Antimicrobial Susceptibility and Characteristics of *Neisseria gonorrhoeae* Isolates from Puerto Rico, 2012-2017

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Objective: Monitoring the susceptibility patterns of *Neisseria gonorrhoeae* is essential for the continuing compliance with current treatment recommendations. Puerto Rico conducts susceptibility tests on *N. gonorrhoeae*; however, trends on antimicrobial resistance in the island have not been reported since the mid 80's.

Methods: We performed a secondary analysis of a national data repository on the antimicrobial susceptibility of *N. gonorrhoeae* isolates between 2012 and 2017; a period of time when the CDC recommended a single dose of ceftriaxone and azithromycin for the treatment of uncomplicated gonorrhea. Data on susceptibility to eight antibiotics using the standard disk diffusion method was obtained for 30.0% (84/276) of the samples collected from the Sexually Transmitted Disease clinics in Puerto Rico. We also performed patient demographic analyses linked to resistance.

Results: Rates of resistance to ceftriaxone and azithromycin were 0% and 4.0% (2/50), respectively. The percentage of isolates resistant to antimicrobials no longer recommended in Puerto Rico, such as tetracycline, ciprofloxacin, and penicillin, was 86.0% (43/50), 76.0% (38/50), and 38.0% (19/50), respectively. Prevalence of resistant *N. gonorrhoeae* was higher among men who have sex with men, MSM (79%, 37/47).

Discussion: Lack of resistance to ceftriaxone and slow emergence of azithromycin resistance was identified from 2012-2017. It is imperative to continue the surveillance for emerging patterns of resistance, especially for ceftriaxone, as it is part of the current treatment guidelines. Therefore, protocols for culture-based surveillance, including sample transport and processing, should be strengthened to ensure quality assured epidemiology of gonococcal resistance in Puerto Rico.

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Key words: Neisseria gonorrhoeae, Antimicrobial resistance, Puerto Rico, Ceftriaxone

The incidence of gonorrhea in the United States (USA) is increasing at an alarming rate (1). Since 1995 (and with the advent of *Chlamydia* as a nationally notifiable disease), gonorrhea has been consistently the second most commonly reported notifiable disease in the United States with 1.57 million incident gonococcal infections among adults aged 15 to 39 years in 2018 with expected lifetime medical costs of $271 million (1). In Puerto Rico, gonorrhea is the second most reported bacterial sexually transmitted disease. From 2012 to 2021, a total of 5,584 cases were reported to the USA Centers for Disease Control and Prevention (CDC), including an unparalleled incidence of 1,013 cases during 2021 (2). The etiological agent in the causation of gonorrhea (*Neisseria gonorrhoeae*) has been reported to progressively develop resistance to almost all antimicrobials previously recommended for treatment of gonorrhea, including penicillins, sulfonamides, tetracyclines, macrolides, and even to extended-spectrum cephalosporins – the last remaining options for empirical therapy (3). Due to the rapidly developed resistance to almost all classes of antibiotics, the CDC has classified gonorrhea as an "urgent threat" (3). Improper treatment due to resistant *N. gonorrhoeae* can lead to serious sequelae, which include sepsis in both sexes, pelvic inflammatory disease, infertility, ectopic pregnancy in women and an increase in a person's risk of acquiring or transmitting HIV (4).

The management of gonorrhea infection in Puerto Rico is based on a syndromic approach. Current treatment consists of a single dose of ceftriaxone 500mg as a first-line drug according to the CDC recommendations in the USA (5). Previous CDC recommendations included the use of azithromycin in dual therapy with ceftriaxone to function as a shield to potentially delay the emergence of resistance to ceftriaxone (6). Both symptomatic and suspected cases are confirmed through nucleic acid amplification test (NAAT).

Monitoring trends in antimicrobial drug–resistant *Neisseria gonorrhoeae* is a critical public health and global health security activity because the number of antimicrobial drugs available to treat gonorrhea effectively is rapidly diminishing. In the United States, the CDC developed a national surveillance system called the Gonococcal Isolate Surveillance System Project (GISP) (7). Surveillance consists of a monthly analysis of demographics and clinical data, including antimicrobial susceptibility gathered from

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selected STD clinics (GISP sentinel sites). Although Puerto Rico is not participating in the GISP, the regional and Central STD clinics at The Puerto Rico Department of Health perform periodic surveillance for antimicrobial resistance with special emphasis on ceftriaxone to ensure continued efficacy. However, no antimicrobial resistance data for *N. gonorrhoeae* isolates from Puerto Rico have been reported since the mid-1980s (8).

The present study reports the results and trends in *N. gonorrhoeae* antimicrobial susceptibility between 2012 and 2017 according to the past recommended antimicrobials for the treatment of gonorrhea.

Methods

We used a nonprobability sampling method to obtain data on *N. gonorrhoeae* antimicrobial resistance from the Public Health Laboratory of the Department of Health of Puerto Rico. Our study included reported cases of *N. gonorrhoeae* infection that were further submitted for confirmatory culture and antimicrobial susceptibility testing between 2012 to 2017. From 2012 to 2017, national surveillance of *N. gonorrhoeae* antimicrobial resistance required the collection of samples from patients presenting the following characteristics: patients with a previous positive NAAT and returning with symptomatic gonococcal disease after treatment, patients with a negative NAAT and presenting symptoms of gonorrhea, HIV-positive patients, and men who have sex with men (MSM). Under those criteria, a total of 429 patients out of a total of 3,017 cases of gonorrhea (14.2%) qualified for culture and antimicrobial testing. Cervical, oral, rectal, and urethral swabs specimens were inoculated directly on JEMBEC agar plates with modified Thayer- Martin (Remel) and placed in a holding candle jar prior to transport to the Reference Public Health Laboratory at the Department of Health of Puerto Rico. At the Reference laboratory, the inoculated plates were incubated at 37°C in CO_2 for daily examination for colonies. Definitive identification for *N. gonorrhoeae* was done by biochemical tests (Gram Stain and oxidase) and enzymatic assay (RapID NH System – Fisher Scientific).

N. gonorrhoeae isolates were tested for susceptibility to antimicrobials by the disc diffusion method (Becton Dickinson, New Jersey, USA, and Oxoid – Thermo Fisher, USA) according to the CDC recommendations (9). The tested antibiotics were: ceftriaxone (Cro), cefixime (Cfm), ciprofloxacin (Cip), tetracycline (Tet), azithromycin (Azm), penicillin (Pen), cefotaxime (Ctx) and cefepime (Fep). *N. gonorrhoeae* ATCC 49226 was used as a control. Antibiotic inhibition zones were interpreted according to the disk diffusion standards as described by the Clinical and Laboratory Standards Institute (CLSI) M100-S23 Vol 33 No1 guidelines (9). For cefotaxime and cefepime, disc diffusion breakpoint values for resistance were not defined by the CLSI at the time of analysis. Assumptions for breakpoint values for resistance were based on the zone diameter criteria for susceptibilty of ≥ 31 mm as per previous CLSI guidelines (10). For azithromycin, a disk diffusion breakpoint value of ≤ 30mm was used as criteria for resistance, as previously described earlier (9). Data to confirm resistance values with methods that determine Minimal Inhibitory Concentration, MIC (e.g. Etest) was unavailable. Demographic and clinical characteristics were available for our study, including age, sex, sexual orientation, HIV status, and anatomical site of infection.

Ethics

This study was revised and approved by the Institutional Review Board of the San Juan Bautista School of Medicine (IRB #EMSJBIRB-6-2022). Informed consent was not necessary since it is a descriptive, noninterventional study involving the use of unidentified data.

Results

Demographics and clinical characteristics of patients with drug-resistant *N. gonorrhoeae*

From 2012 to 2017, 64.3% (276/429) of the patients had positive *N. gonorrhoeae* cultures and tested for antimicrobial susceptibility. Data on antimicrobial susceptibility was available for 84 gonococcal isolates obtained from 47 males and three females (Table 1). Fifty (50) strains showed resistance to at least one antimicrobial, while the remaining (34) were susceptible.

The resistant isolates were predominantly collected from males (94.0%, 47/50), between the age group of 21-30 (60.0%, 30/50) and HIV-negative (74.5%, 35/47). The prevalence of resistant *N. gonorrhoeae* was higher among men who have sex with men, MSM (79%, 37/47). Common sites of specimen collection were the urethra (38.0%, 30/80), pharynx (31.3%, 25/80), and rectum (26.3%, 21/80).

Antimicrobial susceptibility testing

Overall, 86.0% (43/50), 76.0% (38/50), and 38.0% (19/50) of isolates were resistant to tetracycline, ciprofloxacin, and penicillin, respectively (Table 1). Resistance to ceftriaxone and cefixime was not observed between 2012 to 2017.

Inhibition zone sizes of less than 31 mm were detected by the disc diffusion method for cefotaxime and cefepime (Table 1). The results correspond to a unique case of a male of 26 years that presented with a strain resistant to cefotaxime and cefepime in 2014. This strain was not viable for subsequent confirmation. Nevertheless, there is no evidence that such strain has persisted in Puerto Rico.

The rate of resistance to azithromycin, as determined by the disk diffusion method, was 4.0% (Table 1). It corresponds to two azithromycin resistance isolates from two MSM patients between the ages of 30-37 in 2016. However, there were no reports of emergence of azithromycin resistance strains on further analysis nor ceftriaxone treatment failures at the time of the ceftriaxone/azithromycin dual therapy.

Discussion

In the present study, we have retrospectively analyzed the resistance trends of *N. gonorrhoeae* to past and current recommended treatment guidelines to highlight the need for continued and updated surveillance practices. In addition, we describe the demographics of the patients with resistant *N. gonorrhoeae* strains that allow the identification of risk factors predictive for a resistant isolate infection.

Table 1. Demographic, behavioral, and clinical characteristics of patients with resistant *N. gonorrhoeae*

	Third generation cephalosporins			Fourth generation cephalosporin	Other antimicrobials			
	ceftiaxone N (%)	cefixime N (%)	cefotaxime N (%)	cefepime N (%)	ciprofloxacin N (%)	tetracycline N (%)	azithromycin N (%)	penicillin N (%)
Number of resistant isolates	0 (0.0)	0 (0.0)	1 (2.0)	1 (2.0)	38(76.0)	43 (86.0)	2 (4.0)	19 (38.0)
Gender (N)								
Male (N = 47)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	36 (72.0)	38 (76.0)	2 (4.0)	14 (28.0)
Female (N = 3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.0)	3 (6.0)	0 (0.0)	2 (4.0)
Age, years (N)								
14-20 (N = 15)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (73.3)	15 (100.0)	0 (0.0)	1 (6.6)
21-30 (N = 30)	0 (0.0)	0 (0.0)	1 (3.3)	1 (3.3)	23 (76.6)	24 (80.0)	1 (3.3)	14 (4.6)
31-40 (N = 1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)
41 and above (N = 4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (75.0)	2 (50.0)	0 (0.0)	0 (0.0)
HIV status[a]								
HIV + (N = 12)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (66.6)	11 (91.2)	2 (16.7)	3 (25.0)
HIV - (N =35)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	28 (80.0)	27 (77.1)	0 (0.0)	10 (28.6)
Sexual preference/ orientation[b]								
Heterosexual (N = 6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (50.0)	5 (83.3)	0 (0.0)	3 (50.0)
Bisexual (N =3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)	0 (0.0)	1 (33.3)
MSM (N =37)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	28 (75.6)	30 (81.1)	2 (5.4)	11 (29.7)
Anatomical site of infection[c]								
cervical (N = 3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.0)	2 (67.0)	0 (0.0)	1 (33.0)
oral (N = 25)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (44.0)	13 (52.0)	1 (4.0)	6 (24.0)
rectal (N = 21)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (38.1)	13 (61.9)	1 (4.8)	4 (19.0)
urethral (N = 30)	0 (0.0)	0 (0.0)	1 (3.3)	1 (3.3)	17 (56.7)	15 (50.0)	0 (0.0)	8 (26.7)

[a]HIV status was not reported for three patients.
[b]Four patients refused to disclose their sexual preference / orientation.
[c]Some patients presented with infection in more than one anatomic site.

The data collected and analyzed shows that all isolates tested were susceptible to ceftriaxone and cefixime during 2012-2017, a time when recommendations on the use of dual oral therapy (ceftriaxone 250mg plus azithromycin 1g) for uncomplicated gonorrhea infection and cefotaxime for complicated gonorrhea were introduced in USA (6). Although *in vitro* antimicrobial susceptibility testing has shown an absence of ceftriaxone resistance in Puerto Rico, it is important to keep monitoring for changing susceptibility and keeping vigilant for treatment failures as there is concern that isolates with the pblaTEM-135 plasmid, considered to be a precursor to an extended-spectrum beta-lactamase gene may become widespread worldwide (11). According to the CDC's current recommendations, both ceftriaxone and cefixime represent the ultimate first-line and alternative single-dose drug option, respectively, for the treatment of uncomplicated gonorrhea. In addition, the two cases of resistance to azithromycin reported in 2014 and 2016, respectively makes evident the continuous monitoring of emerging resistant isolates as azithromycin 2g is the choice for patients allergic to cephalosporins and those coinfected with *Chlamydia* (5).

This study found that the percentage of *N. gonorrhoeae* isolates resistant to tetracycline, ciprofloxacin, and penicillin was 86.0%, 76.0%, and 38.0%, respectively. The proportion of strains resistant to tetracycline, ciprofloxacin, and penicillin is higher than found in a study from the GISP project in the USA (25.3%, 19.2%, and 16.2%, respectively (12). None of the three antimicrobials are recommended regimens for *N. gonorrhoeae* treatment in Puerto Rico, and thus susceptibility testing is not needed to make recommendations for clinical management.

According to the CDC STD surveillance network of 2010-19, the rates of gonorrhea in the USA are rising rapidly in men who have sex with men, MSM (13). In Puerto Rico, linked gonococcal antimicrobial resistance and patient data from 2012-2017 suggest that the frequency of resistance is highest among MSM group (79%). The prevalence of gonorrhea in MSM is attributed to asymptomatic infection in extragenital anatomic sites (pharyngeal and rectal) and the fact that MSM tend to have higher numbers of sex partners (13). In addition, MSM are less likely to notify an exposed partner to a laboratory-based STI testing for potential treatment. This behavior creates

a scenario in which men with pharyngeal or rectal gonorrhea often go untreated for longer periods of time, promoting a higher reproductive rate for gonorrhea in MSM compared with heterosexuals, independent of the number of sexual partners (13). In our study, pharyngeal and rectal infections within MSM accounted for 58% (46/80) of all infections. Within MSM group, 54.1% of isolates (20/37) displayed resistance to a combination of two antimicrobials (ciprofloxacin / tetracycline, ciprofloxacin / penicillin, tetracycline / penicillin), while 19.0% (7/37) were resistant to three or more antimicrobials (ciprofloxacin / tetracycline / azithromycin; ciprofloxacin / tetracycline / penicillin / cefepime). MSM group could have constituted a risk for the emergence and dissemination of resistance to the past recommended antimicrobials; therefore, continued surveillance of *N. gonorrhoeae* antimicrobial susceptibility within this population is strongly recommended, as rising rates could increase the probability of resistance to the antimicrobials currently in use (14).

The strength of this study is that it is the first national-based system intended to monitor trends in gonococcal resistance in Puerto Rico in nearly four decades. However, the representativeness of the study may be limited because of the following facts: 1) Contrary to the sampling method performed at GISP-selected STD clinics, the island surveillance strategy to ensure that first-line therapies remain effective resulted in a collection of isolates biased towards the following groups: HIV, MSM, NAAT-positive cases with treatment failures and NAAT-negative cases presenting symptoms of gonorrhea. It should be noted that in Puerto Rico; as in most developed countries (15,16), the syndromic management of gonorrhea and the preferential use of nucleic amplification tests over culture for diagnosing gonorrhea has resulted in a decline in the number of gonococcal cultures required for antimicrobial testing. 2) Data on susceptibility was only available for approximately 30 % (84/276) of the positive cultures for this study period. Limitation in logistics, materials, transportation of samples, and storage may contribute to the low estimates of gonorrhea antimicrobial resistance.

In conclusion, emerging resistance to gonorrhea antimicrobials used in Puerto Rico during the period of 2012-2017 reflected a pattern of resistance in compliance with the recommended treatment scheme with an emphasis on the use of ceftriaxone. The findings of our study support the "syndromic-high risk" approach to treatment of gonorrhea in Puerto Rico as it allows for the surveillance of antibiotic susceptibility profiles of high-risk groups, in particular MSM. MSM presents the higher rate of antibiotic-resistant gonorrhea in PR and their unique behavioral characteristics and asymptomatic anatomical sites of infection represents the primary drivers of transmission. However, as antimicrobial resistance *N. gonorrhoeae* continues to emerge, it is recommended to strengthen the gonococcal culture capacity related to proper collection and transport of samples for susceptibility testing. Also, including optimal communication of data related to emerging resistance and contact tracing in populations at higher risk, such as MSM and those at risk for asymptomatic transmission is essential for continued and updated surveillance practices.

Resumen

Objetivos: El monitoreo de los patrones de susceptibilidad de *Neisseria gonorrhoeae* es esencial para el cumplimiento continuo de las recomendaciones de tratamiento actuales. Puerto Rico realiza pruebas de susceptibilidad a *N. gonorrhoeae*; sin embargo, no se han informado tendencias sobre la resistencia a los antimicrobianos desde mediados de los años 80. Métodos: Realizamos un análisis secundario de un repositorio nacional de datos sobre la susceptibilidad a los antimicrobianos de los aislados de *N. gonorrhoeae* entre 2012 y 2017, coincidiendo con el tratamiento recomendado por los CDC de ceftriaxona y azitromicina para ese entonces. Se obtuvieron datos de susceptibilidad a ocho antibióticos utilizando el método estándar de difusión en disco para el 30,0% (84/276) de las muestras recolectadas de las clínicas de Enfermedades de Transmisión Sexual en Puerto Rico. También realizamos análisis demográficos de pacientes relacionados con la resistencia. Resultados: Las tasas de resistencia a ceftriaxona y azitromicina fueron 0% y 4,0% (2/50), respectivamente. El porcentaje de cepas resistentes a antimicrobianos que ya no se recomiendan en Puerto Rico, como tetraciclina, ciprofloxacina y penicilina, fué de 86.0 % (43/50), 76.0 % (38/50) y 38.0 % (19/50), respectivamente. La prevalencia de *N. gonorrhoeae* resistente fué mayor entre hombres que tienen sexo con hombres, HSH (79%, 37/47). Conclusión: Se identificó una ausencia de cepas de *N. gonorrhoeae* resistentes a ceftriaxona y una baja frecuencia de resistencia a azitromicina durante el período de 2012-2017. Es vital continuar con los esfuerzos de vigilar los patrones emergentes de resistencia, y en especial para ceftriaxona, ya que forma parte de las guías de tratamiento recomendadas por el Centro de Control de Enfermedades. Por tal razón, los protocolos para la vigilancia basada en cultivos, transporte y procesamiento de muestras, deben fortalecerse para garantizar una epidemiología de calidad asegurada de la resistencia gonocócica en Puerto Rico.

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