# Fluoroquinolone Resistance in *Neisseria gonorrhoeae*

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Fluoroquinolones and broad-spectrum cephalosporins are the most effective antimicrobial agents for the treatment of gonorrhea. However, clinically significant resistance to fluoroquinolones has emerged in *Neisseria gonorrhoeae*. Fluoroquinolone-resistant strains account for approximately 10% of all gonococcal strains in Hong Kong and the Republic of the Philippines. As many as 50% of strains from some Far Eastern countries exhibit decreased susceptibility (intermediate resistance) to fluoroquinolones. Strains with intermediate resistance and clinically significant resistance are being isolated sporadically in North America, where resistant strains have been associated with an outbreak and with failure of infections to respond to treatment with doses of ciprofloxacin and ofloxacin recommended by the Centers for Disease Control and Prevention; strains exhibiting decreased susceptibility to these agents are endemic in at least one metropolitan area. Monitoring for fluoroquinolone resistance is now critical for ensuring adequate treatment of infections with resistant strains and for maximizing the time during which fluoroquinolones may be used to treat gonorrhea.

Gonorrhea is among the most prevalent sexually transmitted diseases throughout much of the world. The emergence of resistance to antimicrobial agents in *Neisseria gonorrhoeae* is a major obstacle in the control of gonorrhea. In 1989 and 1993, in response to the increasing frequency of isolation of penicillin-, tetracycline-, and spectinomycin-resistant strains of *N. gonorrhoeae* in the United States, the Centers for Disease Control and Prevention (CDC) recommended the use of broad-spectrum cephalosporins or fluoroquinolones for the primary treatment of uncomplicated gonorrhea (1,2). However, resistance to fluoroquinolones has now emerged in *N. gonorrhoeae* (3-8). Because ciprofloxacin and ofloxacin are more frequently used to treat gonorrhea than other fluoroquinolones, this synopsis will focus on these agents.

## Fluoroquinolone Therapeutic Regimens, Therapy Failure, and Susceptibility Tests

In 1993, CDC recommended single-dose, oral therapy with ciprofloxacin (500 mg) or ofloxacin (400 mg) as two of the primary regimens for the treatment of uncomplicated gonorrhea (2). Enoxacin (400 mg), lomefloxacin (400 mg), and norfloxacin (800 mg) were recommended among alternative regimens (2). In some countries, gonococcal infections have been treated with a single, orally administered dose of 250 mg ciprofloxacin (8). The failure of gonococcal infections to respond to treatment with 250 mg ciprofloxacin has been reported in the United Kingdom since 1990 (8-11). The failure of infections to respond to single-dose therapy with 500 mg ciprofloxacin or 400 mg ofloxacin has been reported in the United Kingdom, Australia, Canada, Hong Kong, and the United States (3-7,12).

Different methods for determining in vitro antimicrobial susceptibilities of *N. gonorrhoeae* have been developed in several countries. Differences between these methods—the medium on which the susceptibilities are determined, the concentrations of antimicrobial agents tested, the concentration of antimicrobial agents in disks used to determine zone inhibition diameters, or the inoculum size—complicate the interpretation of susceptibility test results. For example, in the United Kingdom, Australia, and Hong Kong, susceptibilities are usually

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determined on Isosensitest medium (Oxoid, Basingstoke, United Kingdom) (3,8,13-15). In the United States (where criteria for interpreting susceptibilities of *N. gonorrhoeae* are established by the National Committee for Clinical Laboratory Standards [NCCLS]) and Canada, susceptibilities are determined on a Difco or Becton Dickinson base medium (Difco Laboratories, Detroit, MI; Becton Dickinson, Cockeysville, MD) (16-19). Antimicrobial susceptibilities determined on Oxoid base medium may be one concentration lower than those determined on supplemented Difco or Becton Dickinson base media (15). Neither method is right or wrong; nor is one method better than the other; they are different. The method used in different countries is influenced by the commercial availability of the base medium. Thus, criteria for interpreting susceptibilities in the United States and Canada may differ slightly from those used to interpret susceptibilities in the United Kingdom, Australia, and Hong Kong, reflecting the difference in susceptibilities obtained on different media. Similarly, when susceptibilities are measured on media different from those described above it may be necessary to establish "local" criteria using reference strains with known susceptibilities.

Gonococcal strains associated with therapy failure to 250 mg ciprofloxacin have had minimum inhibitory concentrations (MICs) of ≥0.06 to 0.25 µg/ml of ciprofloxacin (8,11); posttreatment strains from infections that failed to respond to treatment with 500 mg ciprofloxacin or 400 mg ofloxacin have had MICs ≥1.0 µg/ml and ≥2.0 µg/ml, respectively (4-6,12,15).

## Criteria for Interpreting Fluoroquinolone Resistance

Before the emergence of fluoroquinolone resistance in *N. gonorrhoeae*, NCCLS established interpretive criteria to differentiate between susceptible strains and those exhibiting decreased susceptibility to selected fluoroquinolones including ciprofloxacin, ofloxacin, lomefloxacin, and enoxacin (16,17). Criteria for the interpretation of clinically significant resistance of gonococcal strains to 500 mg ciprofloxacin and 400 mg ofloxacin have been proposed (6,18).

Criteria for interpreting susceptibilities of *N. gonorrhoeae* to antimicrobial agents should be based on treatment outcome and strain susceptibility data. However, because many infections caused by fluoroquinolone-resistant strains were treated with broad-spectrum cephalosporins and few observations linked fluoroquinolone therapy outcome and antimicrobial susceptibilities (MICs), CDC proposed criteria for interpreting fluoroquinolone resistance in *N. gonorrhoeae* that were based on theoretical predictions of the MICs at which gonococcal infections may fail to respond to CDC-recommended doses of selected fluoroquinolones (18). On the basis of the therapeutic index (calculated by dividing the peak level of the agent in serum by the MIC of the infecting strain), CDC proposed criteria for interpreting susceptibilities to selected fluoroquinolones (Table) (18,20). These criteria were consistent with the MICs of isolates from the observed treatment failures to 500 mg ciprofloxacin and 400 mg ofloxacin documented at the time of the study (4,5,12). Recently, Kam et al. proposed interpretive criteria based on the susceptibilities of many strains that did not respond to treatment with ofloxacin in Hong Kong (Table) (6). CDC and Kam et al. proposed identical MICs for interpreting resistance to ciprofloxacin and ofloxacin: MICs of ≥2.0 µg/ml and ≥1.0 µg/ml of ofloxacin and ciprofloxacin, respectively. These criteria have also been adopted in Australia (15).

In the United Kingdom, where 250 mg ciprofloxacin has been used to treat gonorrhea, strains with MICs of 0.06 to 0.25 µg/ml have been isolated from infections that did not respond to treatment (8,11). Ciprofloxacin in a 250-mg dose produces a peak serum level of approximately 1.2 µg/ml; thus, strains with MICs of ≥0.25 to 0.5 µg/ml would produce therapeutic indices of 4.8:1 and 2.4:1, respectively. These calculated MICs suggest that strains with MICs ≥0.25 µg/ml should be considered resistant to treatment with 250 mg ciprofloxacin. Criteria for interpreting susceptibilities of gonococcal strains to treatment with 250 mg ciprofloxacin have been determined to ciprofloxacin (1 µg disks) or nalidixic acid (30 µg disks) (10,21). Measurement of nalidixic acid resistance may be useful for detecting strains causing infections that may not respond to treatment with 250 mg ciprofloxacin. However, because naladixic acid-resistance indicates decreased susceptibility to ciprofloxacin and ofloxacin, this test does not differentiate between strains with intermediate resistance and clinically significant resistance to treatment with 500 mg ciprofloxacin or 400 mg ofloxacin (18).

Table. Criteria for interpreting susceptibilities of *Neisseria gonorrhoeae* strains to the fluoroquinolones ciprofloxacin, ofloxacin, enoxacin, lomefloxacin, and norfloxacin[a,b]

| Therapeutic agent, Dose | Agent Tested, Disk Content (µg) | Zone Inhibition Diameters[c] nearest whole mm | | | Equivalent MIC[c] (mg/ml) | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | R | I | S | R | I | S | |
| Ciprofloxacin, 250 mg | Cip, 1 | ≤24 | | | ≥0.03 | | | 8 |
| Ciprofloxacin, 500 mg | Cip, 5 | ≤29 | 30-35 | ≥36 | ≥1.0 | 0.13-0.5 | ≤0.06 | 18 |
| | Cip, 5 | ≤22 | | | ≥1.0 | | | 6 |
| Ofloxacin, 400 mg | Ofx, 5 | ≤24 | 25-30 | ≥31 | ≥2.0 | 0.5-1.0 | ≤0.25 | 18 |
| | Ofx, 5 | ≤24 | | | ≥1.0 | | | 6 |
| Enoxacin, 400 mg | Enx, 10 | ≤31 | | ≥32 | ≥1.0 | 0.5 | ≤0.25 | 18 |
| Lomefloxacin, 400 mg | Lom, 10 | ≤26 | 27-35 | ≥36 | ≥2.0 | 0.25-1.0 | ≤0.125 | 18 |
| Norfloxacin, 800 mg | Nor, 5 | ≤32 | 33-35 | ≥36 | ≥1.0 | 0.5 | ≤0.25 | 18 |

[a]These criteria have not been recommended by the National Committee for Clinical Laboratory Standards (NCCLS); they are provided to guide interpretation of susceptibilities until the NCCLS establishes criteria.
[b]Abbreviations: MIC, minimal inhibitory concentration (µg/ml); R, resistant; I, intermediate resistance; S, susceptible; Cip, ciprofloxacin; Ofx, ofoxacin; Enx, enoxacin; Lom, lomefloxacin; Nor, norfloxacin.
[c]With the exception of norfloxacin, criteria for the susceptible categories are those designated by the NCCLS (16,17); criteria for the interpretation of the susceptible category for norfloxacin were proposed by CDC (18).

## Geographic Distribution, Frequency, and Diversity of Fluoroquinolone-resistant *N. gonorrhoeae*

Strains exhibiting intermediate resistance (MICs 0.125 to 0.5 µg/ml of ciprofloxacin; MICs 0.5-1.0 µg/ml of ofloxacin) have been reported from many geographic areas including Australia, Canada, the Canary Islands, Hong Kong, Japan, the Republic of the Philippines, mainland Spain, Thailand, the United Kingdom, the United States, and the West Indies (4,7,9-15,21-32). Fluoroquinolone resistance (MICs of ≥1.0 µg/ml or ≥2.0 µg/ml of ciprofloxacin or ofloxacin, respectively) has been reported most frequently from the Far East, Republic of the Philippines, Hong Kong, Japan) and less frequently from Australia, Canada, Spain, Thailand, United Kingdom, and the United States (3,4,6,7,9,12,15,22-32). Ofloxacin- and ciprofloxacin-resistant strains had been isolated sporadically in Hong Kong, the Republic of the Philippines, and Thailand for some years (22-24). The frequency of fluoroquinolone-resistant strains has increased dramatically since the early 1990s. For example, in Hong Kong, fluoroquinolone-resistant strains were isolated intermittently during 1990 to 1992 (13) but have increased dramatically from an estimated 0.5% in late 1992 to 10.4% in late 1994 (14).

From 1994 to 1995, strains exhibiting decreased susceptibility to ciprofloxacin and ofloxacin accounted for approximately 36%, 54%, and 22% of strains in Hong Kong, the Republic of the Philippines, and Thailand, respectively (13,14,29,31,32). During the same period, fluoroquinolone-resistant strains accounted for approximately 10%, 12%, and 1% of all strains in Hong Kong, the Republic of the Philippines, and Thailand, respectively (13,14,29,31,32). Strains with ciprofloxacin MICs of ≥8.0 µg/ml were first isolated in 1994 (10,12,29,31).

In other geographic areas, strains exhibiting intermediate resistance and resistance have been isolated only sporadically, although with increasing frequency. In Sydney, Australia, fluoroquinolone-resistant strains were isolated infrequently in 1991 to 1994, but with dramatically increasing frequency in early 1995 (15). The pattern of isolation of fluoroquinolone-resistant strains in Australia, i.e., infrequent and sporadic isolations for a number of years followed by increasing frequency of isolation over a short period, may be anticipated in other countries unless the fluoroquinolone-resistant strains are controlled in the Far East, where they are now prevalent. In the CDC-sponsored Gonococcal Isolate Surveillance Project in the United States, the frequency of strains with intermediate resistance has increased significantly from 0.3% (17/5,238) in 1991 to 1.3% (65/4,996) in 1994 ($p \leq 0.001$); however, resistant strains accounted for only 0.04% (2/4,996) of strains in 1994 (33,34). In the United States, the increase in strains with intermediate resistance is associated largely, but not exclusively, with the persistence of such strains in Cleveland, Ohio. First detected in 1992, these strains accounted for 16% to 17.5% of isolates in Cleveland in 1994 (26,35). In addition, a sustained outbreak caused by ciprofloxacin-resistant strains has been reported from Seattle, Washington, in 1995; these strains had MICs of 8.0 µg/ml of ciprofloxacin and ofloxacin (5).

Many different strains, as defined by penicillin/tetracycline resistance phenotype and auxotype/serovar (A/S) class, exhibit intermediate resistance and resistance to fluoroquinolones (4,5,15,25,26,31,32,36). Fluoroquinolone resistance has been identified frequently in strains that produce ß-lactamase and strains exhibiting chromosomally mediated resistance to penicillin and tetracycline and less frequently in strains that are susceptible to penicillin and tetracycline (4,31,32). Fluoroquinolone resistance has not been documented in strains possessing the 25.2-megadalton TetM-containing plasmid (TRNG) alone or in TRNG strains possessing a ß-lactamase plasmid (31). It should not, however, be assumed that these strains may not develop fluoroquinolone resistance, but rather that resistant strains have not been detected at this time. Many strains, as defined by A/S class, are associated with fluoroquinolone resistance; fluoroquinolone resistance is not associated with the epidemic spread of one or two strains (15,31,32). In Australia, strains exhibiting intermediate resistance and resistance to fluoroquinolones have belonged to 27 different A/S classes; strains with MICs 8.0-16.0 µg/ml of ciprofloxacin have belonged to 6 A/S classes (15). In the Republic of the Philippines, strains belonging to 27 A/S classes exhibited decreased susceptibility to ciprofloxacin; strains belonging to 10 A/S classes had MICs ≥1.0 µg/ml of ciprofloxacin (31). In Thailand, strains belonging to 13 A/S classes exhibited decreased susceptibility to ciprofloxacin (32).

The Gonococcal Isolate Surveillance Project monitors the susceptibilities of *N. gonorrhoeae* isolates to fluoroquinolones in the United States and provides prototype reference strains for quality assurance of susceptibility testing of these agents. Information about antimicrobial resistance in *N. gonorrhoeae* in the United States is available on the Internet at http://www.cdc.gov/ncidod/dastlr/gcdir/gono.html.

## Mechanisms of Resistance to Fluoroquinolones

Fluoroquinolones inhibit the replication of DNA; they are believed to bind to the GyrA region of DNA gyrase, which is attached to DNA, and inhibit the enzyme from supercoiling the DNA (37). Resistance to fluoroquinolones in *N. gonorrhoeae* is associated with mutations that result in amino acid changes in the A subunit (GyrA) and the B subunit (GyrB) of the DNA gyrase, and in the *parC*-encoded subunit of topoisomerase IV (37-40). Although mutations in *gyrB* confer low-level resistance to naladixic acid, high-level quinolone resistance is associated with mutations in the quinolone resistance-determining region of *gyrA* (41). Topoisomerase IV, encoded by *parC* and *parE* in *Escherichia coli* and believed to be located in the cytoplasmic membrane, is involved in DNA replication but is not as sensitive to fluoroquinolone inhibition as is DNA gyrase (37). No *parE* analog has been detected in *N. gonorrhoeae* (37). Mutations in *gyrA* and *parC* are most relevant when considering clinically significant levels of fluoroquinolone resistance in *N. gonorrhoeae* (37,39,40). Similar results have been obtained in studies of *gyrA* mutations in both laboratory-adapted strains and clinical isolates (37,39,40): ciprofloxacin-susceptible strains (MICs, <0.03 µg/ml) had no mutations in *gyrA* and strains with MICs, ≥0.5 µg/ml of ciprofloxacin may have changes in nucleotides 272 and 283 of *gyrA*. In addition, strains with MICs ≥2.0 had mutations in *parC*. Mutations in *parC* were observed only in strains with at least one mutation in *gyrA* (37,39) and appeared to be associated with an MIC higher than would be expected with the *gyrA* mutation alone (39). Mutations in *gyrA* and *parC* may be characterized by polymerase chain reaction and DNA sequencing (37,39). The transfer of *gyrA* and *parC* mutations between gonococcal strains has been demonstrated in vitro (37). The presence of transformation sequences just downstream from the *gyrA* sequences suggests that transformation may play a role in the spread of *gyrA* mutations between gonococcal strains in vivo (37). The opportunity for transformation of genes between gonococcal strains, which depends on concurrent infections with multiple strains, has been documented for women and homosexual men (42,43).

In addition to mutations in *gyrA* and *parC*, reduced permeability of the cytoplasmic membrane may contribute to low-level resistance to fluoroquinolones in *N. gonorrhoeae*, e.g., increasing the MIC of one recipient strain from ≤0.002 to 0.06 µg/ml of ciprofloxacin (3,44). This resistance may also be transferred between gonococcal strains by transformation (3).

## Guidance

Continued reports of the isolation of fluoroquinolone-resistant strains of *N. gonorrhoeae* indicate the need for heightened awareness of the potential for increasing prevalence of strains with

clinically significant fluoroquinolone resistance. In areas where a fluoroquinolone is used to treat gonorrhea, the following steps are recommended to monitor and control the spread of fluoroquinolone-resistant strains of *N. gonorrhoeae*.

- Susceptibility testing should be performed to detect fluoroquinolone resistant strains. In geographic areas where interpretive criteria have not been proposed, criteria should be developed for results obtained with local test procedures that use strains with known susceptibilities to ciprofloxacin or ofloxacin.
- Ideally, routine surveillance for emerging fluoroquinolone resistance should be performed in centers where fluoroquinolones are used widely to treat gonorrhea; e.g., a sample of approximately 20 to 50 consecutive isolates should be tested periodically. If ciprofloxacin-resistant isolates are detected, the use of alternative therapies for gonorrhea should be considered; if isolates are ciprofloxacin-susceptible, ciprofloxacin or ofloxacin may be used to treat gonorrhea.
- Susceptibilities of isolates from individual patients whose infections did not respond to treatment with a fluoroquinolone should be determined.
- A cluster of infections unresponsive to fluoroquinolone therapy may indicate an outbreak caused by a resistant strain.
- If treating gonorrhea with a fluoroquinolone, e.g., ciprofloxacin or ofloxacin, never use less than the recommended dose. In the United States, 500 mg of ciprofloxacin or 400 mg ofloxacin should be used.
- Because gonococcal infections caused by fluoroquinolone-resistant strains have been acquired frequently in the Far East, clinicians may wish to treat infections possibly acquired in the Far East with 125 mg ceftriaxone, intramuscularly, or 400 mg cefixime, orally, the current CDC-recommended doses for treating gonococcal infections.

The importance of the emergence and spread of fluoroquinolone resistance in *N. gonorrhoeae* cannot be overstated. Of antimicrobial agents available for treating gonorrhea, broad-spectrum cephalosporins are the only agents to which *N. gonorrhoeae* is not resistant, and exclusive use of these agents, particularly the orally administered cephalosporins such as cefixime, may result ultimately in the emergence of resistance to these agents. Thus, it is critical to take measures to ensure that fluoroquinolones remain effective for the treatment of uncomplicated gonorrhea for as long as possible.

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