorrhoeae* degrades glucose but not maltose, sucrose, fructose or lactose), agglutination assay, biochemical test, NAAT or direct immunofluorescence assay (12,26,27). As culture is the only method allowing AMR testing, it is essential to maintain and, where necessary, strengthen the culture capacity in all countries.

NAATs do not require the presence of viable gonococci; have a superior sensitivity to all other diagnostic methods particularly for pharyngeal and rectal specimens; are less demanding in specimen collection (urine in males, and other non-invasive self-collected samples, such as vaginal swabs in females, can effectively be used), transportation and storage; are rapid and allow automation (12,26-30). NAATs also enable simultaneous detection of several pathogens and, due to the high sensitivity, are effective in detecting both symptomatic and asymptomatic infections. However, importantly NAATs do not allow AMR testing, the most appropriate time for test-of-cure remains unknown and the available NAATs differ in their sensitivity and specificity. Commensal *Neisseria* species present particularly in pharynx and rectum but also more rarely in the urogenital tract, have genetic homology with *N. gonorrhoeae* and might cross-react resulting in false-positive reports (29,30). Consequently, no gonococcal NAATs have yet been licensed by the US Food and Drug Administration (FDA) for diagnosing extragenital gonococcal infections. The main problem is that this slightly suboptimal specificity results in low positive predictive value (PPV – defined as probability of having the disease in case of a positive test result) particularly in low-prevalent populations. If the PPV using NAAT does not exceed 90% in the local setting, it has been recommended that a supplementary NAAT (with different target sequence) should be used for verification of all samples positive with the screening NAAT (12,27). In settings using only NAATs for detection of *N. gonorrhoeae* it is essential to have or be involved in an adequate local, national or international gonococcal AMR surveillance programme. Worryingly, in Poland microscopy of stained smears remains the most frequently used method for detection of *N. gonorrhoeae*, and it is crucial to substantially enhance the capacity for using culture and NAATs for diagnosis of gonorrhoea.

CONCLUSIONS

Gonorrhoea remains a diagnostic and particularly a therapeutic challenge globally, and currently it is feared that gonorrhoea might even become untreatable. In Poland, the officially reported incidence of gonorrhoea is exceedingly low compared to many of the other EU/EEA countries. This might indicate a suboptimal diagnostics and incomplete case reporting and epidemiological surveillance. Accordingly, a well-designed study surveying the laboratory diagnostics for gonorrhoea and the case reporting system, including the epidemiological surveillance, in Poland is recommended. The rapidly increasing resistance to basically all antimicrobial agents in *N. gonorrhoeae* strains globally and the lack of internationally reported AMR data for *N. gonorrhoeae* strains circulating in Poland are grave concerns. Consequently, it is imperative to implement some regular and quality assured antimicrobial susceptibility surveillance for *N. gonorrhoeae* in Poland and to promptly revise the official national Polish treatment guidelines from 1970s.

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