Original Contribution

**Characteristics of Males Infected With Common Neisseria gonorrhoeae Sequence Types in the Gonococcal Isolate Surveillance Project, San Francisco, California, 2009**

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We analyzed 265 urethral Neisseria gonorrhoeae specimens collected from symptomatic males at San Francisco’s municipal sexually transmitted disease clinic, a participant in the Gonococcal Isolate Surveillance Project, during 2009. We used N. gonorrhoeae multiantigen sequence typing to describe characteristics of patients infected with common sequence type families. Specimens were classified into 6 homology-based families and 1 additional family of all other identified strains. Strain family results were combined with results of culture-based antibiotic sensitivity minimum inhibitory concentration, sociodemographic and behavioral risk data collected at the clinic, and presence or absence of the mosaic penicillin-binding protein 2 (penA) allele. Characteristics of patients were compared across strain families through the use of $\chi^2$ statistics. Among men who have sex with men, strain distribution differed by those reporting receptive oral sex as their only urethral exposure ($P = 0.04$), by number of sex partners ($P = 0.03$), and by race/ethnicity ($P < 0.001$); there were no differences by age or human immunodeficiency virus status. Also, among men who have sex with men, strain family distributions differed for culture specimens with reduced susceptibility to a range of antibiotics, as well as with presence of the mosaic penA allele (all $P < 0.001$). The combination of molecular, phenotypic, and epidemiologic data on N. gonorrhoeae infection could help develop a more complete epidemiology of gonorrhea in the United States.

antimicrobial resistance; Neisseria gonorrhoeae; Neisseria gonorrhoeae multiantigen sequence typing; men who have sex with men

Abbreviations: CDC, Centers for Disease Control and Prevention; GISP, Gonococcal Isolate Surveillance Project; MIC, minimum inhibitory concentration; MSM, men who have sex with men; MSW, men who have sex with women; NG-MAST, Neisseria gonorrhoeae multiantigen sequence typing; STD, sexually transmitted disease.

Although Neisseria gonorrhoeae infection is the second most common reportable condition in the United States, with an estimated 700,000 cases each year (1), robust surveillance data to describe its epidemiology are sparse. At the national level, the Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia) is able to describe the gonococcal epidemic in the United States broadly. At the local level, the large burden of disease makes interviewing case patients infeasible; data are therefore often limited to elements easily obtained through laboratory data systems. In light of increasing evidence of transmission of gonorrhea resistant to recommended antibiotic regimens (2), there is a critical need for a clearer understanding of the epidemiology of gonorrhea and more nuanced approaches to its surveillance.

Nationally, case-based surveillance for gonorrhea is often limited to the patient’s age, race/ethnicity, sex, county, and the type of diagnosing provider. Because data on the gender of recent sex partners are not widely collected, describing the epidemiology of gonorrhea by sexual behavior is not currently possible (1). Additional platforms have been developed...
to supplement existing case-based surveillance. The STD Surveillance Network project supports interviews of a random sample of reported gonorrhea cases in 11 health jurisdictions (3, 4). However, the geographical scope of the STD Surveillance Network is limited, interviews are resource intensive, and N. gonorrhoeae case patients are often difficult to locate and interview (3, 5, 6).

*N. gonorrhoeae* has repeatedly developed resistance to antimicrobial agents, leaving only 1 class of antibiotics, cephalosporins, that is now recommended by the CDC for treatment (7). However, cephalosporin resistance has begun to emerge and spread, with treatment failures reported in Asia and Europe and specimens with decreased susceptibility identified in Australia, Europe, and the United States (8). Surveillance for antimicrobial-resistant *N. gonorrhoeae* in the United States is conducted by the Gonococcal Isolate Surveillance Project (GISP), which reports results to the CDC. Although no cephalosporin treatment failures have been identified in the United States, GISP has detected several isolates with decreased susceptibility to ceftriaxone and cefixime, which are injectable and oral cephalosporins, respectively, that are used in combination gonorrhea treatment regimens (1).

To better understand the epidemiology of gonorrhea, innovative surveillance approaches are needed. It could be possible to combine *N. gonorrhoeae* molecular typing data with epidemiologic data to help characterize sexual networks, monitor population-level changes in the organism, and identify changes in antibiotic resistance over time. *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) has been used to group clonally related strains into families (9), and sequencing of the penicillin-binding protein 2 (*penA*) gene has identified a mosaic genotype associated with cephalexin resistance (10). In recent analyses conducted in Europe, possible transmission networks were examined through the use of molecular typing technologies and epidemiologic data (11–13). However, we are not aware of any analysis of *N. gonorrhoeae* molecular typing and epidemiologic data from the United States. Capitalizing on the existing GISP infrastructure and a systematic analysis of specimens with NG-MAST, we aimed to expand traditional person-based surveillance to include *N. gonorrhoeae* families as a way to more closely monitor the local epidemiology of gonorrhea.

**MATERIALS AND METHODS**

GISP began in 1986 as a surveillance platform to monitor resistant urogenital *N. gonorrhoeae* isolates among symptomatic male patients seen at sexually transmitted disease (STD) clinics in the United States (14). San Francisco City Clinic, the only municipal STD clinic in the city of San Francisco, California, has participated in GISP since its inception. The GISP methods have been described previously (15). Briefly, each month, urethral isolates from the first 25 symptomatic males diagnosed with gonorrhea at the clinic were isolated on Modified Thayer-Martin Selective Agar (Becton Dickinson, Sparks, Maryland). Specimens were transported to the San Francisco Department of Public Health Laboratory, where they were incubated, and gonococcal isolates were purified on Chocolate Agar plates (Becton Dickinson). Purified culture samples were split and frozen in tryptic soy broth (Hardy Diagnostics, Santa Maria, California) with 15% glycerol (Hardy Diagnostics); 1 sample was sent to the regional GISP laboratory for analysis, and 1 sample was maintained by the San Francisco Public Health Laboratory for analysis. GISP isolates were analyzed by the University of Washington regional GISP laboratory (Seattle, Washington) with agar dilution, as described in the GISP protocols (15), for minimum inhibitory concentrations (MICs) against the following 7 drugs: penicillin, tetracycline, cefixime, ceftriaxone, ciprofloxacin, cefpodoxime, and azithromycin. MIC results were then reported back to the San Francisco Department of Public Health STD program. The Clinical and Laboratory Standards Institute has established MIC cutpoints for resistance to penicillin, tetracycline, spectinomycin, and ciprofloxacin (16); for the remaining antibiotics (cefixime, ceftriaxone, cefpodoxime, and azithromycin), the CDC has established breakpoint MICs used for surveillance. Because the CDC GISP protocol includes MIC breakpoints for all 7 examined drugs, we used these cutoffs in our analysis. The CDC provides standard MIC cutoffs for each of the 7 drugs that indicate a high likelihood of reduced drug susceptibility; in the present analysis, isolates above the GISP MIC cutoffs are considered to have reduced susceptibility to that specific antibiotic. The GISP cutoffs used in this analysis to indicate a high likelihood of reduced susceptibility are >1 (penicillin), >1 (tetracycline), >0.125 (cefixime), >1 (ceftriaxone), >1 (ciprofloxacin), >0.5 (cefpodoxime), and >2 (azithromycin) (15).

Additional testing of the specimens was conducted at the San Francisco Public Health Laboratory, including NG-MAST DNA sequencing and screening for the mosaic allele of the *penA* gene (10, 17), which has been associated with increased MIC to oral cephalosporins (10). As described by Buono et al. (17), the NG-MAST results were used to classify *N. gonorrhoeae* into 7 broad genotypic families, 6 defined by the homology to a specific NG-MAST strain type and 1 for all other identified strains.

All patients seen at San Francisco City Clinic met with a clinician and underwent a standard behavioral risk assessment before specimen collection, which included questions about recent sexual activity, drug use, and sociodemographics. Males who reported ever having had sex with a male were classified as men who have sex with men (MSM); men who reported both male and female partners were also classified as MSM. Men who reported any female partner and no male partners were classified as men who have sex with women (MSW). A history of gonorrhea was determined through a match with data on all gonorrhea cases reported to the San Francisco Department of Public Health, including all gonorrhea case reports from San Francisco residents as required by the California Health Code. Risk data from those cases were linked to the results of the phenotypic resistance testing from GISP, as well as to the NG-MAST results and polymerase chain reaction for the presence or absence of the mosaic *penA* allele. All isolates that were found to be reactive for the mosaic *penA* allele were subjected to DNA sequencing of the entire *penA* gene to confirm the presence of the mosaic allele.

For all GISP isolates obtained in 2009, the distributions of patient sociodemographic and behavioral risks were compared across NG-MAST families to identify whether certain *N. gonorrhoeae* sequence types were associated with specific risk populations or behaviors. Additionally, we explored
whether GISP drug MICs or the mosaic allele of the \textit{penA} gene was associated with particular \textit{N. gonorrhoeae} strain families. $\chi^2$ statistics were used to compare the distribution of those characteristics across \textit{N. gonorrhoeae} families. Because these were deidentified medical records undergoing retrospective analyses for public health assessment purposes, this study was considered nonresearch in accordance with the Code of Federal Regulations, Title 45.

**RESULTS**

A total of 265 \textit{N. gonorrhoeae} isolates were obtained from male patients diagnosed with gonorrhea at San Francisco City Clinic and selected as part of GISP in 2009, from which 117 different strain types were identified. These individual types were categorized into 6 families, as well as an “other” category for those strains not falling into 1 of the identified families. Of the 265 specimens, 212 (80.0%) were collected from MSM, and the remaining 53 (20.0%) were collected from MSW. Figure 1 describes the distribution of \textit{N. gonorrhoeae} strain families for MSM and MSW. The family distribution was significantly different between MSM and MSW, with 25.5\% of MSM isolates versus 11.3\% of MSW isolates belonging to the 2992 family. More than a third of isolates from MSW belonged to the 4254 family, versus only 2.8\% of isolates from MSM. For both MSM and MSW, nearly half of the examined isolates were considered part of the “other” strain family, which was composed of more than 70 different strain types. Compared with MSW, MSM were more likely to be infected with family 2992 (44.3\% vs. 19.4\%), family 3935 (20.5\% vs. 0\%), family 730 (13.9\% vs. 9.7\%), and family 28 (9.8\% vs. 3.2\%) ($P < 0.001$).

Given the small number of MSW included in the analysis, we restricted further analyses to isolates from MSM ($n = 212$). The epidemiologic characteristics of MSM by the strain family of obtained specimens are shown in Table 1. Although strain family did not differ by the human immunodeficiency virus status of the patient, there were significant differences by race/ethnicity ($P < 0.001$). MSM reporting fellatio as their only urethral exposure in the prior 3 months were more commonly infected with 2992 (37.0\% vs. 20.0\%) and less commonly infected with 730 (0\% vs. 10.7\%) and 28 (1.9\% vs. 7.3\%) than were MSM reporting other types of sex ($P = 0.0361$). Strain families 2992, 3935, and 1407 were associated with MSM reporting more than 3 partners in the prior 3 months. No significant differences were seen with regard to chlamydial coinfection, history of a gonococcal infection, or age.

The distribution of gonococcal strain families was also examined relative to phenotypic and molecular markers of antibiotic resistance (Table 2). Statistically significant differences were seen in the strain distribution for reduced susceptibility to penicillin, tetracycline, cefixime, ciprofloxacin, and cefpodoxime (all $P < 0.0001$); no reduced susceptibility was found for ceftriaxone or azithromycin. Additionally, all 8 of the specimens from strain family 1407 were positive for the \textit{penA} mosaic, whereas no other strain families had specimens positive for a mosaic \textit{penA}. All specimens found positive by polymerase chain reaction for a mosaic \textit{penA} allele were sequences found to possess either the XXXIV or XXXVIII mosaic alleles. Among men who reported only recent oral sex, strain family was associated with reduced susceptibility to tetracycline, cefixime, ciprofloxacin, and cefpodoxime (all $P < 0.05$). In this group, no specimens showed reduced susceptibility to ceftriaxone or azithromycin. For the men who reported sexual exposures other than only oral sex, strain family was associated

![Figure 1. Distribution of \textit{Neisseria gonorrhoeae} strain family by gender of sex partners in urethral isolates from the Gonococcal Isolate Surveillance Project (GISP), San Francisco, California, 2009. $\chi^2$ $P$ value < 0.0001. MSM, men who have sex with men; MSW, men who have sex with women.](https://academic.oup.com/aje/article-abstract/178/8/1289/829196)
with reduced susceptibility to penicillin, tetracycline, and ciprofloxacin (all \( P < 0.05 \)). No specimens among this subgroup had reduced susceptibility to ceftixime, ceftriaxone, or azithromycin. Of note, all 3 specimens with reduced susceptibility to ceftixime and 2 of the 3 specimens with reduced susceptibility to cefpodoxime were from MSM who reported oral sex as their only recent sexual exposure. Additionally, of these 6 specimens, 5 were strain family 1407.

**DISCUSSION**

We examined the distribution of epidemiologic and antibiotic resistance markers by strain families for more than 250 *N. gonorrhoeae* male urogenital specimens collected at our STD clinic through the CDC GISP. We found that specific sociodemographic, behavioral, and phenotypic markers of antibiotic resistance clustered by strain family type. MSM were more likely to be infected with *N. gonorrhoeae* from the 2992 family, whereas heterosexual males were more likely to be infected with 4254. Among MSM, strain family was significantly associated with race/ethnicity, number of sex partners, and reporting oral sex as the only recent urogenital exposure. Additionally, *N. gonorrhoeae* strain family (specifically strain type 1407) was associated with reduced susceptibility to penicillin, tetracycline, ceftixime, cefpodoxime, and ciprofloxacin, as well as a molecular marker for cephalosporin resistance. This sequence type was also previously associated with treatment failures to oral cephalosporins in France.
Spain, and Slovenia (18–20). Additionally, we found that 5 of 6 of the specimens from MSM with reduced susceptibility to cefixime or cefpodoxime were from MSM who reported insertive oral sex as their only recent sexual exposure.

Although *N. gonorrhoeae* infection is the condition second most commonly reported to the CDC, the epidemiologic picture of *N. gonorrhoeae* infections is quite limited. Analyses are largely restricted to age, race/ethnicity, sex, and state or county of residence (1). Supplemental surveillance platforms, such as the STD Surveillance Network, have tried to fill in the gaps in characterizing the epidemiology of *N. gonorrhoeae* but are resource intensive and limited in geographical scope (3). Often, more information is collected from gonorrhea cases diagnosed in STD clinics, where standardized risk and behavioral assessments are the norm. However, given limited resources and the large burden of disease, it is not feasible to conduct extensive interviews with all cases of reported gonorrhea in a locality. Here we show that, among male patients diagnosed with urogenital gonorrhea in a San Francisco STD clinic, certain strain families were strongly associated with being a man who has sex with men, having higher numbers of sex partners, and receiving oral sex as the only urethral exposure during the prior 3 months. With additional resources, it might be possible to expand population-based *N. gonorrhoeae* surveillance to include monitoring of strain distributions. Given the lack of behavioral and other risk information, these genotypic and molecular data could be sufficient proxies for monitoring disease in specific subpopulations such as MSM. Further study on the temporal stability of the associations between NG-MAST family distribution and epidemiologic characteristics could help inform whether molecular analyses such as the one presented might be useful in bolstering national gonorrhea surveillance.

Table 2. Distribution of *Neisseria gonorrhoeae* Strain Families From Urethral Isolates Collected From Men Who Have Sex With Men, the Gonococcal Isolate Surveillance Project, San Francisco, California, 2009

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<th>Strain Family</th>
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<th>2992</th>
<th>3935</th>
<th>4254</th>
<th>730</th>
<th>Other</th>
<th>Total</th>
<th>P Value</th>
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<td>54</td>
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a % represents row percentages.

b The Gonococcal Isolate Surveillance Project cutoffs used in this analysis to indicate a high likelihood of reduced susceptibility are >1 (penicillin), >1 (tetracycline), >0.125 (cefixime), >1 (ceftriaxone), >1 (ciprofloxacin), >0.5 (cefpodoxime), and >2 (azithromycin).
Additionally, NG-MAST data could be useful in understanding the epidemiology of repeat gonorrhea infections and help identify core members facilitating disease transmission. Although just over a quarter of MSM diagnosed with gonorrhea in our analysis reported receiving oral sex as the only urethral exposure during the prior 3 months, differences were seen in the prevalence of receptive oral sex only by strain type. We have previously reported data that suggest that transmission of \(N.\) gonorrhoeae from an infected oropharynx to the male urethra might be more common than previously thought (21, 22). Furthermore, \(N.\) gonorrhoeae DNA can undergo recombination with other commensal \(Neisseria\) species (e.g., \(N.\) meningitidis) in the throat, and the oropharynx might play an important role in the development of antibiotic resistance among \(N.\) gonorrhoeae (8). Data from our analysis support these hypotheses, inasmuch as reduced susceptibility to cefixime or cefpodoxime was associated with a specific strain family (strain family 1407) and was associated with having reported insertive oral sex as the only recent sexual exposure.

Although molecular and phenotypic analyses of \(N.\) gonorrhoeae specimens have been published previously (10, 23–28), few studies have examined the relationship between geno-phenotype characteristics (e.g., resistance phenotypes, genotypic families) and epidemiologic data. Additionally, these mul-
tifactorial epidemiologic investigations have been conducted only in Europe. Bilek et al. (29) demonstrated that NG-MAST could be used to verify epidemiologically linked sexual partners with gonorrhea infections in the United Kingdom. In ana-
lyses of reported gonorrhea in London, England, strain distributions were found to cluster geographically and by epidemiologic subpopulation, notably among MSM (11, 12). In an analysis of STD clinic patients in Glasgow, Scotland, NG-MAST strain types were associated with MSM, as well as coinfection with chlamydia (30). Finally, a recent report from Amsterdam, the Netherlands, showed clustering of \(N.\) gonorrhoeae types by epidemiologic characteristics (13). Our analysis is the first of which we are aware that has examined the relationship of the epidemiologic and genotypic characteristics of \(N.\) gonorrhoeae in the United States.

Several limitations of our analysis deserve mention. GISP includes only symptomatic male urethral specimens collected at STD clinic sites. As a result, we cannot make any inferences about the epidemiology of nongenital \(N.\) gonorrhoeae infections or asymptomatic urogenital infections. The GISP protocol is limited to the collection of urogenital culture specimens. Although NG-MAST can be conducted on nonculture specimens, culture specimens are ideal. An increasing proportion of gonorrhea testing uses nucleic acid amplification testing platforms, which can be conducted with urine, as well as with vaginal or cervical swabs. Because culture capacity declines with increasing access to nucleic acid amplification testing—based diagnostics (31), the utility of NG-MAST – based analyses could be limited. Future studies should explore the molecular epidemiology of nongenital gonococcal infections. Only 1 year of GISP data with NG-MAST results was available for the present analysis; it is possible that genotypic diversity varied over time. Because the gonorrhea epidemic in San Francisco disproportionately affects MSM, we did not have sufficient heterosexual male GISP specimens to allow for a more complex analysis of \(N.\) gonorrhoeae strain variation by sociodemographic and behavioral characteristics. Additionally, the GISP specimens represent a small proportion of overall gonorrhea morbidity in San Francisco.

A more complete understanding of the epidemiology of \(N.\) gonorrhoeae infections has been lacking as a result of large disease burden and limited local resources. The use of genotypic and phenotypic data in analyses of both tuberculosis (32–34) and salmonella (35–37) has helped identify outbreaks and prioritize public health activities. We exploited the robust infrastructure of the GISP project to explore the relationship between \(N.\) gonorrhoeae family and epidemiologic characteristics of case patients. Although our sample is small and limited to males with symptomatic urogenital infections, our analysis identified distinct families of \(N.\) gonorrhoeae that affect subpopulations. Possible avenues for public health action could include active follow-up of persons infected with gonorrhea strain types associated with reduced susceptibility. Although NG-MAST–based analysis will not likely replace phenotypic analyses based on culture specimens, the use of molecular epidemiology studies conducted concur-
rently with phenotypic specimens could be useful in enhancing local understanding of gonorrhea resistance. Furthermore, the role of genotyping to enhance routine disease surveillance in future studies that include pharyngeal and rectal \(N.\) gonorrhoeae specimens and specimens from females should be explored.

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