In Vitro Susceptibility of 120 Strains of Neisseria gonorrhoeae *Isolated in Kyrghyzstan*

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Background: **The World Health Organization has established a worldwide program for gonococcal antimicrobial surveillance, but so far no data on gonococcal susceptibility in Central Asia are available.**

Goal: **The need for biological data on the susceptibility of *Neisseria gonorrhoeae* in Kyrghyzstan, to enable adaptation of the national treatment protocol for gonococcal infections, led Médecins Sans Frontières and Epicentre to conduct a survey in collaboration with the Alfred Fournier Institute in Paris and the health authorities in Bishkek.**

Study Design: **In vitro susceptibility of *N gonorrhoeae* strains was determined with use of the reference agar-plate dilution technique.**

Results: **Results for 11 antibiotics tested on 120 strains of gonococci showed a low proportion (11.7%) of penicillinase-producing *N gonorrhoeae* and high proportions of intermediate or resistant strains to the majority of the antibiotics tested, including fluoroquinolones (≥25% of strains resistant). All the strains were susceptible to spectinomycin, and only two strains had decreased susceptibility to cefixime.**

Conclusion: **The therapeutic choices available in Kyrghyzstan appear to be limited to cephalosporins and spectinomycin.**

AN ESTIMATED 333 MILLION adult cases of curable sexually transmitted infections (STIs; gonorrhea, chlamydia, syphilis, and trichomoniasis) occurred worldwide in 1995, including 18 million in Central Asia and Eastern Europe, where STIs are a major public health problem.[1] The situation is complicated by the decreasing susceptibility of the germs (in particular, *Neisseria gonorrhoeae*) to commonly used antibiotics, especially the penicillin group. The World Health Organization therefore established in 1990 a surveillance program in nine regions of the world (the Gonococcal Antimicrobial Surveillance Program, or GASP), but so far no data on gonococcal susceptibility in Central Asia are available.

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Since 1996, Médecins Sans Frontières has been running a control program for STIs in the Osh region, in the southeast of Kyrghyzstan. In accordance with the World Health Organization standards, Médecins Sans Frontières recommends kanamycin or cotrimoxazole for the treatment of gonococcal infections, although these drugs should be used only in regions where they show continuing efficacy.[2] The national protocol in Kyrghyzstan recommends penicillin as first-line therapy, but in practice several antibiotics are used, including kanamycin at high doses (10 g intramuscularly).

A study was conducted within the Médecins Sans Frontières program between January 1999 and April 2000, in collaboration with the Kyrghyz health authorities, especially the Dermato-Venerological Republican Center, located in the capital (Bishkek), and the Alfred Fournier Institute in Paris. Its objective was to determine the in vitro susceptibility of *N gonorrhoeae*.

Materials and Methods

Subjects included in the study were all male patients presenting with simple acute purulent urethritis. They were all given free treatment with cotrimoxazole or kanamycin, as part of the standard regimen of the World Health Organization.

It is estimated that 100 to 150 gonococcal isolates need to be collected to measure a significant shift in antibiotic resistance between two surveys.[3] It was therefore necessary to include a total of 170 patients, with an expected loss of 40% of strains after thawing.

The *Neisseria* identification was first suspected by direct examination of strains (during which intracellular diplococci were noted) and Gram staining (negative), and it was confirmed by cultures of isolates that were under suspicion after Gram staining and oxidase testing (oxidase-positive).

TABLE 1. Susceptibility of 120 Strains of *Neisseria gonorrhoeae* Isolated in Kyrghyzstan Between January and April 1999

Antibiotic	MIC Reference Values, mg/l	Susceptibility Profile = No. (%) of Isolates		
		Susceptible	Intermediate	Resistant
Penicillin G	≤0.06–≥2.0	13 (10.8)	76 (63.3)	31 (25.9)
Tetracycline	≤0.5–≥2.0	39 (32.5)	42 (35.0)	39 (32.5)
Erythromycin	≤1.0–≥4.0	63 (52.5)	53 (44.2)	4 (3.3)
Chloramphenicol	≤0.5–≥2.0	6 (5.0)	52 (43.3)	62 (51.7)
Ceftriaxone	≤0.25	120 (100.0)	0 (0.0)	0 (0.0)
Cefixime	≤1.0	118 (98.3)	2 (1.7)	0 (0.0)
Spectinomycin	≤32.0–≥128.0	120 (100.0)	0 (0.0)	0 (0.0)
Kanamycin	≤32.0–≥128.0	82 (68.3)	33 (27.5)	5 (4.2)
TMP/SMZ	≤0.5/9.5–≥4.0/76.0	5 (4.2)	113 (94.1)	2 (1.7)
Ciprofloxacin	<0.125–≥1.0	80 (66.7)	28 (23.3)	12 (10.0)
Ofloxacin	<0.5–≥2.0	79 (65.8)	23 (19.2)	18 (15.0)

TMP/SMZ = trimethoprim/sulfamethoxazole.

Several culture media were tested (with addition of glucose, hemoglobin, horse blood, and human blood), on Thayer–Martin medium. Cultures were performed with and without antibiotics, because a proportion of 0.3% to 30.0% of gonococcal strains are naturally resistant to vancomycin.[4] Because of the urogenital origin of samples, carbohydrate degradation tests, which identify the type of *Neisseria*, were not performed, and positive cultures were assumed to be *N gonorrhoeae*. The MIC was determined by means of the reference agar-plate dilution technique, with chocolate agar in tryptic soy broth (Oxoid, Basingstoke, U.K.) supplemented with 5% horse cooked blood and 1% IsoVitaleX.[3] For cotrimoxazole, the medium used was the World Health Organization–recommended diagnostic susceptibility testing agar, with lysed horse blood and Kellogg's supplement.[5] Plasmid-mediated antimicrobial resistance (β-lactamase) was first detected in Kyrghyzstan by the chromogenic method and was confirmed at the Alfred Fournier Institute by the acidimetric method.

The susceptibility of the *N gonorrhoeae* isolates was tested against 11 antibiotics: penicillin G (Diamant), cefixime (Rhône-Poulenc), tetracycline (Roussel–Uclaf), ciprofloxacin (Bayer), ceftriaxone, spectinomycin, kanamycin, ofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole (TMP/SMZ), and erythromycin (Sigma). The strains were considered to be susceptible, intermediate, or resistant according to the interpretative standards and breakpoints defined by the National Committee for Clinical Laboratory Standards for aerobic bacteria, and the findings are included in Table 1.[5] For cefixime, we used the Antibiogramme Committee of the French Society for Microbiology reference values.[6] Three control/reference strains (Canadian origin; LSPQ 2664, 2667, and 2668) were included in the MIC study. The final bacterial inoculum was of 10^4 to 10^5 cfu/ml.

The development of gonococcal resistance to antibiotics may involve either chromosomal or extrachromosomal (plasmid) mechanisms, and for some antibiotics both may be implicated. Chromosomally mediated resistance is generally low (especially against penicillin and tetracycline) and results from modifications in the outer cell membrane and changes in access of the antibiotic to this target site. In addition, gonococcal acquisition of one of several types of plasmid results in high-level resistance to antibiotics.[7]

The strains were kept in a nutrition broth with 10% glycerol and frozen in liquid nitrogen. They were transported to France on dry ice and kept at the Alfred Fournier Institute at −80 °C.

Since Kyrghyzstan has no ethics committee, the protocol was submitted to a French ethics committee, according to CIOMS recommendations.[8] Informed written consent was obtained from patients.

Results

A total of 189 patients were included between January 20 and April 21, 1999, of whom 168 (89%) had a positive culture for *N gonorrhoeae*. The age of patients ranged from 13 to 60 years (median, 24 years). Associated *Trichomonas* was found in 42.9% (72/168).

The medium that produced the "best" cultures (number of strains and legibility) and therefore the most identifiable strains was Thayer–Martin, with supplements I and II and fresh human blood.

Four strains[4] were lost in Kyrghyzstan, but 164 were sent to France, of which 142 have been reinoculated so far; only 7 (5%) did not grow after thawing. MIC determinations were made for the first 120 strains (Table 1).

Among the strains resistant to penicillin, 17 (14.2%) showed a low-level/chromosomic resistance, and 14 (11.7%) were penicillinase-producing *N gonorrhoeae*, as evidence by the acidimetric method, with high-level/plasmidic resistance (MIC, ≥32.0 mg/l). All strains resistant to tetracycline had low-level/chromosomic resistance (MIC, <16.0 mg/l), and for none was the MIC ≥16.0 mg/l, corresponding to a high-level resistance in relation to the

production of a conjugative plasmid. A total of 14 strains (11.7% of the sample) were resistant to both penicillin and tetracycline.

Two strains[2] were intermediate for cefixime (MICs, 2.0 and 4.0 mg/l) and had decreased susceptibility to ceftriaxone (MIC, 0.125 mg/l) but were not penicillinase-producing. With 100% of strains susceptible to spectinomycin, this antibiotic had a better efficacy than kanamycin, to which 38 (31.7%) were intermediate or resistant.

Among the 30 and 32 strains exhibiting decreased susceptibility to ciprofloxacin and ofloxacin, respectively, the MICs for 9 (7.5%) and 18 (15.0%) were quite elevated ($>$1.0 mg/l); for 9 of them, the MICs of both antibiotics were elevated.

Discussion

Our study revealed a high proportion of gonococcal resistance to several antibiotics in our setting. Although decreased susceptibility to an antibiotic can be countered in the early stages with increased dosages (as for kanamycin in Kyrghyzstan), resistance to this antibiotic is expected to occur shortly afterward, therefore preventing its recommendation.

The proportion of penicillinase-producing *N gonorrhoeae* (11.7%, which is relatively low in comparison with that in other Asian countries[9]) is similar to that in France[10] and might be attributed to the limited use of penicillin in Kyrghyzstan over the past few years. The association of chromosomic resistance between penicillin and tetracycline (11.7% of the strains in this study) is frequent and has been described worldwide.[11]

The high proportion of strains intermediate or resistant to chloramphenicol might be the consequence of excessive utilization of this antibiotic for other pathologic conditions, because it is frequently observed in developing countries.

Natural resistance of gonococci to trimethoprim, combined with a high proportion of resistance to sulfonamides, might explain the low activity of TMP-SMZ on *N gonorrhoeae*. Since TMP-SMZ is no longer used for the treatment of gonococcal infections, except in developing countries, no data on the susceptibility of strains are available so far.

The high proportion of strains ($\geq$25.0%) exhibiting decreased susceptibility to fluoroquinolones is similar to that observed in Asia and Southeast Asia[9] and much higher than that observed in Europe, especially in France, where it is $<$3%.[10]

The very low proportion of strains (5%) that did not grow after thawing indicates that the procedures implemented for the conservation and the transport of the strains were adequate. However, the frequent polycontamination of the strains with other pathogens (*Staphylococcus*) observed at the Alfred Fournier Institute is probably due to a lack of asepsis in the laboratory procedures.

Although our results should be confirmed through other drug susceptibility studies conducted in different settings, they clearly indicate a reduction of the therapeutic alternatives for treating gonococcal STIs in Kyrghyzstan to third-generation cephalosporins and spectinomycin. In addition, further studies about mechanisms of resistance, especially to fluoroquinolones, should be conducted.

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