

# Antibiotic resistance and NG-MAST sequence types of *Neisseria gonorrhoeae* isolates in Poland compared to the world

Beata Mlynarczyk-Bonikowska<sup>1</sup>, Magdalena Malejczyk<sup>1</sup>, Sławomir Majewski<sup>2</sup>, Magnus Unemo<sup>3</sup>

<sup>1</sup>Department of Diagnostics of Sexually Transmitted Diseases, Medical University of Warsaw, Warsaw, Poland

<sup>2</sup>Department of Dermatology and Venereology, Medical University of Warsaw, Warsaw, Poland

<sup>3</sup>World Health Organization (WHO) Collaborating Centre for Gonorrhoea and other STIs, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

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## Abstract

Gonorrhoea is one of the most common sexually transmitted infections and in 2012, the World Health Organization estimated about 78 million of new global urogenital cases among adults per year. The main concern during the latest decade has been the emergence and spread of multidrug-resistant strains of *Neisseria gonorrhoeae*. Resistance has emerged internationally to the extended-spectrum cephalosporins, ceftriaxone and cefixime, which are the last remaining options for empiric first-line monotherapy of gonorrhoea. In Poland, the levels of resistance to ciprofloxacin, benzylpenicillin and tetracycline are high, and the prevalence of azithromycin resistance has increased. However, no resistance to ceftriaxone has been identified. The currently spread multidrug-resistant strains frequently represent epidemic clones. The present paper reviews and describes the antimicrobial resistance and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) sequence types of *N. gonorrhoeae* strains spreading in Poland compared to the world.

**Key words:** *Neisseria gonorrhoeae*, gonorrhoea, antimicrobial resistance, ceftriaxone, azithromycin, NG-MAST, sequence type, genogroup.

## Introduction

*Neisseria gonorrhoeae* is the etiological agent of gonorrhoea, one of the most common bacterial sexually transmitted infections. In 2012, the World Health Organization (WHO) estimated about 78 million of new global urogenital cases among adults per year [1]. Laboratory diagnostics of gonorrhoea includes microscopy, nucleic acid amplification tests (NAATs), and *in vitro* culture. Culture is the only method that allows for antimicrobial susceptibility testing and accordingly the culture remains essential [2]. Initially, *N. gonorrhoeae* showed a high susceptibility to many classes of antimicrobials and the treatment of gonorrhoea did not pose any major problems. However, since the first antimicrobials (sulfonamides) were introduced for treatment of gonorrhoea in the mid-1930s, *N. gonorrhoeae* has shown an extraordinary capacity to develop or acquire resistance to all therapeutic antimicrobials recommended. At present, internationally there is a high prevalence of *N. gonorrhoeae* strains with resistance to majority of antimicrobials earlier and currently

widely used for treatment (e.g., sulfonamides, penicillins, earlier generation cephalosporins, tetracyclines, earlier generation macrolides and fluoroquinolones) [3]. In the mid-1990s, the first strains resistant to ceftriaxone and cefixime, the last remaining options for empiric first-line monotherapy of gonorrhoea, emerged in Japan [4], and subsequently started to spread internationally. In the recent decade, decreased susceptibility or resistance to ceftriaxone and cefixime has been reported in most countries worldwide [5]. This development is a major public health concern and in 2017, *N. gonorrhoeae* was included on the WHO list of 12 antibiotic-resistant pathogens that pose a global threat to human health and require priority efforts towards developing new antibiotics [6].

## Antimicrobial resistance of *N. gonorrhoeae* isolates in Poland

In Poland, no gonococcal isolates with resistance to ceftriaxone have been identified [7–10]. However, the

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**Address for correspondence:** Prof. Sławomir Majewski MD, PhD, Department of Dermatology and Venereology, Medical University of Warsaw, 82 A Koszykowa St, 02-008 Warsaw, Poland, phone: +48 22 502 13 13, fax: +48 22 502 13 13, e-mail: [slawomir.majewski@wum.edu.pl](mailto:slawomir.majewski@wum.edu.pl)

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proportion of isolates with decreased susceptibility to these antibiotic has increased, including the rate of isolates with a ceftriaxone minimum inhibitory concentration (MIC) of 0.125 mg/l (exactly at the resistance breakpoint) [8, 9]. In 2016 resistance to cefixime was detected first time (5.3% of isolates from Poland in Euro-GASP). The prevalence of azithromycin-resistant isolates in 2010–2015 ranged from 5.4% to 19.0% [7, 8, 10, 11]. Some azithromycin-resistant strains belonging to the *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) genogroups G1407, G1478, and G21 exhibited a C2611T mutation in all the four 23S rRNA alleles, which result in an azithromycin MIC of 4–32 mg/l [12]. The prevalence of ciprofloxacin-resistant isolates in 2010–2015 varied from 50.0% to 71.7% [7, 8, 10, 13]. Most isolates were resistant or intermediately resistant, with only 2.8% and 1.7% of strains susceptible, to benzyl penicillin, in 2012 and 2013, respectively [8, 9]. The prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG) strains has been mainly low [8, 9, 14] illustrating that most of the resistance to benzyl penicillin is chromosomally-mediated. The prevalence of tetracycline-resistant isolates in 2010–2013 ranged from 38.0% to 50.8% [8, 15].

#### Antimicrobial resistance of *N. gonorrhoeae* isolates all over the world

In the recent decade, *N. gonorrhoeae* isolates with decreased susceptibility or resistance to ceftriaxone and cefixime have been identified in most countries globally [5]. The first gonococcal strain with high-level resistance to ceftriaxone (MIC = 2 mg/l) and cefixime (MIC = 4 mg/l) was isolated in Kyoto, Japan, in 2009 [16, 17]. Another strain with high-level resistance to ceftriaxone and cefixime (MICs of 1 mg/l and 2 mg/l, respectively) was isolated in France in 2010 [18], and two isolates similar to the French strain were later identified in Spain in 2012 [19]. None of these strains appear to have spread further [5, 10, 20–26], which is likely due to a suboptimal biological fitness of these strains [27]. Subsequently, additional sporadic ceftriaxone-resistant isolates with a ceftriaxone MIC of  $\geq 0.5$  mg/l have also been characterized in more detail during the latest years, for example, isolates from Australia in 2013 and 2017 [28, 29], Argentina in 2014 [30], Japan in 2014 and 2015 [31, 32], Denmark in 2017 [33], and Canada in 2017 [34]. Retrospective studies in China [35–37] and Japan [38] have also identified relatively high proportions of ceftriaxone-resistant isolates. It is a grave public health concern that the first three *N. gonorrhoeae* isolates globally with resistance to ceftriaxone combined with high-level resistance to azithromycin (MIC  $\geq 256$  mg/l) were identified at the beginning of 2018 in the United Kingdom (1 case) [39] and a few months later in Australia (2 cases) [40]. These gonococcal strains pose a main threat to the first-line dual antimicrobial therapy (ceftriaxone of 250–500 mg administered intramuscu-

larly plus azithromycin of 1–2 g administered orally) recommended in Europe [41] and other settings worldwide.

In the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP), 26 (0.26% of 9910 isolates) ceftriaxone-resistant isolates were reported from 2011 to 2015 (10 in 2011), 3 in 2012, 7 in 2013, 5 in 2014 and 1 isolate in 2015). These ceftriaxone-resistant isolates were cultured in Greece ( $n = 4$ ), Spain ( $n = 6$ ), Germany ( $n = 10$ ) and Austria ( $n = 3$ ), and one isolate each in Norway, Ireland, and Slovenia [10, 21–25]. Resistance rates to cefixime have been substantially higher and, in 2012–2014, cefixime-resistant isolates were found in most European countries; in 2013, their prevalence was highest in Spain (15.1%) and Greece (14.5%), and in 2014, in Belgium (12.1%). Resistance to azithromycin has also been found in nearly all European countries (except for Estonia and Iceland), with the highest prevalence in Greece (39.6%) and Ireland (37.6%) in 2014, and in Greece (22.7%) in 2013. Resistance to ciprofloxacin was found in all European countries, with the highest rates (more than 70%) in Austria, Greece, and Germany, and the lowest in Estonia (2.0%); PPNG strains were most common in Austria, Sweden, and Norway [10, 21–24].

The prevalence of resistance to cefixime, azithromycin, ciprofloxacin in various countries stratified by years is presented in Table 1. For countries continuously participating in Euro-GASP, the presented resistance data span 2012–2015 [10, 21–25]. Moreover, resistance data from some non-EU/EEA WHO European region countries (Belarus and Russia) have been included [42–45].

#### Molecular epidemiological typing of *N. gonorrhoeae*

Molecular epidemiological typing, particularly linked to antimicrobial resistance and epidemiological data of patients, can be exceedingly valuable. The typing can identify the spread of antimicrobial resistant strains, potentially more virulent strains, and describe changing strain distributions overall and among risk groups [46, 47].

For *N. gonorrhoeae*, NG-MAST has been the most frequently used molecular typing method [8, 12, 46–48]. NG-MAST includes sequencing of parts of two more variable genes: *porB* (encoding the transmembrane channel protein porin B (PorB)) and *tbpB* (encoding the transferin-binding protein B (TbpB)). The sequences are subsequently assigned allele numbers and, at present, more than 9800 *porB* alleles and 2,700 *tbpB* alleles have been described. The combination of the *porB* and *tbpB* allele number is assigned an NG-MAST sequence type (ST). At present, approximately 16,800 NG-MAST STs have been described ([www.ng-mast.net](http://www.ng-mast.net)).

The proportions of different NG-MAST STs in different countries have been summarized in Table 2 [8, 35, 36, 42–44, 49–57]. Briefly, in 2009–2010, the most common STs in Europe were as follows: ST1407 (15.6%) of genogroup G1407;

**Table 1.** Antimicrobial resistance of *Neisseria gonorrhoeae* in the European Union (EU)/European Economic Area (EEA) and some non-EU/EEA WHO European region countries (Belarus and Russia)

Country	Year	% of resistance			PPNG (%)
		CFX	AZT	CIP	
Austria*	2012/13/14/15	5/6/0/0	3/6/2/3	74/72/53/66	32/26/24/32
Belarus	2012/13	0/3	1/0	21/28	0/0
Belgium*	2012/13/14/15	1/6/12/11	2/2/4/3	56/56/58/50	10/16/19/14
Croatia*	2015	0	0	38	13
Cyprus*	2015	0	0	67	0
Denmark*	2012/13/14/15	12/12/6/0	14/9/4/3	59/58/33/31	18/8/9/9
Estonia*	2014/15	0/0	0/0	2/28	0/0
France*	2012/13/14/15	2/4/1/0	0/0/11/6	39/45/51/42	7/33/ND/ND
Germany*	2012/13/14/15	6/13/0/2	2/4/3/2	74/63/63/62	19/17/13/16
Greece*	2012/13/14/15	6/15/5/11	6/23/40/22	69/72/70/77	12/3/6/11
Hungary*	2012/13/14/15	6/7/1/2	0/2/1/5	66/68/55/53	1/7/14/14
Iceland*	2013/14/15	0/0/0	0/0/0	40/58/29	ND/22/0
Ireland*	2012/13/14/15	4/0/0/1	9/3/38/18	23/26/35/46	3/5/2/11
Italy*	2012/13/14/15	6/0/2/0	2/1/6/2	65/63/78/71	9/9/8/7
Latvia*	2012/13/14/15	3/3/0/0	5/16/14/0	39/26/19/11	0/13/0/0
Malta*	2012/13/14/15	0/0/0/0	0/0/5/14	56/36/57/66	19/6/10/24
Netherlands*	2012/13/14/15	0/0/2/1	1/1/2/4	34/35/32/37	ND/ND/ND/ND
Norway*	2012/13/14/15	6/5/6/1	13/11/5/4	56/80/74/59	27/29/29/25
<b>Poland*</b>	<b>2012/13/14/15</b>	<b>0/ND/0/0</b>	<b>10/19/9/5</b>	<b>69/62/65/57</b>	<b>4/9/0/5</b>
Portugal*	2012/13/14/15	0/0/0/0	2/18/17/17	41/47/36/37	8/6/7/8
Russia	2012/2015	ND/ND	17/2	26/40	0/0
Slovakia*	2012/13/14/15	4/5/1/4	3/2/4/2	54/47/68/54	6/4/25/26
Slovenia*	2012/13/14/15	4/1/1/0	15/0/2/0	40/63/45/35	9/14/22/5
Spain*	2012/13/14/15	15/15/0/2	10/8/7/3	58/66/68/65	15/13/19/20
Sweden*	2012/13/14/15	0/0/0/0	6/9/4/14	57/60/57/45	31/18/19/13
UK*	2012/13/14/15	0/1/0/0	2/0/1/13	28/32/33/40	5/10/9/19
EU/EEA**	2012/13/14/15	4/5/2/2	5/5/8/7	50/53/51/49	13/13/14/15

\*Countries participating in Euro-GASP included in the EU/EEA average, CFX – cefixime (MIC > 0.125 mg/l), AZT – azithromycin (MIC > 0.5 mg/l), CIP – ciprofloxacin (2012: MIC > 0.5 mg/l; 2013–2015: MIC > 0.064 mg/l), PPNG – penicillinase-producing *Neisseria gonorrhoeae*, ND – not done.

ST2992 (7.1%) of G2992; and ST225 (4.7%) of G225. ST1407 predominated in the European countries – Austria, Belgium, Italy, the Netherlands, Portugal, Romania, Slovenia, Spain, Switzerland, the United Kingdom [58], but also in Canada [50, 51] and the USA [56, 59]. ST2992 predominated in Ireland and Norway, while ST225 predominated in Denmark, Sweden, and on Malta [58]. In 2013, the most frequent STs in Europe remained ST1407 (7.6%) and ST2992 (6.7%), with an increase in the rates of ST2400 (3.9%, genogroup G2400). ST1407 predominated in Belgium, Hungary, Norway, Portugal, Slovenia, Spain and Switzerland. ST2992 predominated in Ireland, Italy, on Malta, in the Netherlands, and the United

Kingdom. ST2400 was detected in eight countries but was not the predominant ST in any of them [55].

In recent years, whole genome sequencing (WGS) has been increasingly used for epidemiological typing of *N. gonorrhoeae*. Compared to NG-MAST, WGS has a substantially higher and more accurate resolution and can identify multidrug-resistant clades linked to risk groups and additionally predict antimicrobial resistance with a relatively high sensitivity and specificity [12, 52, 59, 60]. The WGS will likely be the method of choice for molecular epidemiology and in antimicrobial resistance surveillance of *N. gonorrhoeae* soon. However, appropriate funding,

**Table 2.** Prevalence of NG-MAST sequence types in Europe and selected non-European countries

Country	Year	Sequence type (ST)	% ST	Country	Year	Sequence type (ST)	% ST
Australia	2011–2013	758/9716/7126	22/13/11	Latvia	2009–2010 2013	3227/5185/5340/225 5/10828/21/4269	41/10/7/7 37/13/8/8
Austria	2010 2013	1407/387/359/5600 3785/11575/4995/387/225	32/10/8/8 17/11/7/7/7	Malta	2009–2010 2013	225/5463/1407/4949 2992/484/10788/	46/12/6/6 33/14/14
Belarus	2010–2013	1993/807/285/9735	28/7/4/4	Netherlands	2010 2013	1407/2992/5402/2400 2992/2400/8919	16/7/7/6 10/10/6
Belgium	2010 2013	1407/387/2992 1407/387/2992	14/12/8 16/15/15	Norway	2009–2010 2013	2992/1407/4347 1407/4275/2400	23/14/10 9/7/6
Canada	2010 2011 2012 2013 2014	1407/3150/3158 1407/3307/3550 1407/2400/3150 2400/9663/5985 2400/5985/10451	13/12/9 15/10/6 11/7/7 12/7/6 14/14/10	Pakistan	2012–2014	338/3328/10876/10886	3/3/3/3
China	2012–2013	2318/1866/4846	3/2/1	<b>Poland</b>	<b>2010</b> <b>2011</b> <b>2012</b>	<b>1405/5421/225</b> <b>1407/2992/8379</b> <b>1407/8391/1861/2992</b>	<b>18/18/14</b> <b>13/13/9</b> <b>43/7/5/5</b>
Denmark	2010 2013	225/3158/1407 1993/1407/2400	12/12/6 18/13/7	Portugal	2009–2010 2013	1407/1034/2992 1407/7445/2	14/8/8 16/11/5
France	2010 2013	2/2992/1407 645/11352/225/2400/2992/ 3149/ 4995/5624/11357	14/14/8 9/5/4/4/4/ 4/4/4/4	Romania	2010	1407/4120/3952/5330	22/22/11/11
Germany	2010 2013	25/1407/5425 4995/25/359/5441/9500	32/28/6 8/6/6/6/6	Russia	2011–2012 2015	807/5714/228 9476/807/1544/5714/12531	8/6/3 11/8/5/5/5
Greece	2010 2013	5405/5505/1407 3128/225/4730/11055	16/16/12 19/10/10/10	Slovakia	2010 2013	437/5595/5598 1407/359/11042	26/20/10 14/14/13
Greenland	2012–2013	210/9816/2400	50/26/10	Slovenia	2009–2010 2013	1407/225/5570 21/10801/10800/10798	28/16/10 13/13/9/9
Hungary	2010 2013	5332/356/1407/5333 1407/995/387/8115/11046	29/12/12/12 21/13/6/6/6	Spain	2009–2010 2013	1407/2992/292/3149/1861 1407/7232/2992/21	28/9/4/4/4 11/8/7/7
Ireland	2010 2013	2992/384/1407 2992/384/21/437/10843/649/ 2400/4643/10846	32/12/6 16/11/4/4/ 4/4/4/4/4	Sweden	2010 2013	225/2992/5175/5177/5188/5210 5445/7445/7164	8/6/6/6/6/6 10/6/6
Italy	2010 2013	1407/2992/995 2992/6360/2400/1407	34/10/10 18/12/12/10	Switzerland	2009 2011	1407/2058 1407/2992	38/25 22/22
				United Kingdom	2010 2013	1407/2/51 2992/51/4995	14/6/6 10/10/9
				USA	2011	1407/7268/5895	4/2/1

NG-MAST – *Neisseria gonorrhoeae* multiantigen sequence typing.

capacity building and quality assurance remain essential for this transition.

### Molecular epidemiological typing of *N. gonorrhoeae* isolated in Poland

In Poland, *N. gonorrhoeae* isolates cultured in 2010–2012 have been examined by NG-MAST [8]. The most common STs in 2010 were ST5421 (17.9%; G1479), ST1405 (17.9%; G1405), and ST225 (14.3%; G225). In 2011, the most prevalent STs were the multidrug-resistant ST1407 (13.0%; G1407), ST2992 (13.0%; G2992), and ST8379 (8.7%; G8379). In 2012, nearly half of the isolates were assigned as ST1407 (44.3%; G1407), followed by ST8391 (6.5%; G5043), ST2992 (4.6%; G2992), ST1861 (4.6%; G1861), ST21 (3.7%; G21), and ST8392 (3.7%; G8392) [8].

### Conclusions

The extensive spread of the multidrug-resistant *N. gonorrhoeae* NG-MAST G1407 in Europe, including Poland, has been raising major concerns. However, fortunately the prevalence of G1407 significantly decreased from 2009/2010 to 2013 in Europe [60] and no isolates resistant to ceftriaxone have been detected in Poland so far. Nevertheless, isolates with ceftriaxone MIC exactly at the ceftriaxone resistance breakpoint (MIC = 0.125 mg/l) have been identified in Poland and ceftriaxone-resistant isolates have been cultured in neighboring countries. In 2016 the first cefixime-resistant isolates were observed in Poland. Furthermore, sporadic ceftriaxone-resistant isolates belonging to other NG-MAST genogroups and with a ceftriaxone MIC of  $\geq 0.5$  mg/l have also been identified and characterized in more detail during the latest years, e.g. isolates from Australia [28, 29], Argentina [30], Japan

[31, 32], Denmark [33] and Canada [34]. Recently the first three *N. gonorrhoeae* isolates in the world with resistance to ceftriaxone combined with high-level resistance to azithromycin (MIC  $\geq$  256 mg/l) have been identified. Consequently, it is a major concern that *N. gonorrhoeae* strains with resistance to ceftriaxone will be introduced and spreading in Poland in the near future. An enhanced, quality assured surveillance of antimicrobial resistance and antimicrobial resistance determinants in *N. gonorrhoeae* is essential in Poland, as well as in most other countries.

### Conflict of interest

The authors declare no conflict of interest.

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