Antimicrobial susceptibility of *Neisseria gonorrhoeae* in Greece: data for the years 1994–2004

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**Objectives**: Surveillance data concerning antimicrobial susceptibilities of *Neisseria gonorrhoeae* isolated in Greece during the 11 year period 1994–2004 are presented.

**Methods**: Antimicrobial susceptibilities of all gonococcal isolates received by the Greek National Reference Center for *N. gonorrhoeae* during the study period were determined in terms of MICs using Etest. Trends in yearly isolation frequencies by susceptibility category were estimated for defining significant changes in overall susceptibility figures.

**Results**: Cefotaxime and spectinomycin retained undiminished activity against all isolates throughout the study period. High rates of resistance and intermediate susceptibilities were noticed for penicillin, tetracycline and erythromycin, and even for norfloxacin and ciprofloxacin. A substantial portion (16.5%) of the gonococcal samples consisted of multiresistant strains exhibiting resistance to two or more agents of different antibiotic classes. Although annual rates of low-level chromosomal resistance decreased, high-level resistance owing to the presence of penicillin- and tetracycline-resistance plasmids increased. Fluoroquinolone resistance also showed a significant increasing trend after 1996, reaching a peak rate of 11.3% in 2004.

**Conclusion**: Third-generation cephalosporins and spectinomycin should be considered as first-choice drugs for the empirical treatment of gonorrhoea in Greece.

Keywords: *N. gonorrhoeae*, gonorrhoea, antibiotic resistance, fluoroquinolones

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**Introduction**

The importance of monitoring antimicrobial resistance in *Neisseria gonorrhoeae* for disease management and control is well established.¹⁻³ The recent emergence of gonococci with reduced susceptibility to the currently recommended antibiotics for gonorrhoea therapy, including fluoroquinolones and some cephalosporins, reinforces the need for continuous surveillance of the susceptibility trends in *N. gonorrhoeae*.

The Greek National Reference Center for *N. gonorrhoeae* (NRCNG) has been conducting continuous surveillance on microbiological characteristics of gonococcal infection since 1984, and in previous publications has reported antibiotic susceptibility data for *N. gonorrhoeae* strains isolated in Greece up to 1993.⁴ In this article, we present data for the years 1994–2004.

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**Materials and methods**

A total of 1027 gonococcal strains submitted to the Greek NRCNG between January 1994 and December 2004 were included in the study. The strains were non-repetitive (one per patient) and represented 71.8% of all 1431 gonorrhoea cases reported to the NRCNG during this 11 year period; the remainder of the reported cases escaped culture, relying for diagnosis on symptoms and Gram-staining. Of the strains, 908 (88.4%) were consecutively isolated from the cases seen in the outpatient clinic of the ‘Andreas Sygros’ Hospital, Athens, which is the largest clinic in Greece for venereal diseases, receiving the main bulk of gonorrhoea cases throughout the country. The remaining 119 strains (11.6%) were sporadically isolated, most of them from occasional culturing of the cases seen in the Venereal Hospital of Thessaloniki, North...
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<td>2</td>
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Antimicrobial susceptibility of *N. gonorrhoeae* in Greece

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<th>MIC50 (mg/L)</th>
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<td>Nalidixic acid</td>
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Greece, and some from sporadic cases encountered in other non-STD-specialized hospitals located in Athens, Piraeus and Ioannina. This strain collection represented the total of the viable isolates obtained in all reporting hospitals without any kind of selection. Of the isolates, 1006 were from males and 21 from females. The limited number of strains from females probably reflects, at least in part, a preference among women to attend private medical practitioners, scarcely reporting gonorrhoea cases to the NRCNG. 

*N. gonorrhoeae* identification was performed with conventional procedures, including Gram-staining, oxidase reaction and carbohydrate utilization (*Neisseria* 4H, Amersham-BioRad-Diagnostics Pasteur). The MICs of penicillin, cefotaxime, spectinomycin, tetracycline, erythromycin, ciprofloxacin and norfloxacin were determined using Etest according to the instructions of the manufacturer (AB Biodisk, Sweden). Tests were performed on GC agar supplemented with Vitox (Oxoid). The plates were inoculated with a bacterial suspension of turbidity equal to 0.5 on the McFarland scale and incubated at 35°C in the appropriate conditions of CO₂ (5%) and humidity (70%). The *N. gonorrhoeae* strains WHO A to WHO D were used as quality control standards. For susceptibility categorization, the breakpoints proposed by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) were used, when available. Generally accepted breakpoints were adopted for erythromycin (MIC ≥ 2 mg/L for resistance and ≤0.25 mg/L for susceptibility) and norfloxacin (MIC ≥ 1 mg/L for resistance and ≤0.25 mg/L for susceptibility). For categorization of susceptibility results according to these breakpoints, MIC values lying between marginal 2-fold concentrations were rounded to the nearest higher concentration. Penicillinase-producing (PPNG) strains were detected using a nitrocefin assay. The presence of Tet-M plasmids in highly tetracycline-resistant gonococci (TRNG) was confirmed by amplification of the tetM gene using PCR as described previously. The characterization of strains as quinolone resistant (QRNG) was based upon growth on GC agar containing 64 mg/L nalidixic acid, indicative of gyrA or gyrB plus parC mutations.

Statistical analysis based on least squares (linear model) was carried out to define trends in yearly isolation frequencies by susceptibility category.

### Results and discussion

During the 11 year period of the study, the NRCNG received a total of 1027 isolates, with annual numbers ranging between 55 in 1996 and 142 isolates in 2004. Increments in the total number of isolates collected each year did not reflect any significant change in the relative incidence of gonorrhoea cases, which maintained its low rate over the study period.

Results from antimicrobial susceptibility testing of gonococcal strains isolated annually are presented in Table 1. Cefotaxime and spectinomycin retained undiminished activity against all the isolates throughout the study period, with only one strain, isolated in 1997, presenting intermediate susceptibility to spectinomycin. Ciprofloxacin, followed by norfloxacin, was active against the majority of the isolates, but a significant trend (P < 0.001) to increasing incidence of fluoroquinolone-resistant gonococci was noticed. Penicillin, tetracycline and erythromycin exhibited poor in vitro activities, with MIC₅₀ values in the intermediate range and MIC₉₀ values steadily in the resistance range. High rates of both plasmid-mediated and low-level chromosomally-mediated resistance contributed to this figure, but the most pronounced...
between the years 1991 and 1998 (Figure 1). The rates of multidrug-resistant outbreak strains documented in Greece were resistant and an additional 61% intermediately susceptible to tetracycline and much less pronounced for penicillin.

The level of resistance to penicillin exhibited by PPNG strains ranged from 2 to >256 mg/L and that of plasmid-mediated resistance to tetracycline exhibited by TRNG strains from 6 to 128 mg/L. Notably, the lower levels of plasmid-mediated resistance to penicillin and tetracycline, exhibited by the so-called CMRNG isolates, accounted for 3.6% of the total gonococcal isolates. In contrast to plasmid-mediated resistance, the frequency of isolates chromosomally resistant to either agent decreased (P < 0.01), probably reflecting a reduction in the presence of multidrug-resistant outbreak strains documented in Greece between the years 1991 and 1998 (Figure 1). The rates of chromosomal resistance were greater for erythromycin than for tetracycline and much less pronounced for penicillin.

An additional portion of the gonococcal population studied, consisting of 180 (17.5%) of the total isolates, were chromosomally resistant to penicillin, tetracycline or erythromycin, with half of them (90 isolates) exhibiting cross-resistance to one or both of these agents (data not shown). Combined resistance to penicillin and tetracycline, exhibited by the so-called CMRNG isolates, accounted for 3.6% of the total gonococcal sample. In contrast to plasmid-mediated resistance, the frequency of isolates chromosomally resistant to either agent decreased (P < 0.01), probably reflecting a reduction in the presence of multidrug-resistant outbreak strains documented in Greece between the years 1991 and 1998 (Figure 1).

The most important observation in the present study is the establishment of QRNG strains in the Greek community. QRNG strains were only sporadically isolated in Greece before 1994 and were absent in the first 2 years of the study period. In the years 1997 and 1998, the annual MIC\textsubscript{90} values of fluoroquinolones were slightly increased, mainly due to the presence of isolates intermediately susceptible to these agents. Apart from this event, which has been attributed to clonal spread of an outbreak strain, QRNG strains with high levels of fluoroquinolone resistance were isolated in progressively increasing frequencies after 1996, reaching a rate of 11.3% in the year 2004 (P < 0.001) (Table 1 and Figure 1). Similar findings have been reported recently from most other countries in Europe and worldwide.

Irrespective of the resistance type and pattern, 35.5% (364 strains) of all gonococci isolated during the study period were resistant and an additional 61% intermediately susceptible to at least one of the antibiotics tested. Moreover, approximately half of the resistant strains (16.5% of all isolates) showed multiple-resistance patterns, including two or more different antibiotic classes (data not shown).

In conclusion, antimicrobial susceptibility of N. gonorrhoeae isolated in Greece during the past decade was characterized by high rates of resistance and intermediate susceptibility to penicillin, tetracycline, erythromycin and fluoroquinolones. To our knowledge, none of these agents is currently used as first-line therapy for gonorrhoea, at least in the clinical settings reporting to the NRCNG. However, resistance profiles documented here cannot be easily interpreted in relation to antimicrobial policies, since national guidelines do not exist. Different prescribing policies may be followed depending on each hospital’s directives and probably based on international guidelines combined with local clinical experience. Moreover, the unregulated use of various antibiotics, including penicillins, macrolides and fluorinated quinolones, that are available over the counter in Greece may have contributed to the establishment of resistance. Results of this study illustrate that third-generation cephalosporins and spectinomycin must be considered as first-choice drugs for the empirical treatment of gonorrhoea in Greece.

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Transparency declarations

None to declare.

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