

The Russian gonococcal antimicrobial susceptibility programme (RU-GASP) – national resistance prevalence in 2007 and 2008, and trends during 2005-2008

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Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a major problem worldwide. In the former Soviet countries including Russia, the knowledge regarding AMR has been highly limited. However, in 2004 the Russian gonococcal antimicrobial susceptibility programme (RU-GASP) was initiated. The aims of this study were to examine and describe the prevalence of *N. gonorrhoeae* AMR in 2007 and 2008 in Russia, and reveal trends in the period from 2005 to 2008. Gonococcal isolates (660 in 2007 and 900 in 2008) from 36 surveillance sites were examined using agar dilution method. From 2005 to 2008, the proportion of isolates resistant to spectinomycin increased from 0% to 7.2%, and remained high for those resistant to ciprofloxacin (approximately 49%). The resistance to azithromycin was 2.3% and 0.4% in 2007 and 2008, respectively. All isolates between 2005 and 2008 were susceptible to ceftriaxone. In conclusion, the AMR of *N. gonorrhoeae* in Russia is high, as in most countries in the European Union, and ceftriaxone should be the first line for treatment. If there is no access to ceftriaxone or in the presence of severe beta-lactam antimicrobial allergy, spectinomycin should be used; however, the resistance to spectinomycin has increased. Regular, quality-assured national and international surveillance of AMR in *N. gonorrhoeae* is crucial globally for public health.

Introduction

Gonorrhoea remains one of the most common sexually transmitted infections (STIs) in most countries [1]. In Russia, the estimated gonorrhoea incidences were 60.8 and 56.4 cases per 100,000 inhabitants in 2007 and 2008, respectively. However, the incidence varied substantially in the seven federal districts (FDs) of Russia. The incidence in Russia remains high. Nevertheless, the incidence from 1993 to 2008, with exception of the years 1999 and 2000, decreased from 230.9 to 56.4 cases per 100,000 inhabitants [2].

The impact of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* on effective treatment and, accordingly, control of gonorrhoea is of long-standing concern. Emergence and transmission of resistance in *N. gonorrhoeae* to most antimicrobials used for treatment are major problems globally [3-14], and the World Health Organization (WHO) has recently published initiatives to enhance global *N. gonorrhoeae* AMR surveillance that focus on multidrug- and extensively drug-resistant *N. gonorrhoeae* [3]. Expanded, but quality-assured and quality-controlled, AMR surveillance worldwide is crucial to inform STI management and treatment guidelines and, accordingly, for public health [3,4,8,13,14].

In the European Union (EU) countries, a *N. gonorrhoeae* AMR surveillance programme, the European gonococcal antimicrobial susceptibility programme (EURO-GASP), has been running since 2004 [11]. However, in eastern Europe and the former Soviet countries, including Russia and its highly diverse federal districts (FDs), the knowledge regarding AMR in *N. gonorrhoeae*, which is crucial for the empirical treatment, has been highly limited [15-18]. However, in 2004 the national Russian GASP (RU-GASP), coordinated at the State Research Center of Dermatology and Venereology of the Russian Ministry of Health (SRCDV), Moscow, was initiated with the main objective to inform the STI management and treatment guidelines in Russia. In this programme, SRCDV initially implemented optimised and quality-assured systems for collection, storage and transportation of clinical specimens and *N. gonorrhoeae* cultures from the different FDs of Russia to SRCDV. In 2008, RU-GASP published the first ever international report that described the *N. gonorrhoeae* AMR in 2005 and 2006 in Russia [10]. The aims of the present study were to examine and describe the prevalence of *N. gonorrhoeae* antimicrobial resistance in 2007 and

2008 in Russia, including all seven FDs, and to reveal trends in the resistance from 2005 to 2008.

Materials and methods

Study population

As previously described [10], dermato-venereological clinics situated all over Russia are surveyed in RU-GASP. In the present study, *N. gonorrhoeae* isolates from 36 surveillance sites, which were selected to represent all the FDs of Russia, were examined and the results were compared to the previously published results from 2005 and 2006 [10].

Representative, i.e. mainly consecutive, culture-positive patients attending the clinics from January 2007 to December 2008 were included. The inclusion criterion was: male or female patient (12 to 60 years of age), with diagnosed (clinically and using culture) symptomatic uncomplicated gonorrhoea. Exclusion criteria were: i) refusing participation and ii) presence of serious somatic pathology or disease of the central nervous system.

Diagnostics, culture conditions and preservation of *N. gonorrhoeae* isolates

A clinical examination was performed, and specimens (urethral and cervical from females, and urethral from males) were collected.

All specimens were cultured on selective culture media and the *N. gonorrhoeae* isolates were species-verified, preserved in cryomedium, and transported to SRCDV as previously described [10].

Antimicrobial susceptibility testing

At SRCDV, the susceptibility to ciprofloxacin, spectinomycin, ceftriaxone, and also azithromycin (not included before 2007) was determined using agar dilution method, according to the recommendations of Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) [19]. Accordingly, the minimum inhibitory concentration (MIC) breakpoints for susceptibility or resistance were $\leq 0.06/\geq 1$ for ciprofloxacin, $\leq 32/\geq 128$ for spectinomycin and ≤ 0.25 (susceptible) for ceftriaxone [19]. For azithromycin, CLSI does not describe any breakpoints, and the MIC breakpoints ($\leq 0.25/\geq 1$) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.esamid.org/

research_projects/eucast) were used. The susceptibility to penicillin G and tetracycline was also analysed using the CLSI method [19]. However, because penicillin G and tetracycline are not recommended for treatment of gonorrhoea, the susceptibility to these was not comprehensively analysed. For quality control, the CLSI-recommended *N. gonorrhoeae* reference strain ATCC 49226 was examined in each run [19]. However, during 2009, the 2008 WHO *N. gonorrhoeae* reference strains intended for quality assurance and quality control of gonococcal AMR surveillance [4] were also included in the quality control. Beta-lactamase production was identified using nitrocefin discs, according to the manufacturer's instructions (Cefinase discs; Becton Dickinson).

Results

Patient characteristics

N. gonorrhoeae isolates (one isolate per patient) from 660 patients in 2007 (594 males and 66 females) and 900 patients in 2008 (766 males and 134 females) were examined. The mean ages of the men were 25 years (median age: 27 years; range: 15 to 45 years) and 22 years (median age: 23 years; range: 14 to 57 years) in 2007 and 2008, respectively. The mean ages of the women were 21 years (median age: 23 years; range: 12 to 35 years) and 19 years (median age: 20 years; range: 12 to 60 years) in 2007 and 2008, respectively. In order to reveal any trends, the results of the AMR testing for these patients were compared to previously published results from 2005 and 2006 [10]. The gender distribution and age distribution were relatively similar during the four years compared.

Antimicrobial susceptibility of *N. gonorrhoeae* isolated in Russia in 2007 and 2008

The proportions of the *N. gonorrhoeae* isolates that displayed resistance and intermediate susceptibility to the four antimicrobials used in the recommended gonorrhoea treatment in 2007 and 2008 are described in Table 1.

Briefly, the proportions of isolates displaying resistance in 2007 and 2008, respectively, were: 49.6% and 49.1% for ciprofloxacin, 2.3% and 0.4% for azithromycin, 0.9% and 7.2% for spectinomycin, and 0% and 0% for ceftriaxone (Table 1). Of the *N. gonorrhoeae* isolates, 0.0% and 2.2% were beta-lactamase-producing

TABLE 1

Proportion of *Neisseria gonorrhoeae* isolates in Russia displaying resistance and intermediate susceptibility to the four antimicrobials used in the recommended gonorrhoea treatment, Russia, 2007 (n=660) and 2008 (n=900)

	Proportion of isolates (%)			
	Intermediate susceptible		Resistant	
	2007	2008	2007	2008
Ciprofloxacin (S \leq 0.06 mg/l; R \geq 1 mg/l)	5.5	7.5	49.6	49.1
Spectinomycin (S \leq 32; R \geq 128)	3.6	1.1	0.9	7.2
Ceftriaxone (S \leq 0.25)	0	0	0	0
Azithromycin (S \leq 0.25; R \geq 1)	7.4	4.8	2.3	0.4

R: resistant; S: susceptible

in 2007 and 2008, respectively. The proportions of isolates displaying resistance or intermediate susceptibility to penicillin G and tetracycline in 2007 and 2008 (in parenthesis) were: 72.4% (81.3%) and 67.2% (85.5%), respectively. The susceptibility to these antimicrobials was not further evaluated, because they are not recommended for treatment. From 2005 to 2008, the proportion of isolates resistant to spectinomycin increased significantly from 0% to 7.2%, and remained high to ciprofloxacin (at approximately 49%) [10]. All isolates (100%) from these four years were susceptible to ceftriaxone (Table 1; [10]). Nevertheless, examining the MIC distribution of ceftriaxone in general, the MIC values of ceftriaxone increased in the period from 2005 to 2008, and isolates at the breakpoint (especially in 2007: MIC=0.25 mg/l, n=14) were increasingly identified (data not shown).

Furthermore, multiple resistance to several of the four antimicrobials used in the recommended treatment of gonorrhoea was common (Table 2). In the period from 2007 to 2008, the level of *N. gonorrhoeae* isolates

TABLE 2

Proportion of *Neisseria gonorrhoeae* isolates in Russia displaying multiple resistance to several of the antimicrobials used in the recommended gonorrhoea treatment, Russia, 2007 (n=660) and 2008 (n=900)

Year	CIP+SPM	CIP+SPM+AZM
2007 (n=660)	0 (4.5)	0 (2.6)
2008 (n=900)	6.0 (6.5)	0.4 (1.5)

AZM: azithromycin; CIP: ciprofloxacin; SPM: spectinomycin. Resistance (resistance or intermediate susceptibility) are shown. All isolates were susceptible to ceftriaxone.

TABLE 3

Proportion of *Neisseria gonorrhoeae* isolates displaying resistance or intermediate susceptibility to the four antimicrobials used in the recommended gonorrhoea treatment in 2007 and 2008 in all the seven federal districts (FDs) of Russia

Federal district	Year (no. of isolates)	Ciprofloxacin (S≤0.06 mg/l; R≥1 mg/l)	Spectinomycin (S≤32; R≥128)	Ceftriaxone (S≤0.25)	Azithromycin (S≤0.25; R≥1)
Central	2007 (n=99)	52.5	5.1	0	9.0
	2008 (n=210)	58.8	6.0	0	10.1
North-western	2007 (n=185)	51.5	2.9	0	10.0
	2008 (n=112)	53.8	12.1	0	9.6
Southern	2007 (n=75)	24.0	4.0	0	8.0
	2008 (n=115)	34.0	8.7	0	5.4
Volga	2007 (n=198)	60.3	2.7	0	10.0
	2008 (n=256)	50.7	9.6	0	3.4
Urals	2007 (n=10)	40.0	10.0	0	0
	2008 (n=86)	83.3	6.5	0	0
Siberian	2007 (n=47)	73.7	13.2	0	21.0
	2008 (n=121)	71.4	6.0	0	0
Far-eastern	2007 (n=46)	71.1	0	0	0
	2008 ^a	nd ^a	nd ^a	nd ^a	nd ^b

nd: not done; R: resistant; S: susceptible.

^a Unfortunately, it was not possible to receive any viable *N. gonorrhoeae* isolates from the Far-eastern federal district in 2008.

resistant to ciprofloxacin+spectinomycin and to ciprofloxacin+spectinomycin+azithromycin increased from 0% to 6.0% and from 0% to 0.4%, respectively.

The levels of resistance or intermediate susceptibility to the antimicrobials in the seven FDs of Russia in 2007 and 2008 are summarised in Table 3.

Substantial regional differences regarding prevalence of gonococcal AMR in the different FDs of Russia were identified. The levels of resistance or intermediate susceptibility to ciprofloxacin were high in all the FDs. Most disquieting, resistance or intermediate susceptibility to spectinomycin and azithromycin was found in six and five, respectively, of the seven FDs.

Discussion

This study is the second ever international report from RU-GASP that annually, since the programme's initiation in 2004, surveys the antimicrobial resistance of *N. gonorrhoeae* in Russia. The present multicentre study comprehensively describes the antimicrobial resistance of *N. gonorrhoeae* in 2007 and 2008, including the trends during the period from 2005 to 2008, in all seven highly diverse FDs of the Russian Federation.

In Russia, the level of resistance of *N. gonorrhoeae* to all antimicrobials used in the traditional gonorrhoea treatment (penicillins, tetracycline and ciprofloxacin) is exceedingly high. However, between 2005 and 2008, all Russian isolates were susceptible to ceftriaxone and still no gonorrhoea treatment failures using ceftriaxone of appropriate quality and dosage has been described for urogenital gonorrhoea worldwide [3]. Nevertheless, in Russia and in many other countries [3] the MIC values of ceftriaxone have increased. Resistance to azithromycin was also identified, dispersed in five of the

seven FDs (range: 3.4% to 21% during 2007 and 2008), which may reflect the fact that azithromycin is commonly used in these FDs. The resistance to spectinomycin, which had not been identified in Russia in 2005 [10] and is rare internationally, also increased during 2007 and 2008 (0.9%-7.2%), which may reflect a frequent use [20]. A representative selection of these isolates was also confirmed as resistant using Etest and/or genetic methods; however, they only displayed a low level of resistance. The 2008 WHO *N. gonorrhoeae* reference strains intended for quality assurance and quality control of gonococcal AMR surveillance [4], which were implemented for quality control in Russia in 2009, will from now on confirm the validity of all the AMR results in RU-GASP.

Major longitudinal trends of the *N. gonorrhoeae* AMR in the different FDs of Russia remain difficult to interpret due to the limited and divergent sample sizes from each FD and the short time period for studying dynamics. In RU-GASP, efforts are continuously made to increase the representativeness and number of examined isolates, and the number has increased by 77% from 2005 (n=509 [10]) to 2008 (n=900).

A main objective of the RU-GASP, as for all AMR surveillance programmes, is to form the basis for continuous revision and updating of the Russian STI management and treatment guidelines. As previously stated [10], the RU-GASP has clearly highlighted that penicillins and tetracycline, as well as the fluoroquinolones used frequently since the 1990s should not be used for empirical gonorrhoea treatment. Furthermore, oral azithromycin is not recommended in empirical treatment because it needs to be administered in doses of 2 g to avoid treatment failures, i.e. doses that commonly give adverse gastro-intestinal effects, and resistant strains are spreading in Russia and increasingly in many other countries, including high-level resistance in England, Wales, Scotland and Italy [3,7,11,12,21-23]. Fluoroquinolones and azithromycin are not recommended for use in the gonorrhoea treatment unless MIC results are available for the specific isolates. The recommended first-line antimicrobial should be ceftriaxone (250 mg, 1×intramuscularly) and, if there is no access to ceftriaxone or in the presence of severe allergy to beta-lactam antimicrobials, spectinomycin (2 g, 1× intramuscularly) should be used. However, increasing levels of resistance to spectinomycin have been observed in Russia, and adequate monitoring of its use and of the treated patients is crucial.

RU-GASP has implemented optimised, harmonised and quality-assured culture diagnostics, as well as quality-assured and quality-controlled AMR testing in Russia, in accordance with national and international recommendations [17,19, 24-33]. In addition, the 2008 WHO *N. gonorrhoeae* reference strains intended for quality assurance and quality control of gonococcal AMR surveillance [3,4], a prerequisite for any global WHO AMR surveillance programme for *N. gonorrhoeae*,

were implemented during 2009 in quality assurance and control. The further rationale and applications for, and uses of, these reference strains are provided in WHO documents elsewhere [13]. These are used for the provision of internationally valid and comparable phenotypic and genetic AMR data worldwide. Genetic typing of resistance mechanisms, an additional aim for RU-GASP, is becoming increasingly relevant for surveillance of resistance to antimicrobials, especially for the expanded-spectrum, third generation cephalosporins [3,4,34,35], even though current testing remains based on MIC determinations.

Conclusion

In conclusion, the present national RU-GASP survey emphasises that the antimicrobial resistance of *N. gonorrhoeae* across Russia is exceedingly high and ceftriaxone should be the first-line antimicrobial for gonorrhoea treatment. If there is no access to ceftriaxone or in cases of severe beta-lactam antimicrobial allergy, spectinomycin should be used. Continuous and quality-assured local, national and international surveillance of *N. gonorrhoeae* antimicrobial susceptibility/resistance is crucial for public health purposes. It is fundamental to establish, quality-assure and quality-control regional and national GASP networks in many of the other eastern European countries, something that is presently in progress under WHO protocols [3,4,13].

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