Summary and Trends of the Russian Gonococcal Antimicrobial Surveillance Programme, 2005 to 2016

Alexey Kubanov,^a Viktoria Solomka,^a Xenia Plakhova,^a Aleksandr Chestkov,^a Natalya Petrova,^a Boris Shaskolskiy,^b Ekaterina Dementieva,^b Arvo Leinsoo,^b Dmitry Gryadunov,^b Dmitry Deryabin^a

^aState Research Center of Dermatovenereology and Cosmetology, Moscow, Russia

^bEngelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

ABSTRACT The Russian Gonococcal Antimicrobial Surveillance Programme (RU-GASP) was established in 2004 and operated continuously during the years from 2005 to 2016. The aims of this study were to summarize the RU-GASP results over this 12-year period and evaluate the trends in *Neisseria gonorrhoeae* antimicrobial resistance in Russia. In total, 5,038 verified *N. gonorrhoeae* isolates from 40 participating regions were tested for susceptibility to six antimicrobials via an agar dilution method. DNA loci involved in antimicrobial resistance were identified via minisequencing or DNA microarray techniques. From 2005 to 2016, increasing susceptibility to penicillin G (from 22.6% to 63.0%), tetracycline (from 34.8% to 53.0%), and ciprofloxacin (from 50.6% to 68.6%) was observed, but resistance to these drugs remained high. The proportions of isolates nonsusceptible to azithromycin and spectinomycin peaked in 2011 and decreased thereafter. Of the isolates, only 6 and 23 were identified as nonsusceptible to ceftriaxone according to the CLSI definitions and EUCAST breakpoint (0.57% of the total population), respectively. Comparison of *N. gonorrhoeae* antimicrobial resistance genetic determinants in 2005 versus those in 2016 showed a significant decrease in the number of isolates carrying chromosomal mutations. The proportion of isolates with wild-type genotypes increased from 11.7% in 2005 to 30.3% in 2016. Thus, the RU-GASP can be considered a successful gonorrhea surveillance program, and the current state of *N. gonorrhoeae* antimicrobial resistance in Russia is less serious than that in other WHO GASP regions.

KEYWORDS *Neisseria gonorrhoeae*, Russian Federation, antimicrobial resistance, drug susceptibility testing, gonorrhea morbidity, surveillance program

Due to widespread antimicrobial resistance (AMR) in *Neisseria gonorrhoeae*, a bacterial pathogen of global priority (1), management and control of gonococcal infection remain problematic. *N. gonorrhoeae* resistance has developed over the past 70 years since the advent of antibiotics, and resistance to penicillins, tetracyclines, and fluoroquinolones successively emerged following the introduction of these antimicrobials into clinical practice (2). The rapid decrease in antimicrobial susceptibility of *N. gonorrhoeae* to macrolides (azithromycin), aminocyclitols (spectinomycin), and especially extended-spectrum cephalosporins is an important modern public health problem associated with the risk of untreatable gonorrhea (3).

The notable ability of *N. gonorrhoeae* to develop AMR is determined by the acquisition of numerous chromosomal mutations and internal recombinations or the horizontal transfer of external AMR determinants using transformation and plasmid-mediated mechanisms (4). For example, mutations in the chromosomal *penA* and *ponA* genes result in the decreased affinity of penicillin-binding proteins (5), while horizontal transfer of the *bla*_{TEM} plasmid increases resistance to penicillins through the acquisition of beta-lactamase activity (6). Resistance to tetracyclines involves V57M or V57L amino

Citation Kubanov A, Solomka V, Plakhova X, Chestkov A, Petrova N, Shaskolskiy B, Dementieva E, Leinsoo A, Gryadunov D, Deryabin D. 2019. Summary and trends of the Russian Gonococcal Antimicrobial Surveillance Programme, 2005 to 2016. J Clin Microbiol 57:e02024-18. <https://doi.org/10.1128/JCM.02024-18>.

Editor Erik Munson, Marquette University
Copyright © 2019 American Society for Microbiology. All Rights Reserved.

Address correspondence to Dmitry Deryabin, dgderabin@yandex.ru.

Received 18 December 2018

Returned for modification 16 January 2019

Accepted 13 March 2019

Accepted manuscript posted online 20 March 2019

Published 24 May 2019

acid substitutions in ribosomal protein S10 (the *rpsJ* gene product) (7) and the plasmid-mediated *tetM* determinant (8). Resistance to fluoroquinolones is caused by mutations in the *gyrA* and *parC* genes encoding the gyrase and topoisomerase proteins that form a “quinolone pocket” (quinolone-resistant *N. gonorrhoeae* [QRNG] region) (9). Acquired resistance to spectinomycin and azithromycin is determined by mutations of the *rrs* (16S RNA) and *rrl* (23S RNA) genes, respectively (10, 11), which is accompanied by deletions or amino acid alterations in some ribosomal proteins. Nonspecific AMR mechanisms include mutations in amino acids 120 and 121 of *porB1b* (12) that decrease cell wall permeability as well as mutations in the promoter or coding region of the *mtrR* repressor that result in overexpression of the MtrCDE efflux pump (13); together, these mutations decrease the antibiotic concentration in the bacterial cell and contribute significantly to multidrug resistance.

Because commonly used molecular diagnostic tests for *N. gonorrhoeae* do not provide AMR results at the time of treatment and rapid AMR genetic kits (microarray-based assays [14] and multiplex bead suspension arrays [15]) are still commercially unavailable, clinical practitioners are required to treat gonococcal infection according to global and local treatment guidelines. The World Health Organization (WHO) recommends the use of an antimicrobial for gonococcal infection treatment when more than 95% of circulating *N. gonorrhoeae* isolates are susceptible to the drug (16). This approach requires continuous AMR monitoring with a standardized laboratory protocol.

Accordingly, the Gonococcal Antimicrobial Surveillance Programme (GASP) was initiated in 1992 to monitor gonococcal AMR in the Southeast Asia region, and GASP activities were subsequently extended to other WHO regions (17). The Russian Gonococcal Antimicrobial Surveillance Programme (RU-GASP) was established in 2004 following the establishment of the Euro-GASP (18). The RU-GASP is part of a worldwide laboratory network coordinated by the European WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections (19).

The main RU-GASP targets were as follows: (i) annual control of the emergence and spread of AMR in *N. gonorrhoeae*, i.e., trend analysis of AMR; (ii) the development of new molecular technologies for AMR genetic determinants to improve AMR surveillance data; and (iii) the presentation of AMR data for the revision of national gonorrhea treatment guidelines. Previous RU-GASP data have been presented in numerous international publications (20–23).

The aims of this study were to summarize the RU-GASP results over a 12-year period and to evaluate the trends in *N. gonorrhoeae* AMR in Russia in the context of changes in the recommended antimicrobial therapy regimes.

MATERIALS AND METHODS

Collection of *N. gonorrhoeae* isolates. In total, 5,038 *N. gonorrhoeae* isolates collected from January 2005 to December 2016 were included in this study. For each sample (one isolate from one patient), the following data were available: the patient's sex, age, and sexual orientation; the specimen collection date and site; and concurrent sexually transmitted infections (STIs) diagnosed during that episode. The isolates were obtained from 4,096 males (81.3%) and 994 females (19.7%), reflecting the sex distribution of the gonococcal infection incidence in Russia. The median age of the patients was 29 years (range, 12 to 64 years); 47% were aged 25 years or younger and 53% were older than 25 years. One characteristic of the RU-GASP was the lack of information on high-risk status, which patients did not indicate or withheld in their personal information, and we could not clarify this parameter by other approaches. Moreover, although the RU-GASP protocol allows *N. gonorrhoeae* isolation from several sites (urethra, cervix, pharynx, rectum, and other anogenital sites), only urethral specimens from males and cervical/urethral specimens from females were provided by the participating regional clinics.

Clinical specimens were cultured on GC-based agar supplemented with 1% IsoVitalEx enrichment and 1% vancomycin, colistin, amphotericin, and trimethoprim (VCAT) selective supplement (bioMérieux, Marcy l'Etoile, France). The primary identification of *N. gonorrhoeae* was performed at participating regions and included Gram staining and rapid oxidase reaction. For centralized testing, Gram-negative, oxidase-positive diplococcal cultures 18 to 24 h old were frozen in cryomedium-Trypticase soy broth with 20% glycerol (Becton, Dickinson BBL, Sparks, MD, USA) and transported on dry ice to the RU-GASP laboratory center at the State Research Center of Dermatovenerology and Cosmetology in Moscow, Russia. Furthermore, all isolates were analyzed via a sugar utilization test with a *Neisseria/Haemophilus* (NH) ID card for the Vitek 2 compact analyzer (bioMérieux, Marcy l'Etoile, France), and the samples with

“excellent” (96 to 99%) and “very good” (93 to 95%) results were verified as *N. gonorrhoeae*. Biochemically atypical isolates with “low discrimination” results were further checked via matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis using a MALDI Biotyper system (Bruker Daltonics, Bremen, Germany).

During the study period, the number of isolates obtained per year varied from 622 to 268, corresponding to the decreased dynamics of gonorrhea incidence in Russia and representing approximately 1% of the total number of gonococcal infection cases.

All collected *N. gonorrhoeae* isolates were preserved at -70°C and were then used for DNA extraction and antimicrobial susceptibility testing.

***N. gonorrhoeae* antimicrobial susceptibility testing.** Centralized susceptibility testing was performed using an agar dilution method that allows isolates to be categorized as susceptible (S), intermediate resistant (I), or resistant (R) based on the MIC value for each antimicrobial (all antimicrobials were purchased from Sigma-Aldrich, St. Louis, MO, USA). During 2005 through 2016, the tested antimicrobials included those previously used for gonorrhea treatment (penicillin G, tetracycline, and ciprofloxacin) and those currently recommended in Russia (ceftriaxone and spectinomycin). The breakpoints for these antimicrobials were interpreted using the recommendations of the U.S. Clinical and Laboratory Standards Institute (CLSI) (24). Considering the international guideline recommendations for dual therapy with ceftriaxone and azithromycin for gonorrhea, susceptibility testing to azithromycin began in 2007, and MIC breakpoints were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org).

The MICs (mg/liter) of the antimicrobials were determined on GC-based agar enriched with 1% IsoVitalEx (Becton, Dickinson, Sparks, MD, USA) according to CLSI recommendations (24). Since 2005, two replicates of the CLSI-recommended *N. gonorrhoeae* ATCC 49226 reference strain were included for quality assurance and quality control. Moreover, since 2009, the WHO *N. gonorrhoeae* reference strains (25) have been included in the quality control program for gonococcal AMR surveillance.

Detection of AMR genetic determinants in *N. gonorrhoeae*. For the 414 *N. gonorrhoeae* isolates obtained in 2005, the nucleotide polymorphisms in the chromosomal *penA*, *ponA*, *rpsJ*, *gyrA*, *parC*, *rrl*, and *rrs* genes and the *mtrR* gene promoter were detected by minisequencing reactions followed by MALDI-TOF MS analysis using a Reflex IV MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) as described previously (26). Targeted sequencing of internal regions of the *porB* gene (490 bp) was performed with the Sanger method using an ABI Prism 3100 genetic analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The presence of the plasmid-mediated *bla*_{TEM} and *tetM* genes was determined by PCR.

For the 268 *N. gonorrhoeae* isolates collected in 2016, detection of the DNA loci involved in AMR was performed via hybridization on a low-density oligonucleotide hydrogel microarray (biochip), which was developed by the authors (14) and has been included in the RU-GASP protocol since 2015 (23). Briefly, the procedure comprised three steps: (i) multiplex PCR to amplify the target DNA loci in the *penA*, *ponA*, *bla*_{TEM}, *rpsJ*, *tetM*, *gyrA*, *parC*, *rrl*, *rrs*, and *mtrR* gene segments; (ii) hybridization of the fluorescently labeled single-stranded PCR products with immobilized specific oligonucleotides corresponding to wild type or AMR determinants in *N. gonorrhoeae*; and (iii) analysis of fluorescence signals of biochip elements to define the presence or absence of AMR determinants in *N. gonorrhoeae*.

Sequencing of the *porB* gene was performed according to a conventional protocol (27) using a 3730xl genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis. The trend analysis to estimate changes in *N. gonorrhoeae* AMR during the study period used the regression method, and the standard deviation of the regression was calculated. Trends with a *P* of <0.05 were defined as statistically significant. The distribution of ceftriaxone MICs against *N. gonorrhoeae* in 2005 versus that in 2016 was analyzed by analysis of variance (ANOVA). The data on AMR genetic determinants were analyzed for two different years—2005 and 2016—using the two-tailed Fisher’s exact test.

RESULTS

Changes in *N. gonorrhoeae* susceptibility during the years 2005 through 2016.

The 2005 analysis of *N. gonorrhoeae* susceptibility revealed an extremely high resistance level for three antimicrobials previously recommended for gonorrhea treatment (Fig. 1). Of the total 444 isolates, 287 (64.6%) showed intermediate resistance to penicillin G and 60 (13.5%) were resistant; in summary, less than one-quarter of the *N. gonorrhoeae* population showed susceptibility to this antibiotic. Only one-third of the isolates were susceptible to tetracycline, whereas 154 (34.7%) were evaluated as intermediate resistant and 111 (25%) as resistant. Approximately one-half of the isolates (218 of 444; 49%) were resistant to ciprofloxacin. Based on initial AMR surveillance results, national guideline revisions in the following year stated that penicillins, tetracyclines, and fluoroquinolones, used frequently since the 1990s, should no longer be recommended for empirical gonorrhea treatment; however, the monitoring of susceptibility to these antimicrobials was continued.

During the study period, the proportion of nonsusceptible isolates to penicillin G and tetracycline increased until 2008, whereas resistance to ciprofloxacin peaked in

FIG 1 Proportions of nonsusceptible *N. gonorrhoeae* isolates (intermediate resistant, yellow sections of the bars; resistant, blue sections of the bars) to penicillin G (PEN), tetracycline (TET), ciprofloxacin (CIP), azithromycin (AZM), and spectinomycin (SPT) in Russia from 2005 to 2016. For PEN, TET, and CIP, statistically significant ($P < 0.05$) trends and standard deviations are indicated. The banners in the upper row indicate the year (2006) of PEN, TET, and CIP exclusion from the gonorrhea treatment recommendations. The banners on the SPT histogram show the year (2006) of SPT introduction for alternative gonorrhea treatment and the years (2012, 2015) of treatment recommendation changes according to the RU-GASP results.

2010 (Fig. 1). In recent years, partial recovery of penicillin G, tetracycline, and ciprofloxacin susceptibility was observed; by 2016, the proportions of nonsusceptible isolates to these antimicrobials decreased approximately 2-fold from those in 2005, when the RU-GASP was initiated. Among the 268 tested isolates, only 21 (7.8%) were resistant and 78 (29.1%) were intermediate resistant to penicillin G. The prevailing change in susceptibility to tetracycline decreased the number of resistant isolates to 51 (19%), whereas 75 isolates (28%) showed intermediate susceptibility. Furthermore, resistance to ciprofloxacin was detected in 83 of the 268 isolates (31%), whereas only two isolates (0.74%) showed intermediate susceptibility to this antimicrobial. Moreover, the isolates displayed trends toward decreased resistance to penicillin G ($P = 0.0008$), tetracycline ($P = 0.0093$), and ciprofloxacin ($P = 0.0057$) (Fig. 1). The long-term trend analysis predicted the continuation of this tendency; however, full recovery of susceptibility to penicillin G, tetracycline, and ciprofloxacin cannot be accurately predicted.

Analysis of *N. gonorrhoeae* susceptibility to azithromycin started in 2007, when only 1.3% of isolates were found to be resistant (Fig. 1). However, during the next 4 years, the proportions of nonsusceptible isolates changed very rapidly. By 2011, 14.4% of the isolates were resistant, and 10.9% showed intermediate resistance, perhaps due to the massive use of azithromycin in urology and gynecology, although national guidelines did not recommend this antimicrobial for the treatment of gonococcal infection. In turn, by 2016, azithromycin susceptibility was partially recovered in the *N. gonorrhoeae*

FIG 2 Distribution of the ceftriaxone MIC against *N. gonorrhoeae* isolates in Russia: 2005 (green bars) versus 2016 (purple bars).

population, to 91.4% (245 of 268 total isolates). The reasons for this decrease in resistance are less clear, however, as the use of azithromycin continued in related fields, a complete reversion did not occur, and susceptibility to this antimicrobial is still below the 95% threshold.

Very similar changes to those described above were found in the proportion of *N. gonorrhoeae* isolates susceptible to spectinomycin (Fig. 1). In 2005, only 6 of 444 total isolates (1.4%) showed intermediate resistance to this antimicrobial. Hence, in 2006, the national guidelines recommended spectinomycin (2 g, 1×, intramuscularly) as an alternative for gonorrhea treatment in patients without access to ceftriaxone or with a severe allergy to beta-lactam antimicrobials. Subsequently, the proportion of nonsusceptible isolates increased more than 10-fold relative to that in 2005. In 2011, 10% of isolates were already resistant and 8.8% were intermediate resistant. According to these data, the national guidelines were revised in 2012, and spectinomycin was recommended only in the RU-GASP participating regions with a proven 95% susceptibility rate. Thereafter, the high level of AMR was reduced very rapidly. Since 2013, more than 95% of isolates have been found to be susceptible to spectinomycin, and since 2015, this antimicrobial has again been recommended for use throughout Russia.

Ceftriaxone (250 mg, 1×, intramuscularly) has been recommended as a first-line antimicrobial for gonococcal infection treatment since 2006. According to CLSI definitions, sporadic *N. gonorrhoeae* nonsusceptibility to ceftriaxone was detected in Russia within the study period as follows: 1 isolate in 2005, 4 isolates in 2007, and 1 isolate in 2008. According to the EUCAST breakpoint, we further analyzed the isolates with MICs of >0.125 mg/liter as *N. gonorrhoeae* isolates with signs of emerging resistance to ceftriaxone. We identified 23 such isolates as follows: 2 in 2005, 2 in 2007, 13 in 2009, 3 in 2010, 2 in 2011, and 1 in 2015. In summary, the proportion of nonsusceptible isolates to ceftriaxone was only 0.57% (29 of 5,038), and no noticeable changes were observed during the study period. Thus, we compared the individual MICs of ceftriaxone in *N. gonorrhoeae* populations isolated in 2005 versus those isolated in 2016 (Fig. 2). This analysis identified a small proportion of nonsusceptible isolates (0.2% according to CLSI guidelines and 0.4% according to the EUCAST breakpoints) in 2005; however, in 2016, no nonsusceptible isolates were identified. In addition, the proportion of highly susceptible isolates with MICs of ≤0.002 mg/liter doubled during the study period,

increasing from 34.7% to 65.4%. However, ANOVA showed no significant differences in the ceftriaxone MIC distribution in 2005 versus that in 2016 ($P > 0.05$). Although the RU-GASP results indicated no emerging resistance to ceftriaxone, based on international data, the recommended dose for empirical gonorrhea treatment was doubled (500 mg, 1×, intramuscularly) in the 2015 national guideline revision, and ceftriaxone remains the first-line antimicrobial for gonorrhea treatment. The administration of cefixime (400 mg, 1×, *per os*) is also acceptable, but this antimicrobial has not extended to routine clinical practice in Russia.

Thus, the RU-GASP data from 2005 to 2016 showed partial recovery of *N. gonorrhoeae* susceptibility to antimicrobials previously used for gonococcal infection therapy (penicillin G, tetracycline, and ciprofloxacin). Similarly, the data indicated an absence of emerging AMR to the currently recommended agents ceftriaxone and spectinomycin. In summary, in 2005, 82.2% of *N. gonorrhoeae* isolates were nonsusceptible or resistant to one or more antimicrobials, whereas in 2016, the proportion of nonsusceptible isolates decreased significantly to 57.5% ($P < 0.001$). Correspondingly, the proportion of isolates that showed susceptibility to all tested antimicrobials increased from 17.8% in 2005 to 42.5% in 2016 ($P < 0.001$).

Comparison of *N. gonorrhoeae* AMR genetic determinants: 2005 versus 2016.

To compare AMR genetic determinants from 2005 and 2016, corresponding genomic loci related to resistance were analyzed in every isolate obtained in 2005 and 2016. The frequencies of resistance-associated determinants in the 2005 and 2016 isolates are summarized in Table 1.

In the isolates included in the study in 2005, the D345A insertion in the *penA* gene was the most predominant determinant, followed by the *ponA* L421P mutation. Double mutations in these loci were found in 298 isolates (67.1%). In contrast, 79 isolates (17.8%) carried a single resistance determinant, and 68 isolates (15.3%) were wild type on *ponA* and *penA* genes. In 2016, *penA* and *ponA* mutant alleles were less common than in 2005, and *ponA* mutations always occurred in combination with *penA* mutations. Hence, the percentage of wild-type isolates increased proportionally (67.9%; $P < 0.001$). This finding agrees with the trend of decreasing resistance to penicillin G in the *N. gonorrhoeae* population and the changes in the ceftriaxone MIC distribution. However, the proportion of isolates harboring the *bla*_{TEM} plasmid-borne gene did not significantly change, and these isolates were highly resistant to penicillin G.

Concerning the tetracycline-resistant isolates, the V57M substitution in the *rpsJ* gene was the sole mutation detected in 2005, whereas in 2016, the proportion of V57M mutants was decreased and few isolates with the V57L mutation were first identified. Moreover, an increase in the proportion of *tetM*-positive isolates with high-level resistance to tetracyclines was observed in 2016.

Eight different combinations of determinants associated with fluoroquinolone resistance were identified. In 2005, mutations in the *gyrA* and *parC* genes were identified in 334 (75.2%) and 173 (40%) isolates, respectively. Isolates with high-level resistance typically bore the double mutation S91F/D95G in *gyrA*, complemented by the S87R substitution in *parC*. The wild-type genotype was detected in 222 (50.0%) of the *N. gonorrhoeae* isolates. In 2016, the predominant high-level resistance genotype was the combination of S91F/D95N in *gyrA* and S87R in *parC* (this triple mutation was found in 23 of the 268 isolates [8.6%]). Comparison of the 2005 and 2016 data showed a significant decrease in the proportions of isolates harboring the S91Y, D95G, and S87R mutations, whereas other mutations persisted or were initially identified (e.g., E91K in the *parC* gene). The wild-type target genes were found in 163 (60.8%; $P = 0.005$) of the 2016 modern *N. gonorrhoeae* isolates.

Among the 2005 isolates, the A2059G mutation in the *rrl* gene responsible for resistance to macrolides and the *rrs* C1192T mutation associated with aminocyclitol resistance were detected sporadically in 3 and 2 isolates (0.7% and 0.5%), respectively. However, in 2016, these AMR determinants were not found.

Finally, we analyzed mutations in the *porB1b* and *mtrR* genes that led to reduced susceptibility to multiple antimicrobials. In 2005, either the G120K or A121D substitu-

TABLE 1 AMR determinants in *N. gonorrhoeae* populations, 2005 versus 2016

Trait and gene	AMR determinant	No. of isolates (%) according to yr:		P value
		2005 (n = 444)	2016 (n = 268)	
Resistance to beta-lactams				
<i>penA</i>	D345a	376 (84.7)	176 (65.7)	<0.001
<i>ponA</i>	L421P	301 (67.8)	86 (32.5)	<0.001
<i>bla</i> _{TEM}	Beta-lactamase	22 (5.0)	15 (5.6)	0.729
Resistance to tetracyclines				
<i>rpsJ</i>	V57M	335 (75.4)	110 (42.1)	<0.001
	V57L	0	3 (1.1)	0.052
<i>tetM</i>	tetM protein	6 (1.4)	15 (5.6)	0.002
Resistance to fluoroquinolones (ciprofloxacin)				
<i>gyrA</i>	S91F	101 (22.7)	78 (29.2)	0.061
	S91Y	33 (7.5)	0	<0.001
	S91T	0	1 (0.4)	0.376
	D95G	133 (30.0)	22 (8.2)	<0.001
	D95N	67 (15.0)	44 (16.5)	0.67
<i>parC</i>	S87N	1 (0.2)	3 (1.1)	0.152
	S87R	165 (37.2)	52 (19.5)	<0.001
	E91G	7 (1.6)	14 (5.2)	0.009
	E91K	0	4 (1.5)	0.019
Resistance to macrolides (azithromycin)				
<i>rrl</i>	A2059G	3 (0.7)	0	0.294
Resistance to aminocyclitols (spectinomycin)				
<i>rrs</i>	C1192T	2 (0.5)	0	0.529
Decrease in membrane permeability				
<i>porB1b</i>	G120D	38 (8.6)	17 (6.3)	0.313
	G120K	180 (40.5)	31 (11.6)	<0.001
	G120N	14 (3.2)	6 (2.2)	0.64
	G120R	2 (0.5)	0	0.529
	G120T	0	3 (1.1)	0.052
	A121D	130 (29.3)	23 (8.6)	<0.001
	A121G	14 (3.8)	10 (3.8)	0.673
	A121N	59 (13.3)	10 (3.8)	<0.001
Overexpression of the MtrCDE efflux pump				
<i>mtrR</i>	−35 delA	157 (35.4)	52 (19.6)	<0.001

tion was found in 110 (24.8%) isolates, and the G120K and A121N substitutions were found together in 56 (12.6%) isolates. The −35 delA alteration in the *mtrR* gene promoter was found in 157 of the 444 isolates (35.4%), and the simultaneous presence of *porB1b* and *mtrR* mutant alleles was detected in 128 (28.8%) isolates. In 2016, the combination of G120K and A121D substitutions remained prevalent (17 isolates; 6.3%). A significant decrease in the proportions of G120D, G120K, A121D, and A121N substitutions was identified in the 2016 *N. gonorrhoeae* population relative to these proportions in the 2005 population. Moreover, the proportion of isolates carrying the −35 delA mutation in the *mtrR* gene promoter was decreased 2-fold in 2016, and only 27 isolates (10.0%; $P < 0.001$) carried the *porB1b* and *mtrR* mutations simultaneously.

In summary, from 2005 to 2016, the proportion of isolates with a wild-type genotype increased more than 2-fold (11.7% to 30.3%; $P < 0.001$), complementary to the increasing trend in the proportion of susceptible isolates among the modern *N. gonorrhoeae* population in Russia.

DISCUSSION

Since the RU-GASP was established in 2004, gonorrhea susceptibility testing results and epidemiological surveillance data have been published regularly in a timely

manner (20–23). In this article, we summarize the RU-GASP results over a 12-year period and reveal long-term changes in *N. gonorrhoeae* AMR occurring during this period.

Based on data for 5,038 isolates identified and tested at our institution between 2005 and 2016, we report the decreasing trend in AMR to agents used in traditional gonorrhea treatment (penicillin G, tetracycline, and ciprofloxacin), which had been extremely high in previous years. Moreover, we found significant changes in the *N. gonorrhoeae* genotypes because of the decreasing frequency of chromosomal AMR determinants (including single-nucleotide polymorphisms or deletions in the *penA*, *ponA*, *rpsJ*, *gyrA*, and *parC* genes) accompanied by the increasing frequency of the wild-type *porB1b* gene and *mtrR* promoter recovery. Currently, whether reverse mutations in the *N. gonorrhoeae* genome or intensive distribution of the wild-type genotype caused these changes is unclear. Moreover, in the absence of selection due to the action of antimicrobial agents, these mutations harm bacterial fitness (28), thus leading to the elimination of these mutations from the *N. gonorrhoeae* population. Surprisingly, the *bla*_{TEM} and *tetM* determinant proportions did not change from 2005 to 2016, indicating that plasmids in *N. gonorrhoeae* play a more diverse role than horizontal transfer of AMR determinants and, therefore, persist in *N. gonorrhoeae* even when selective antimicrobial pressure is relieved (29).

Emerging resistance to spectinomycin was described during the study period, with maximum resistance noted in 2011. To prevent further increases in resistance to this important antimicrobial, spectinomycin was recommended for alternative gonorrhea treatment only in regions with proven *N. gonorrhoeae* susceptibility. As shown by the current results, this crisis has been overcome, and susceptibility to spectinomycin recovered to a minimum of 95%. Indeed, in 2016, we found no mutation profiles in the *rrs* gene that determine high-level resistance to this antimicrobial.

Similar dynamics were found for azithromycin resistance, although complete reversal did not occur, and these changes cannot be explained by national guideline revisions.

During the study period, only a few *N. gonorrhoeae* isolates resistant to ceftriaxone were detected, and a comparison of the MIC distributions in 2005 and 2016 did not show emerging resistance to this antimicrobial. Moreover, the comparison of the MIC distribution in the 2005 and 2016 populations indicated increased susceptibility to ceftriaxone. Thus, our data, in agreement with recently published Euro-GASP results, show that the resistance levels to the first-line antimicrobials used to treat gonorrhea infection are, encouragingly, decreasing (30).

The incoming surveillance results show that the current AMR status in Russia is less serious than that in other WHO GASP regions and that several national features are important. First, intramuscularly administered ceftriaxone was widely used for empirical gonorrhea therapy, whereas orally administered cefixime was not introduced in medical practice. Perhaps this pattern prohibited the formation of resistance to third-generation cephalosporins because cefixime usage was associated with unsuccessful gonorrhea treatment cases worldwide (2, 31). Second, azithromycin has never been recommended for empirical gonorrhea treatment in Russia (except for cases with proven chlamydiosis). Simultaneous administration of azithromycin and ceftriaxone is usually justified by the slowed development of *N. gonorrhoeae* resistance, but no evidence-based arguments have been made. Moreover, bacterial biofilm overgrowth occurred with sub-MIC azithromycin concentrations (32), which may be a condition for AMR development in pharyngeal or rectal gonorrhea cases. Thus, the nonuse of azithromycin in Russia could also prevent the development and dissemination of *N. gonorrhoeae* resistant to first-line antimicrobials. Third, limited cross-border migration determines the geographically restricted gonorrhea epidemiology, where some sequence types autochthonous for Russia and susceptible to all antimicrobials (807, 1544, 1993 et al.) dominate while the international spread of multidrug-resistant genotypes (1407 et al.) is detected only sporadically (23).

The limiting factor of the RU-GASP from 2005 to 2016 was the impossibility of selective analyses of *N. gonorrhoeae* isolates from high-risk groups, which perhaps

develop AMR most readily (33, 34). The current aim is to improve the program protocol in accordance with WHO standards, although data from the total population also reflect the situation in risk groups to a certain extent. Another limitation was the testing of approximately 1% of the total *N. gonorrhoeae* population in Russia, which is comparable to the coverage in the United Kingdom and France, according to the 2016 Euro-GASP report (30), but requires a coverage increase of at least 5%. Improvement of these parameters and continuation of the annual control of *N. gonorrhoeae* AMR emergence and spread will provide efficient gonococcal infection control in Russia and minimize the risk of untreatable cases of this disease.

ACKNOWLEDGMENTS

This study was supported by a government contract of the Ministry of Health of the Russian Federation (project no. 056-00015-18-00) and Russian Science Foundation grant no. 17-75-20039 (analysis of resistance determinants using microarrays).

REFERENCES

- World Health Organization. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organization, Geneva, Switzerland.
- Unemo M, Shafer WM. 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 27:587–613. <https://doi.org/10.1128/CMR.00010-14>.
- Unemo M, Nicholas RA. 2012. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. *Future Microbiol* 7:1401–1422. <https://doi.org/10.2217/fmb.12.117>.
- Shaskolskiy B, Dementieva E, Leinsoo A, Runina A, Vorobyev D, Plakhova X, Kubanov A, Deryabin D, Gryadunov D. 2016. Drug resistance mechanisms in bacteria causing sexually transmitted diseases and associated with vaginosis. *Front Microbiol* 7:747. <https://doi.org/10.3389/fmicb.2016.00747>.
- Lindberg R, Fredlund H, Nicholas R, Unemo M. 2007. *Neisseria gonorrhoeae* isolates with reduced susceptibility to cefixime and ceftriaxone: association with genetic polymorphisms in *penA*, *mtrR*, *porB1b*, and *ponA*. *Antimicrob Agents Chemother* 51:2117–2122. <https://doi.org/10.1128/AAC.01604-06>.
- Muhammad I, Golparian D, Dillon JA, Johansson A, Ohnishi M, Sethi S, Chen SC, Nakayama S, Sundqvist M, Bala M, Unemo M. 2014. Characterisation of *bla*_{TEM} genes and types of beta-lactamase plasmids in *Neisseria gonorrhoeae*—the prevalent and conserved *bla*_{TEM-135} has not recently evolved and existed in the Toronto plasmid from the origin. *BMC Infect Dis* 14:454. <https://doi.org/10.1186/1471-2334-14-454>.
- Hu M, Nandi S, Davies C, Nicholas RA. 2005. High-level chromosomally mediated tetracycline resistance in *Neisseria gonorrhoeae* results from a point mutation in the *rpsJ* gene encoding ribosomal protein S10 in combination with the *mtrR* and *penB* resistance determinants. *Antimicrob Agents Chemother* 49:4327–4334. <https://doi.org/10.1128/AAC.49.10.4327-4334.2005>.
- Turner A, Gough KR, Leeming JP. 1999. Molecular epidemiology of *tetM* genes in *Neisseria gonorrhoeae*. *Sex Transm Infect* 75:60–66. <https://doi.org/10.1136/sti.75.1.60>.
- Vernel-Pauillac F, Hogan TR, Tapsall JW, Goarant C. 2009. Quinolone resistance in *Neisseria gonorrhoeae*: rapid genotyping of quinolone resistance-determining regions in *gyrA* and *parC* genes by melting curve analysis predicts susceptibility. *Antimicrob Agents Chemother* 53:1264–1267. <https://doi.org/10.1128/AAC.01104-08>.
- Galimand M, Gerbaud G, Courvalin P. 2000. Spectinomycin resistance in *Neisseria* spp. due to mutations in 16S rRNA. *Antimicrob Agents Chemother* 44:1365–1366. <https://doi.org/10.1128/AAC.44.5.1365-1366.2000>.
- Jacobsson S, Golparian D, Cole M, Spiteri G, Martin I, Berghem T, Borrego MJ, Crowley B, Crucitti T, Van Dam AP, Hoffmann S, Jeverica S, Kohl P, Mlynarczyk-Bonikowska B, Pakarna G, Stary A, Stefanelli P, Pavlik P, Tzelepi E, Abad R, Harris SR, Unemo M. 2016. WGS analysis and molecular resistance mechanisms of azithromycin-resistant (MIC >2 mg/L) *Neisseria gonorrhoeae* isolates in Europe from 2009 to 2014. *J Antimicrob Chemother* 71:3109–3116. <https://doi.org/10.1093/jac/dkw279>.
- Olesky M, Zhao S, Rosenberg RL, Nicholas RA. 2006. Porin-mediated antibiotic resistance in *Neisseria gonorrhoeae*: ion, solute, and antibiotic permeation through PIB proteins with *penB* mutations. *J Bacteriol* 188:2300–2308. <https://doi.org/10.1128/JB.188.7.2300-2308.2006>.
- Veal WL, Nicholas RA, Shafer WM. 2002. Overexpression of the *MtrC-MtrD-MtrE* efflux pump due to an *mtrR* mutation is required for chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. *J Bacteriol* 184:5619–5624. <https://doi.org/10.1128/JB.184.20.5619-5624.2002>.
- Kubanov AA, Leinsoo AT, Chestkov AV, Dementieva EI, Shaskolskiy BL, Solomka VS, Gryadunov DA, Deryabin DG. 2017. Drug resistance mutations and susceptibility phenotypes of *Neisseria gonorrhoeae* isolates in Russia. *Mol Biol (Mosk)* 51:431–441. (In Russian.) <https://doi.org/10.7868/S0026898417030119>.
- Balashov S, Mordechai E, Adelson ME, Gyax SE. 2013. Multiplex bead suspension array for screening *Neisseria gonorrhoeae* antibiotic resistance genetic determinants in noncultured clinical samples. *J Mol Diagn* 15:116–129. <https://doi.org/10.1016/j.jmoldx.2012.08.005>.
- World Health Organization. 2012. Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*. World Health Organization, Geneva, Switzerland.
- Tapsall J, World Health Organization. 2001. Antimicrobial resistance in *Neisseria gonorrhoeae*. World Health Organization, Geneva, Switzerland.
- Cole MJ, Unemo M, Hoffmann S, Chisholm SA, Ison CA, van de Laar MJ. 2011. The European gonococcal antimicrobial surveillance programme, 2009. *Euro Surveill* 16:pii=19995.
- Unemo M, Ison CA, Cole M, Spiteri G, van de Laar M, Khotenashvili L. 2013. Gonorrhoea and gonococcal antimicrobial resistance surveillance networks in the WHO European region, including the independent countries of the former Soviet Union. *Sex Transm Infect* 89:iv42–iv46. <https://doi.org/10.1136/sextrans-2012-050909>.
- Kubanova A, Frigo N, Kubanov A, Sidorenko S, Pripitnevich T, Vachnina T, Al-Khafaji N, Polevshikova S, Solomka V, Domeika M, Unemo M. 2008. National surveillance of antimicrobial susceptibility in *Neisseria gonorrhoeae* in 2005–2006 and recommendations of first-line antimicrobial drugs for gonorrhoea treatment in Russia. *Sex Transm Infect* 84:285–289. <https://doi.org/10.1136/sti.2007.029033>.
- Kubanova A, Frigo N, Kubanov A, Sidorenko S, Lesnaya I, Polevshikova S, Solomka V, Bukanov N, Domeika M, Unemo M. 2010. The Russian gonococcal antimicrobial susceptibility programme (RU-GASP)—national resistance prevalence in 2007 and 2008, and trends during 2005–2008. *Euro Surveill* 15:pii=19533.
- Kubanova A, Kubanov A, Frigo N, Solomka V, Semina V, Vorobyev D, Khairullin R, Unemo M. 2014. Russian gonococcal antimicrobial susceptibility programme (RU-GASP)—resistance in *Neisseria gonorrhoeae* during 2009–2012 and NG-MAST genotypes in 2011 and 2012. *BMC Infect Dis* 14:342. <https://doi.org/10.1186/1471-2334-14-342>.
- Kubanov A, Vorobyev D, Chestkov A, Leinsoo A, Shaskolskiy B, Dementieva E, Solomka V, Plakhova X, Gryadunov D, Deryabin D. 2016. Molecular epidemiology of drug-resistant *Neisseria gonorrhoeae* in Russia (current status, 2015). *BMC Infect Dis* 16:389. <https://doi.org/10.1186/s12879-016-1688-7>.

24. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing; 28th informational supplement. CLSI M100. Clinical and Laboratory Standards Institute, Wayne, PA.
25. Unemo M, Fasth O, Fredlund H, Limnios A, Tapsall J. 2009. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. *J Antimicrob Chemother* 63:1142–1151. <https://doi.org/10.1093/jac/dkp098>.
26. Ilina EN, Vereshchagin VA, Borovskaya AD, Malakhova MV, Sidorenko SV, Al-Khafaji NC, Kubanova AA, Govorun VM. 2008. Relation between genetic markers of drug resistance and susceptibility profile of clinical *Neisseria gonorrhoeae* strains. *Antimicrob Agents Chemother* 52: 2175–2182. <https://doi.org/10.1128/AAC.01420-07>.
27. ECDC. 2012. Molecular typing of *Neisseria gonorrhoeae*—results from a pilot study 2010–2011. ECDC, Stockholm, Sweden.
28. Unemo M, Althaus CL. 2017. Fitness cost and benefit of antimicrobial resistance in *Neisseria gonorrhoeae*: multidisciplinary approaches are needed. *PLoS Med* 14:e1002423. <https://doi.org/10.1371/journal.pmed.1002423>.
29. Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW. 2009. Genomic islands: tools of bacterial horizontal gene transfer and evolution. *FEMS Microbiol Rev* 33:376–393. <https://doi.org/10.1111/j.1574-6976.2008.00136.x>.
30. ECDC. 2018. Gonococcal antimicrobial susceptibility surveillance in Europe, 2016. ECDC, Stockholm, Sweden.
31. Lewis DA. 2014. Global resistance of *Neisseria gonorrhoeae*: when theory becomes reality. *Curr Opin Infect Dis* 27:62–67. <https://doi.org/10.1097/QCO.0000000000000025>.
32. Mart'ianov SV, Zhurina MV, El'-Registan GI, Plakunov VK. 2015. Activation of formation of bacterial biofilms by azithromycin and prevention of this effect. *Mikrobiologiya* 84:27–36. (In Russian.)
33. Mason PR, Gwanzura L, Latif AS, Marowa E, Ray S, Katzenstein DA. 1997. Antimicrobial resistance in gonococci isolated from patients and from commercial sex workers in Harare, Zimbabwe. *Int J Antimicrob Agents* 9:175–179.
34. Kirkcaldy RD, Zaidi A, Hook EW, 3rd, Holmes KK, Soge O, del Rio C, Hall G, Papp J, Bolan G, Weinstock HS. 2013. *Neisseria gonorrhoeae* antimicrobial resistance among men who have sex with men and men who have sex exclusively with women: the gonococcal isolate surveillance project, 2005–2010. *Ann Intern Med* 158:321–328. <https://doi.org/10.7326/0003-4819-158-5-201303050-00004>.