Fluoroquinolone Resistance in *Neisseria gonorrhoeae* After Cessation of Ciprofloxacin Usage in San Francisco:

Using Molecular Typing to Investigate Strain Turnover

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Background: Ciprofloxacin resistance (CipR) among gonococcal strains in San Francisco (SF) increased between 2001 and 2006 and decreased between 2007 and 2009. Molecular typing of isolates obtained from 2005 to 2009 was performed to elucidate changes in CipR prevalence.

Methods: A total of 2526 samples were collected at the SF City Clinic between 2001 and 2009. Minimum inhibitory concentrations to ciprofloxacin were obtained by agar dilution. Prevalences of CipR strains were determined, with corresponding confidence intervals (CIs). Between 2005 and 2009, 460 isolates were selected for molecular typing using *Neisseria gonorrhoeae* multiantigen sequence typing.

Results: Between 2001 and 2006, the prevalence of CipR increased from 3.4% (95% CI, 1.3%–5.4%) to 44% (95% CI, 39%–50%). However, in 2007 prevalence began to decrease, reaching 9.6% (95% CI, 6.0%–13%) by 2009. Of the 203 strain types identified between 2005 and 2009, 126 genogroups of closely related strain types were formed (varying by ≤1% at both target loci). Levels of CipR within the data set correlate with the prevalence of 3 major genogroups (G): G437, G1407, and G3112.

Conclusions: Molecular typing reveals that CipR within the tested population is maintained by strain turnover between resistant genogroups. Despite early recommendation in 2002 to stop ciprofloxacin use in California, CipR in SF increased through 2006. The subsequent decrease in CipR corresponds with the 2007 national recommendation to cease ciprofloxacin treatment of gonorrhea, which suggests that national recommendations are potentially more effective at reducing CipR than regional recommendations in areas with high strain turnover.

Gonorrhea is currently the second most commonly reported sexually transmitted disease in the United States, with an estimated 820,000 new cases occurring each year.[1,2] *Neisseria gonorrhoeae* (*GC*), the causative agent of gonorrhea, is highly transmissible, has an impressive capacity for gaining resistance to antibiotics, and, when left untreated, can facilitate HIV transmission and cause other serious sequelae.[1,3–5]

Since the 1940s, *GC* has gained resistance to nearly all classes of antibiotics used against it, including sulfonamides, penicillins, tetracyclines, and fluoroquinolones.[6–11] Ciprofloxacin, a second-generation fluoroquinolone, became the recommended treatment of *GC* infections in 1993, as it was determined to be safe, efficacious, and cost-effective.[8] Ciprofloxacin-resistant (CipR) *GC* was reported first in Hawaii, followed by California, then among men who have sex with men (MSM), and eventually the rest of the United States.[12] Because of the growing prevalence of resistant isolates, ciprofloxacin recommendations were stopped in California and Hawaii in 2002, among the MSM population in 2004, and in 2007 for the rest of the country.[8,13,14] The development of fluoroquinolone resistance resulted in the availability of a single class of antibiotics that meet the Centers for Disease Control and Prevention's (CDC) standards for treatment of gonorrhea—the cephalosporins.[14] However, there is now growing evidence for *GC* resistance to cephalosporins within the United States as well.[9,15–17]

Because of the rise in cephalosporin resistance, we sought to examine the current state of ciprofloxacin susceptibility within San Francisco (SF). Surveillance of antibiotic resistance in *GC* is monitored nationally by the CDC Gonococcal Isolate Surveillance Project (GISP). GISP data collected at the SF City Clinic were used in our study to examine the prevalence of ciprofloxacin susceptibility from 2001 to 2009. Despite regional recommendations to stop the use of fluoroquinolones, CipR increased between 2001 and 2006 and decreased beginning in 2007, when national recommendations were implemented.[12] The purpose of this study was to examine potential factors that attributed to the CipR trends in SF.

To investigate the observed changes in CipR prevalence, we used molecular typing to determine strain type (ST) identities for GISP isolates collected at the SF City Clinic. Combining ST identities with GISP susceptibility data allowed us to determine which STs were responsible for resistance within the population from year to year. The observation

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of strain turnover within the population is also permitted. These data helped to characterize the tested population and elucidate the changing CipR levels observed within SF. The method used herein of combining genetic-based surveillance with phenotypic surveillance provides a powerful tool for understanding trends in drug resistance. With the growing threat of cephalosporin-resistance in the United States, there is an urgent need to better understand the epidemiology of resistance in *GC*.

MATERIALS AND METHODS

GC Isolate Collection

From 2001 to 2009, 2526 urogenital *GC* isolates were collected from symptomatic males at the SF City Clinic. All cultures were isolated on Modified Thayer Selective Agar (Becton Dickinson, Sparks, MD) and immediately incubated for 24 hours in 10% CO_2-enriched atmosphere at 35 °C. Plates with growth were transferred to the SF Public Health Laboratory and identified via microscopy after Gram staining and oxidase test. Cultures were purified on Chocolate Agar and incubated for 24 hours in 10% CO_2-enriched atmosphere at 35 °C. Pure cultures were frozen as paired samples in tryptic soy broth with 1% glycerol. One set of samples was shipped to a CDC GISP laboratory at the University of Washington, Seattle, and the second set was stored at −30 °C.[18] Not all samples survive culturing, transport, and/or storage; thus, in some years, there were less than 300 isolates available from the SF City Clinic.

Drug Susceptibility Testing

Isolates sent to the University of Washington GISP Laboratory were tested for ciprofloxacin susceptibility using the agar dilution method as outlined by the CDC GISP protocol.[19] The CDC defines CipR as a minimum inhibitory concentration (MIC) ≥ 1.0 μg/mL.[20]

Monitoring Drug Susceptibility

The prevalence of CipR *GC* isolates collected at the SF City Clinic was obtained from the 2001–2009 GISP Annual Reports.[12] Ciprofloxacin resistance prevalence was calculated as the number of CipR isolates divided by the total number of isolates collected. Corresponding 95% confidence intervals (CI) were calculated. Statistics were performed using STATA 12.[21]

A comparison of susceptibility to third-generation cephalosporins among genogroups 437, 1407, and 3112 was performed using a *t* test of the mean MIC to third-generation cephalosporins. The *F* test was used to determine whether the *t* test assumed equal or unequal variance. Comparisons were found to be statistically significant for 2-tailed *P* values less than 0.05. Descriptive and statistical analyses were performed using Microsoft Excel 2011 and StatPlus:mac 2009.

GC Multiantigen Sequence Typing

Of the 2526 clinical samples obtained at the SF City Clinic from 2001 to 2009, 1370 were collected after 2005. All isolates collected in 2009 were sequenced as part of another study.[22] Using the complete information available from 2009, we randomly selected 60 isolates per year from 2005 to 2008 for molecular typing using *GC* multiantigen sequence typing (NG-MAST). Not all samples amplified because many were inviable. We did not replace samples that did not amplify, which resulted in the following number of samples available for molecular typing each year: 45 from 2005, 56 from 2006, 54 from 2007, and 45 from 2008. Random selection was done without replacement from the CipR population and from the ciprofloxacin-sensitive population to maintain proportions of resistance that were observed among the entire population collected at the SF City Clinic. Isolates collected before 2005 were not available for molecular typing because the archived nature of the samples made them unviable for amplification.

Multiantigen sequence typing was performed using a modification from the original protocol for NG-MAST.[18] The *por*B gene fragment was amplified using a single round of polymerase chain reaction (PCR), whereas the *tbp*B gene fragment was amplified using 2 rounds of nested PCR on the GSTORM 482 thermocycler. Amplification of the *por*B gene fragment was performed in reaction volumes of 20 μL. Each PCR contained 0.5 μM of each primer, 1× buffer (New England Biolabs), 200 μM dNTPs (New England Biolabs), 5 μL template DNA, 0.4 U of Phusion DNA Polymerase (New England Biolabs), and water to a volume of 20 μL. The PCR cycle involved an initial denaturation of 3 minutes at 95 °C, followed by 35 cycles of 30 seconds at 95 °C, 30 seconds at 64 °C, 2 minutes at 72 °C, followed by a final extension of 5 minutes at 72 °C and cooling to 10 °C. Polymerase chain reaction amplification of the *tbp*B gene fragment was performed using 0.2 μM of each primer, 1× buffer (Life Technologies), 1.5 mM $MgCl_2$ (Life Technologies), 200 μM dNTPs (New England Biolabs), 0.08 μL Platinum Taq DNA Polymerase (Life Technologies), and water to a volume of 20 μL. For the external round of PCR, 5 μL of template DNA was used; for the internal round, a 1-μL sample from the external round was used as template DNA. Thermocycling conditions were done by the same method, but with an annealing temperature of 56 °C. Sequences of primers are specified in Table 1. The amplified regions of the *por*B and *tbp*B genes were prepared for 4 sequencing reactions: 2 with a forward primer (*por*B-NGS2 or *tbp*B-NGS1) and 2 with a reverse primer (*por*B-NGS1 or *tbp*B-NGS2). Amplicon sizes for *por*B and *tbp*B were 490 and 390 nucleotides, respectively. Sequences were obtained using the 3730xl DNA Analyzer (Applied Biosystems). ClustalW2 was used to generate sequence alignments, and consensus sequences were obtained using the scoring matrix EDNAFULL. Consensus sequences for each isolate were submitted to the NG-MAST database (www.ng-mast.net) and assigned allele and ST identities. Closely related STs (differing by <1% DNA sequence of the *por*B and *tbp*B gene) were grouped into genogroups, as previously described in Buono et al.[22] and Hess et al.[23]

RESULTS

Prevalence of CipR Isolates

Among the 2526 SF City Clinic *GC* samples tested for susceptibility to ciprofloxacin between 2001 and 2009, 506 were resistant to ciprofloxacin (MIC ≥ 1.0 μg/mL). The prevalence of

TABLE 1. Sequences of Primers

Primer	Sequence
por-NGP1	CCAAGGCAGAAGTGCGTTTGGAGAAG
por-NGP2	TCGCCGACTTCGGTTCAAAAATCGGC
por-NGS1	CCGACAACCACTTGGT
por-NGS2	CAAGAAGACCTCGGCAA
tbpB-NGP1	GGAATTGGGTTTCCGCTTTTTGAGCCAC
tbpB-NGP2	AGATAAACGACTTTTTGTTCTTTTGG
tbpB-NGS1	CGTTGTCGGCAGCGCGAAAAC
tbpB-NGS2	TTCATCGGTGCGCTCGCCTTG

Primers were obtained from Integrated DNA Technologies.

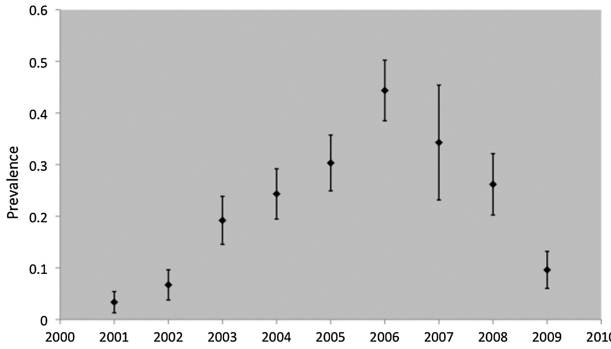

Figure 1. Prevalence of CipR *GC* isolates with corresponding 95% CIs: SF City Clinic 2001–2009.

CipR isolates increased from 3.4% (95% CI, 1.3%–5.4%) in 2001 to 44% (95% CI, 39%–50%) in 2006. However, in 2007, the prevalence of CipR isolates decreased to 34% (95% CI, 23%–45%) and further dropped to 9.6% (95% CI, 6.0%–13%) by 2009 (Fig. 1). Figure 1 shows the prevalence of CipR for all years from 2001 to 2009, with corresponding 95% CIs.

NG-MAST Analysis of *N. gonorrhoeae* Isolates

Between 2005 and 2009, 460 isolates underwent molecular typing: 45 from 2005 (n = 300), 56 from 2006 (n = 299), 54 from 2007 (n = 300), 45 from 2008 (n = 211), and 260 from 2009 (n = 260). The results of NG-MAST from this period revealed 203 unique STs among the 460 isolates. Within these 203 STs, 126 genogroups were defined. Three genogroups were notable for their high prevalence among the CipR population: G437, G1407, and G3112. G437 was the largest genogroup in this study and included 18 different STs (ST437, ST225, ST6179, ST7546, ST7543, ST7545, ST892, ST7544, ST7542, ST7449, ST4534, ST7452, ST7538, ST1132, ST7475, ST1861, ST891, and ST7539). G1407 included ST1407, ST3431, and ST4533, whereas G3112 only included ST3112. Between 2005 and 2009, all STs within G437, G1407, and G3112 were consistently observed to have a CipR phenotype. Antibiogram data for these genogroups are presented in Figure 2.

Strain Turnover Within the Resistant Population

Among the tested population, the prevalence of G437 increased from 15% (7/45) in 2005 to 43% (24/56) in 2006. In 2007, the prevalence decreased to 7.4% (4/54) and by 2009 dropped to 1.5% (4/260) (Fig. 3). A similar trend is seen for G437 among the CipR isolates. In 2005 and 2006, G437 made up 39% (7/18) and 71% (24/34) of the resistant population. However, from 2007 to 2009, the prevalence of G437 declined to 24% (5/21), 15% (4/26), and 16% (4/25), respectively (Fig. 4).

In 2005 and 2006, the prevalence of G1407 within the population is 0.0% (0/45, 0/56). However, G1407 appears in 2007 at a prevalence of 13% (7/54) and remains in the population in 2008 and 2009 at 8.9% (4/45) and 3.5% (9/260), respectively (Fig. 3). Likewise, G1407 is absent from the CipR population in 2005 and 2006 (0/18, 0/34), but makes up 33% (7/21) of the resistant isolates in 2007. The prevalence of G1407 drops in 2008 to 15% (4/26) but increases again in 2009 to 36% (9/25) (Fig. 4).

In both the total isolate population and the CipR population, G3112 is absent between 2005 and 2007 and in 2009. However, in 2008 G3112 makes up 16% (7/45) of the tested population (Fig. 3) and 27% (7/26) of the CipR isolates (Fig. 4).

Third-Generation Cephalosporin Sensitivity Among CipR Genogroups

CDC regional guidelines for SF suggested a switch from ciprofloxacin to oral third-generation cephalosporins as the recommended treatment of gonorrhea in 2002.[10,11] For cefixime (cefixime testing was not performed on SF GISP isolates in 2008), only G1407 had isolates at the alert value MIC of 0.25 μg/mL, with 3 of 10 tested isolates at the alert value (Fig. 5A). The average MIC to cefixime of the tested G1407 was 0.12 μg/mL (Table 2). None of the 37 tested isolates in G437 had an MIC greater than 0.25 μg/mL, with an average MIC of 0.024 μg/mL (Table 2). The difference in cefixime MIC between G437 and G1407 was statistically significant with a *P* value of 0.013 (Table 3).

Furthermore, we examined the MIC of the injectable third-generation cephalosporin—ceftriaxone. No isolates from any of the CipR genogroups displayed an alert level MIC to ceftriaxone (≥0.125 μg/mL) (Fig. 5B). The average MICs to ceftriaxone for G437, G1407, and G3112 were 0.013, 0.016, and 0.011 μg/mL,

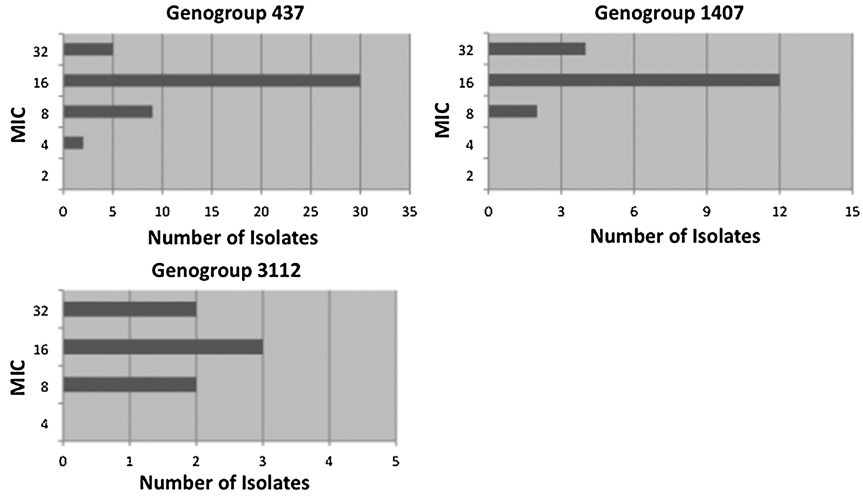

Figure 2. Histograms showing frequency of ciprofloxacin MIC values for G437, G1407, and G3112: SF City Clinic 2005–2009.

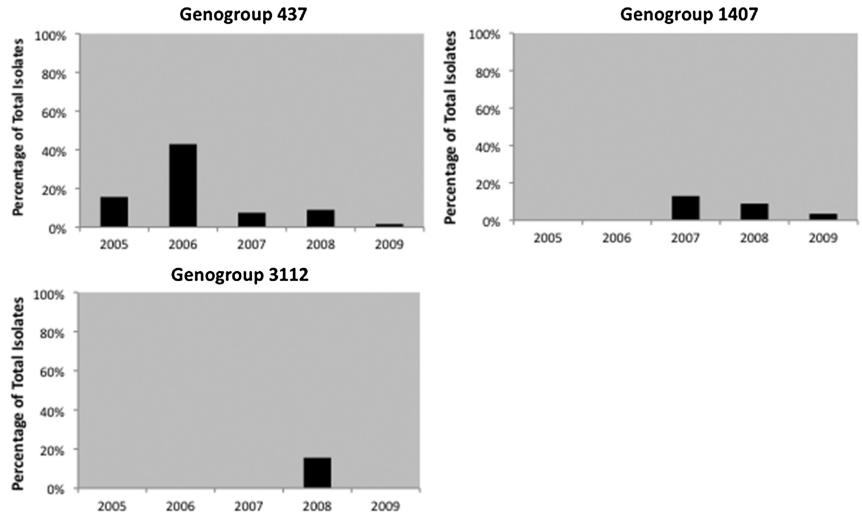

Figure 3. Percentage of G437, G1407, and G3112 within the tested isolate population (n = 460) from 2005 to 2009.

respectively (Table 2). None of the ceftriaxone MICs were statistically significant among G437, G1407, and G3112 (Table 3). Lastly, Table 2 includes all average MIC data and SDs for antibiotics tested as part of GISP.

DISCUSSION

Decreased Susceptibility to Third-Generation Cephalosporins Is Likely One of Many Factors Driving Strain Turnover

During the period examined in this study, third-generation oral cephalosporins were the recommended front-line therapy, which replaced ciprofloxacin as the recommended front-line therapy in 2002.[10,11] G1407 does display decreased susceptibility to oral cephalosporins and has CipR. G1407 is a successful international ST associated with treatment failures to oral cephalosporins[8,9,15,22] and is known to be multidrug resistant including ciprofloxacin. It is likely that the decreased susceptibility to oral cephalosporins in G1407 was a driving factor for the establishment of that strain in SF. However, the other 2 major CipR genogroups in our study, G437 and G3112, had no evidence of decreased susceptibility to oral cephalosporins. Thus, although resistance to current frontline therapies is surely an important driver in strain turnover, it cannot be the sole factor.

Decline in CipR Coincides With Changes to National Treatment Recommendations

We determined the ciprofloxacin MIC for the gonococcal isolates from the first 25 symptomatic males attending the SF City Clinic each month between 2001 and 2009. Susceptibility testing of the 2526 isolates indicated an increase in resistance to ciprofloxacin among *GC* isolates from 2001 to 2006, followed by a subsequent decline from 2007 to 2009. Although California stopped the recommendation of ciprofloxacin as a first-line treatment of

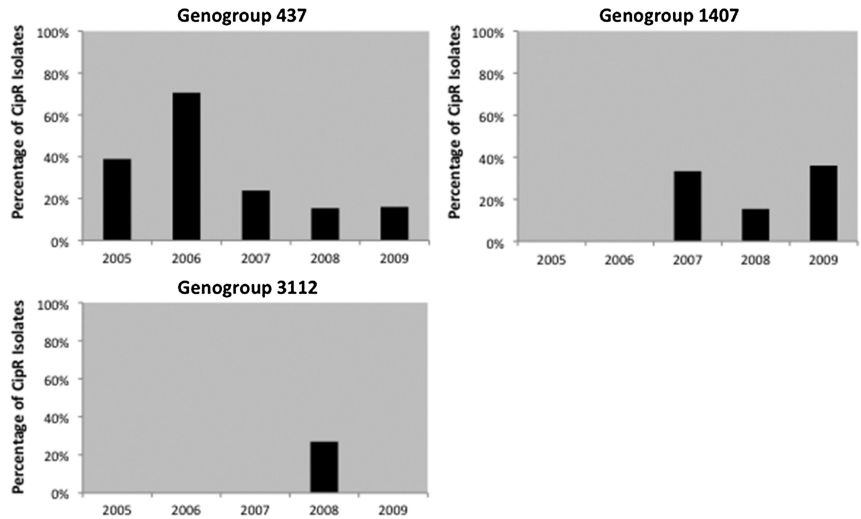

Figure 4. Percentage of G437, G1407, and G3112 within the tested CipR isolate population (using 1.0 μg/mL as the cutoff for resistance), n = 124.

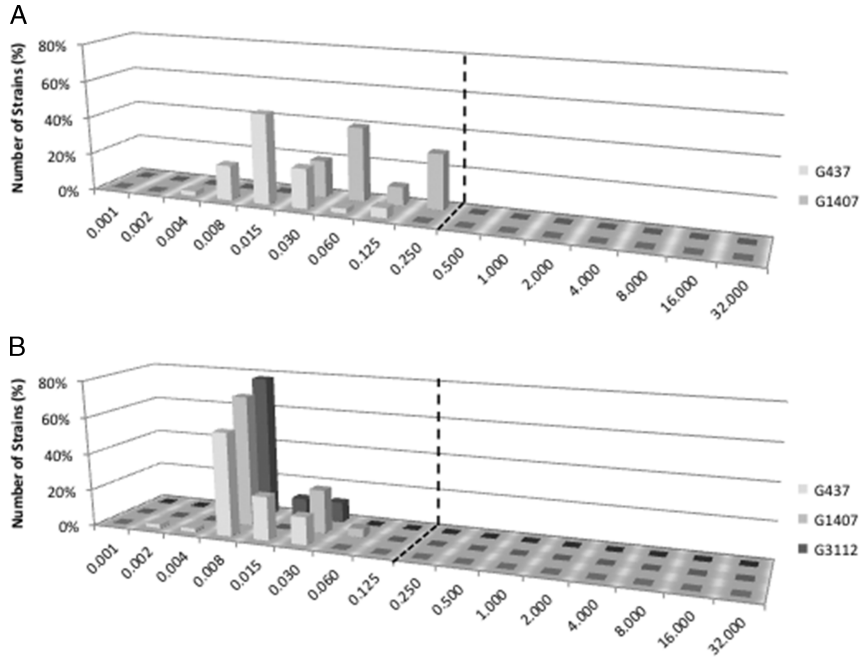

Figure 5. A, Percentage of G437 and G1407 isolates with given MIC values to cefixime (MIC values to cefixime for G3112 were not determined); alert MIC value (0.250 μg/mL) shown by dashed line. B, Percentage of G437, G1407, and G3112 isolates with given MIC values to ceftriaxone; alert MIC value (0.125 μg/mL) shown by dashed line.

gonorrhea in 2002, a decline in resistance was not observed until 2007. This decrease coincides with a national CDC recommendation in 2007 to cease the use of ciprofloxacin for treatment of gonorrhea.

Molecular Typing Reveals High Levels of Strain Turnover in SF

The observed trends in resistance and the NG-MAST data suggest that strain turnover, driven by the importation of new strains, is maintaining levels of CipR in SF. The NG-MAST data obtained between 2005 and 2009 show rapid strain turnover of *GC* STs from year to year in SF, particularly within the CipR population. Although G437 was dominant among the CipR population in 2005 and 2006 (39% and 71%, respectively), its prevalence begins to decline quickly in 2007. However, CipR within the entire population is maintained by the importation of other resistant strains. In 2007, when G437 suddenly appears at a much lower prevalence of the CipR population (24%), there is an increase in the prevalence of G1407 from 0.0% in 2006 to 33% in 2007. Similarly, G3112 is absent from the population between 2005 and 2007, yet comprises 27% of resistant strains in 2008. The data describing G437, G1407, and G3112 demonstrate that resistant strains are replacing other resistant strains within the CipR population from year to year and thus maintaining CipR levels. This high rate of strain turnover among resistant strains could be prolonging the prevalence of CipR after the California recommendation to cease ciprofloxacin treatment of gonorrhea in 2002. This, in conjunction with the dramatic decrease in CipR and corresponding national guideline in 2007, suggests that the levels of CipR in SF are influenced by the importation of strains from outside SF. Persistence of CipR may also be influenced by increased fitness due to known compensatory mutations for gyrA mutations, as reported in Kunz et al.[24]

Reversion of Resistance Mutations Does Not Explain the Observed Decline in Resistance

We explored the possibility that the decline in CipR after 2007 was due to reversion mutations, causing existing strains to return to a susceptible phenotype. Studies have shown that *GC* STs acquire CipR through mutations in the *gyrA* gene.[25] All STs within G437, G1407, and G3112 revealed *gyrA* mutations by molecular testing and phenotypic resistance by agar dilution across all years. This suggests that reversion of mutations conferring CipR is not occurring. If there were reversion, STs with a resistant

TABLE 2. Mean MIC Values With Corresponding SDs for Genogroups 437, 3112, and 1407 to a Panel of Antibiotics

	MIC Values (μg/mL)					
Genogroup	**CFX**	**CRO**	**CIP**	**PEN**	**TET**	**AZI**
437	0.024 ± 0.027	0.013 ± 0.0081	16 ± 6.9	2.4 ± 1.3	2.7 ± 1.2	0.32 ± 0.15
3112	n.d.	0.011 ± 0.0074	14 ± 12	2.7 ± 1.3	1.8 ± 0.44	0.14 ± 0.088
1407	0.12 ± 0.10	0.016 ± 0.014	20 ± 8.4	1.9 ± 1.2	3.3 ± 3.2	0.49 ± 0.20

CFX indicates cefixime; CR0, ceftriaxone; CIP, ciprofloxacin; PEN, penicillin; TET, tetracycline; AZI, azithromycin; n.d., no data.

TABLE 3. Comparison of Mean MIC Values of Third-Generation Cephalosporins Among Genogroups 437, 1407, and 3112

Genogroups Compared	Antibiotic	*P**
G437 vs. G1407	Cefixime	0.013†
G437 vs. G3112	Ceftriaxone	0.59‡
G437 vs. G1407	Ceftriaxone	0.38†
G3112 vs. G1407	Ceftriaxone	0.37‡

*$P < 0.05$ considered significant.
†Assumed unequal variance.
‡Assumed equal variance.

phenotype would be observed in later years with a susceptible phenotype, as ST identity is determined by *por*B and *tbp*B genes and would not be altered by a mutation in *gyrA*. Our data demonstrate that the decline in CipR among the SF isolate population was not due to reversion, further suggesting that changes in CipR prevalence were due to strain turnover and importation.

A number of limitations of our analysis are worth noting. Isolates collected before 2005 were unavailable for molecular typing; therefore, strain turnover could not be determined for isolates collected between 2001 and 2004. In addition, approximately 70% of the GISP isolates from SF were collected from MSM[12]; however, information to distinguish between MSM and men who have sex with women GISP participants was not available for the 460 isolates that underwent molecular typing. As a result, the data are unable to differentiate between the MSM and men who have sex with women populations in SF. Moreover, GISP solely accounts for male urethral infections[26] and neglects pharyngeal and rectal gonoccocal infections, areas that play a potential role in antimicrobial resistance development and ongoing transmission. Therefore, urethral screening alone compared with screening the rectum and pharynx may have missed an additional number of gonococcal infections.[27,28] This can lead to an underestimation of resistant cases in GISP, resulting in inaccurate resistance data. Moreover, GISP fails to assess women with gonorrhea infections (i.e., cervical gonoccocal infections); therefore, sentinel surveillance may not be representative of all patients with gonorrhea. Finally, as only isolates from SF were available for assessment, analysis of isolates from other areas of the United States would be necessary to determine if strains were imported into SF from outside SF.

Gonorrhea is one of the most prevalent sexually transmitted diseases in the United States, and strain turnover, as well as the importation of strains, seems to be influencing the levels of CipR within the SF population. Our findings suggest that regional treatment recommendations for gonorrhea are potentially less effective at lowering CipR than national recommendations in an area with high strain turnover. These findings and other studies demonstrate that genetic-based surveillance, when combined with phenotypic surveillance, provides a powerful tool for public health efforts seeking to understand trends in drug resistance.[22,23,29] Therefore, it is vital to improve and continue the monitoring of *GC* antimicrobial susceptibility throughout the United States to delay the emergence of future drug-resistant strains. Such data can provide health departments with the information to make better strategic choices and policy decisions regarding surveillance and treatment of gonorrhea.

REFERENCES

1. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2009. Atlanta, GA: US Department of Health and Human Services; 2010.
2. Centers for Disease Control and Prevention. Gonorrhea CDC Fact Sheet. July 1, 2014. Available at: http://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm. Accessed July 14, 2014.
3. Kirkcaldy RD, Ballard RC, Dowell D. Gonococcal resistance: Are cephalosporins next? Curr Infect Dis Rep. 2011; 13:196–204.
4. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: The contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 1999; 75:3–17.
5. Tapsall JW, Ndowa F, Lewis DA, et al. Meeting the public health challenge of multidrug- and extensively drug-resistant *Neisseria gonorrhoeae*. Expert Rev Anti Infect Ther 2009; 7:821–834.
6. Centers for Disease Control and Prevention. Increases in fluoroquinolone-resistant *Neisseria gonorrhoeae*—Hawaii and California, 2001. MMWR 2002; 51:1041–1044.
7. Centers for Disease Control and Prevention. CDC grand rounds: The growing threat of multi-drug resistant gonorrhea. MMWR 2013; 62:103–106.
8. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases treatment guidelines, 2006: Flouorquinolones no longer recommended for treatment of gonococcal infections. MMWR 2007; 56:332–336.
9. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 2010; 59 (No. RR-12): 1–110.
10. Bolan GA, Sparling PF, Wasserhelt JN. The emerging threat of untreatable gonococcal infection. N Engl J Med 2012; 366:485–487.
11. Unemo M, Shafer WM. Antibiotic resistance in *Neisseria gonorrhoeae*: Origin, evolution, and lessons learned for the future. Ann N Y Acad Sci 2011; 1230:E19–E28.
12. Centers for Disease Control and Prevention. Gonococcal Isolate Surveillance Project (GISP) Profiles and Annual Reports, All Years. February 26, 2014. Available at: http://www.cdc.gov/std/gisp/gisp-stats-all-years.htm. Accessed July 14, 2014.
13. Centers for Disease Control and Prevention. Antibiotic-Resistant Gonorrhea (ARG) Basic Information. November 18, 2013. Available at: http://www.cdc.gov/std/gonorrhea/arg/basic.htm. Accessed July 14, 2014.
14. Centers for Disease Control and Prevention. 2011 Sexually Transmitted Diseases Surveillance, Gonorrhea. December 13, 2012. Available at: http://www.cdc.gov/std/stats11/gonorrhea.htm. Accessed July 14, 2014.
15. Whiley D, Namraj G, Lahra M, et al. The ticking time bomb: Escalating antibiotic resistance in *Neisseria gonorrhoeae* is a public health disaster in waiting. J Antimicrob Chemother 2012; 67:2059–2061.
16. Centers for Disease Control and Prevention. Cephalosporin susceptibility among *Neisseria gonorrhoeae* isolates—United States, 2000–2010. MMWR 2011; 60:873–877.
17. Katz A, Komeya A, Soge O, et al. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: Case report of the first isolate identified in the United States. Clin Infect Dis 2011; 54:841–843.
18. Martin IM, Ison CA, Aanensen DM, et al. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis 2004; 189:1497–505.
19. Centers for Disease Control and Prevention. Gonococcal Isolate Surveillance Project (GISP) Protocol. July 2010. Available at: http://www.cdc.gov/std/gisp/GISP-Protocol07-15-2010.pdf. Accessed July 14, 2014.
20. Kirkcaldy RD, Zaidi A, Edward HW, et al. *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 2013; 158: 321–328.
21. Stata Statistical Software [computer program]. Version 12. College Station, TX: StataCorp LP; 2011.
22. Buono S, Wu A, Hess D, et al. Using the *Neisseria Gonorrhoeae* multiantigen sequence typing (NG-MAST) method to assess strain diversity and antibiotic resistance in San Francisco, CA. Microb Drug Res 2012; 18:510–517.
23. Hess D, Wu A, Golparian D, et al. Genome sequencing of a *Neisseria gonorrhoeae* isolate of a successful international clone with decreased susceptibility/resistance to extended-spectrum cephalosporins. Antimicrob Agents Chemother 2012; 55:5633–5641.

24. Kunz AN, Begum AA, Wu H, et al. Impact of fluoroquinolone resistance mutations on gonococcal fitness and in vivo selection for compensatory mutations. J Infect Dis 2012; 205: 1821–1829.
25. Deguchi T, Yasuda M, Asano M, et al. DNA gyrase mutations in quinolone-resistant clinical isolates of *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 1995; 39:561–563.
26. Centers for Disease Control and Prevention. Gonococcal Isolate Surveillance Project (GISP). July 2, 2014. Available at: http://www.cdc.gov/std/gisp/. Accessed July 14, 2014.
27. Marcus JL, Bernstein KT, Kohn RP, et al. Infections missed by urethral-only screening for chlamydia or gonorrhea detection among men who have sex with men. Sex Transm Dis 2011; 38:922–924.
28. Park J, Marcus JL, Pandori M, et al. Sentinel surveillance for pharyngeal chlamydia and gonorrhea among men who have sex with men—San Francisco, 2010. Sex Transm Dis 2012; 39:482–484.
29. Martin I, Sawatzky P, Allen V, et al. Emergence and characterization of *Neisseria gonorrhoeae* isolates with decreased susceptibilities to ceftriaxone and cefixime in Canada: 2001–2010. Sex Transm Dis 2012; 39:316–323.