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Authors	Hovhannisyan, G; von Schoen-Angerer, T; Babayan, K; Fenichiu, O; Gaboulaud, V
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## Antimicrobial Susceptibility of *Neisseria gonorrhoeae* Strains in Three Regions of Armenia

GAYANE HOVHANNISYAN, MD,\* TIDO VON SCHOEN-ANGERER, MD,† KAREN BABAYAN, MD, PhD,‡  
OLIVIER FENICHIU, MD,§ AND VALERIE GABOULAUD, MD¶

**Objective:** There are no data available on gonococcal susceptibility in the Caucasus region. We aimed to determine in vitro antimicrobial susceptibility of *Neisseria gonorrhoeae* in Armenia in order to update the national treatment protocol.

**Methods:** Isolates from men with urethral discharge presenting at 3 STI clinics in 3 different sites of Armenia were used to determine susceptibility of *N. gonorrhoeae* strains for 11 antimicrobials using the disc diffusion technique.

**Results:** Among the 101 isolates tested the susceptibility rate for penicillin, doxycycline, and kanamycin were 37.6, 25.7, and 80.2%, respectively. Sensitivity to quinolones was 95% for both ofloxacin and ciprofloxacin. All strains were susceptible to third-generation cephalosporins and to spectinomycin. Only 11% of strains were susceptible to all antibiotics tested.

**Conclusion:** Third-generation cephalosporins and spectinomycin are suitable first-line regimens. Quinolones are not advisable as first-line treatment given current borderline susceptibility, known tendency for rapid resistance development in this class, and frequent over-the-counter use of this antibiotic in Armenia.

ONE OF THE CONSEQUENCES of the collapse of the Soviet Union, with its ensuing socioeconomic upheaval and widespread collapse of the health care system, has been the alarming rise of sexually transmitted infections in Eastern Europe and Central Asia.<sup>1</sup> STIs have become a major public health problem in this part of the world<sup>2</sup> and concern is further fuelled by the quickly spreading HIV epidemic. In Armenia, as in other former Soviet Union countries, poor health care seeking and underreporting are common with reported prevalence of STI being but the tip of the iceberg.<sup>3</sup> Furthermore, self-treatment is regular practice and antibiotics are easily available over the counter. Nevertheless, there are indications that STIs are already widespread.<sup>4</sup>

Increasing antibiotic resistance of *Neisseria gonorrhoeae* is a widespread problem. In much of the world, gonococci are already resistant to penicillins and tetracyclines, and resistance to multiple agents is common.<sup>5</sup> Resistance of *N. gonorrhoeae* to ciprofloxacin is also being detected and is spreading rapidly.<sup>6</sup> While there is some data from Russia and Kyrgyzstan showing high resistance to a number of antimicrobials, there is still a paucity of resistance

From the \*Princess Margaret Hospital, Toronto, Canada; †Médecins sans frontières, Geneva, Switzerland; ‡Medical-Scientific Center for Dermatology and STI, Yerevan, Armenia; §Médecins sans frontières, Brussels, Belgium; and ¶Epicentre, Paris, France

data in the Commonwealth of Independent States (CIS).<sup>7–9</sup> To date, there is no data on gonococcal antimicrobial resistance in the Caucasus, including Armenia.

This study was conducted to determine in vitro susceptibility of *N. gonorrhoeae* in Armenia to update the national STI protocols for gonorrhea treatment.

### Methods

A multicenter study was carried out in 3 STI clinics located in different sites of Armenia: the Medical-Scientific Center for Dermatology and STI in the capital Yerevan; a STI clinic run by Médecins sans frontières (MSF) in Gyumri, the second largest city; and the MSF STI clinic in Bagratashen, a village located at the Armenian-Georgian border and site of a large cross-border market. Subjects included were all consecutive adult men presenting with clinically confirmed, simple acute urethritis. Patients with complicated gonorrhea infection or antibiotic intake within the previous 3 weeks were excluded from the sample. All 3 clinics provided anonymous and free-of-charge treatment for the patients. Ethical approval was received by the Ethical Review Board of MSF and written permission was obtained from the Ministry of Health of Armenia.

The WHO recommendation of a minimum of 100 isolates was used as a sample size estimate for the assessment of local patterns of antibiotic resistance.<sup>5</sup> Consecutive specimens were collected between October 2003 and August 2004.

Immediate microscopic examination of Gram-stained urethral specimens was followed by culture if Gram-negative intracellular diplococci were identified. Selective modified Thayer-Martin medium was used to isolate *N. gonorrhoeae*. Inoculated plates were incubated for 24 to 48 hours in a CO<sub>2</sub> enriched environment (3–7%) at 35 to 37°C and 70% humidity. The presumptive identification of *N. gonorrhoeae* was done with oxidase test, and positive cultures were transported at 25–40°C and in a CO<sub>2</sub> enriched atmosphere (Anaerocult® C mini, Merck) to the reference laboratory in Yerevan, where the presence of *N. gonorrhoeae* was confirmed by monoclonal phadebact GC technique. The nonselective modified Thayer-Martin medium was used to subculture positive colonies and for the sensitivity analyses. The susceptibility of *N.*

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Correspondence: Gayane Hovhannisyanyan, MD, Princess Margaret Hospital, 610 University Avenue, Room 9-414, Toronto, ON M5G2M9. E-mail: Gayane.Hovhannisyanyan@uhnres.utoronto.ca

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TABLE 1. Susceptibility Results of 101 Strains of *N. gonorrhoeae* Isolated in Armenia Between October 2003 and August 2004

Antibiotic (Disc Content in $\mu\text{g}$ )	Inhibition Zone Diameter (mm)			Susceptible <i>n</i> (%)	Intermediate <i>n</i> (%)	Resistant <i>n</i> (%)
	S	I	R			
Azithromycin (30 $\mu\text{g}$ )	$\geq 26$	—	$\leq 25$	101 (100)	0	0
Cefixime (30 $\mu\text{g}$ )	$\geq 32$	—	—	101 (100)	0	0
Ceftriaxone (30 $\mu\text{g}$ )	$\geq 32$	—	—	101 (100)	0	0
Chloramphenicol (60 $\mu\text{g}$ )	$\geq 30$	27–29	$\leq 26$	101 (100)	0	0
Ciprofloxacin (10 $\mu\text{g}$ )	$\geq 36$	30–35	$\leq 29$	96 (95.0)	1 (1.0)	4 (4.0)
Doxycycline (80 $\mu\text{g}$ )	$\geq 36$	27–35	$\leq 26$	26 (25.7)	72 (71.3)	3 (3.0)
Erythromycin (78 $\mu\text{g}$ )	$\geq 30$	—	$\leq 29$	101 (100)	0	0
Kanamycin (100 $\mu\text{g}$ )	$\geq 28$	24–27	$\leq 23$	81 (80.2)	16 (15.8)	4 (4.0)
Ofloxacin (10 $\mu\text{g}$ )	$\geq 32$	25–31	$\leq 24$	96 (95.0)	0	5 (5.0)
Penicillin G (5 $\mu\text{g}$ )	$\geq 34$	23–33	$\leq 22$	38 (37.6)	57 (56.4)	6 (5.9)
Spectinomycin (200 $\mu\text{g}$ )	$\geq 23$	20–22	$\leq 19$	101 (100)	0	0

*gonorrhoeae* isolates was tested against 11 antibiotics using the disc diffusion method (Neo-Sensitabs®, Rosco, Denmark): azithromycin, cefixime, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, kanamycin, ofloxacin, penicillin, and spectinomycin. Clinical and Laboratory Standards Institute (CLSI), former NCCLS, susceptibility breakpoints were used for interpretation of inhibition zone diameters when available, and azithromycin, chloramphenicol, doxycycline, erythromycin, and kanamycin were interpreted using the Rosco guidelines.<sup>10,11</sup> Quality controls were performed using a gonococcal reference strain of known sensitivity (ATCC 49226, Becton Dickinson).

Data were entered in Epi-info 6.04 days (CDC, Atlanta, GA), and analysis was performed on Intercooled Stata 7.0 (Stata corporation, TX). Comparison between the percentages was made using a  $\chi^2$  test. For comparison of susceptibility patterns between study sites, Fisher exact test was used.

### Results

A total of 539 patients with acute urethral discharge was seen in the study sites between October 2003 and August 2004; 407 of them were eligible and consented for participation. On direct microscopy Gram-negative diplococci were identified in 129 patients (31.7%); 28 of these were lost due to various reasons (most commonly media contamination and transportation problems), and the total number of sensitivity analyzes successfully performed was 101. The approximate number of patients excluded from the

study, but later identified as having gonorrhea, was 6 and was evenly distributed between the 3 sites.

Table 1 presents the susceptibility for the 11 antibiotics tested. The lowest susceptibility was reported for penicillin and doxycycline. No resistance to erythromycin, chloramphenicol, ceftriaxone, cefixime, and spectinomycin was found. Borderline susceptibility of 95% to quinolones (ciprofloxacin, ofloxacin), recommended in the national protocol as first-line antibiotics for gonorrhea treatment, was reported. There was decreased susceptibility to kanamycin as well.

Decreased susceptibility or resistance to more than 1 antimicrobials was detected in 90 (89.1%) of the strains. In addition, 58 (57.4%) strains showed decreased susceptibility to 2 and 3 antimicrobials, the most common combination being penicillin and doxycycline. Most (4 out of 6) strains that had reduced susceptibility to quinolones also had reduced susceptibility to kanamycin, penicillin, and doxycycline. Only 11 (10.9%) strains were sensitive to all 11 antibiotics tested.

Table 2 summarizes the distribution and the susceptibility characteristics of the examined strains in 3 study sites. The susceptibility of gonococcal strains was not significantly different between study sites ( $P > 0.05$ ).

### Discussion

To our knowledge, this study was the first in vitro sensitivity study on *N. gonorrhoeae* carried out in Armenia and the Caucasus in general. In the absence of comprehensive antimicrobial surveillance in the

TABLE 2. Susceptibility Distribution of *N. gonorrhoeae* Strains in Armenia by the Study Sites Between October 2003 and August 2004

	Penicillin	Kanamycin	Doxycycline	Ofloxacin	Ciprofloxacin
Bagratashen ( <i>n</i> = 17)					
Susceptible	8 (47.1)	14 (82.3)	4 (23.5)	16 (94.1)	16 (94.1)
Intermediate	8 (47.1)	2 (11.8)	13 (76.5)	0 (0.0)	0 (0.0)
Resistant	1 (5.8)	1 (5.9)	0 (0.0)	1 (5.9)	1 (5.9)
Gumri ( <i>n</i> = 24)					
Susceptible	12 (50.0)	21 (87.5)	5 (20.8)	23 (95.8)	23 (95.8)
Intermediate	10 (41.7)	2 (8.3)	18 (75.0)	0 (0.0)	0 (0.0)
Resistant	2 (8.3)	1 (4.2)	1 (4.2)	1 (4.2)	1 (4.2)
Yerevan ( <i>n</i> = 60)					
Susceptible	18 (30.0)	46 (76.7)	17 (28.3)	57 (95.0)	57 (95.0)
Intermediate	39 (65.0)	12 (20.0)	41 (68.3)	0 (0.0)	1 (1.7)
Resistant	3 (5.0)	2 (3.3)	2 (3.3)	3 (5.0)	2 (3.3)

former Soviet Union, our data from Armenia contribute important information to understanding the susceptibility patterns of gonorrhea in this part of the World as during Soviet times treatment protocols were the same throughout the entire Soviet Union.

Emerging resistance to quinolones (ciprofloxacin and ofloxacin) in Armenia is evident, indicating that the WHO target of greater than 95% efficacy for drugs of the first-line therapy is no longer achievable for this class. Given the known tendency for rapid resistance development in this quinolones class<sup>6</sup> and frequent over-the-counter sale of antibiotics in Armenia, further rapid increase of resistance is a likely scenario. Higher resistance could have been expected if men with previous antibiotic use or complicated infection would have been included in the study as well. This is clearly not the first report of decreased quinolone susceptibility in the former Soviet Union: ciprofloxacin susceptibility in the Kyrgyzstan was <75%<sup>7</sup> and decreased quinolone susceptibility and occurrence of high-level resistance has been reported from Russia.<sup>9,12,13</sup>

The high level of resistance to penicillin and tetracycline found in our study is not surprising as this has been widely reported throughout the world including Russia and Kyrgyzstan.<sup>7,9</sup> Of note is the decreased susceptibility for kanamycin, which was a first-line treatment option in the Armenian national protocol before this study but has since been removed.

Armenia is a small country and the similar findings in the 3 sites indicate that sites were comparable, and thus the results give a good overview of the current situation in the country. However, caution has to be exercised when extrapolating results to the entire country as the study did not include patients from the south of the country. Furthermore, the results could underestimate the real trend of resistance as we excluded pretreated and complex cases of gonorrhea infection.

We used the disc diffusion method to determine inhibitory zones, as it is relatively cheap and easy to apply. The agar dilution for MIC, which is the WHO reference method for resistance detection, can only be performed in specialized laboratories and was not feasible in our setting. Nevertheless, our results seem to be in accordance with the current trends of resistance for *N. gonorrhoeae*. For susceptibility testing, nonselective modified Thayer-Martin medium was used as it was easier to obtain and it performed well when tested against the CLSI recommended Quality Control strain *N. gonorrhoeae* ATCC 49226. The Neo-sensitabs disks, which were considered as antimicrobials of choice in our setting, as they can be kept at room temperature, complied with Q.C zone diameters, recommended for tests on gonococcus agar when tested against *N. gonorrhoeae* ATCC 49226 on modified Thayer-Martin.<sup>11</sup>

The proportion of penicillin resistance due to the  $\beta$ -lactamase production is not known as due to the technical problems the  $\beta$ -lactamase testing was discontinued. Another limitation of the current study was the use of only 1 control strain. Nalidixic acid could have been tested to ensure that resistance to all quinolones is detected. However, it should be kept in mind that the study was conducted in a developing country where susceptibility testing is not done on a routine basis.

The lack of resistance to third-generation cephalosporines (cefixime and ceftriaxone) in our study make these drugs excellent choices as first-line treatment in Armenia. Spectinomycin, an antibiotic almost exclusively used for the treatment of gonorrhea and no detected resistance in our study is a good first-line choice too.

Following the recommendations from this study, cefixime was included as an alternative first-line treatment in the Armenian national protocol. This was also warranted because resistance to ciprofloxacin is approaching 5%, and lack of resources in Armenia will make regular surveillance unlikely. At present, due to cheaper price, ciprofloxacin remained first-line treatment for simple gonococcal infections, and spectinomycin was not included as first-line due to price. Future studies to assess the resistance trends in Armenia and to allow timely revision of treatment protocols are highly recommended, and donors should be encouraged to fund such studies.

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