Molecular Characterization of Ciprofloxacin-Resistant *Neisseria gonorrhoeae*. Is Ciprofloxacin a Suitable Alternative for Precision Treatment in the STI Clinic Setting?

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INTRODUCTION

Gonorrhea is the second most commonly reported bacterial sexually transmitted infection (STI) worldwide. In 2017, 555,608 cases of gonorrhea were reported to the Centers for Disease Control and Prevention (CDC), a 67% increase from 2013. Gonorrhea infections have serious sequelae among women: pelvic inflammatory disease, tubal factor infertility, chronic pelvic pain, ectopic pregnancy, and can increase HIV transmission 3-5 fold. The most vulnerable groups for STIs and their sequelae are women, adolescents, and racial and ethnic minorities.

Neisseria gonorrhoeae (NG), the causative agent of gonorrhea, has progressively developed resistance to all commonly-prescribed antimicrobials and the threat of untreatable gonorrhea is a global health concern. The World Health Organization (WHO) has identified several strategies to control the spread of antimicrobial resistant (AMR) NG, including improved methods for diagnosis and characterization/monitoring of antimicrobial resistance. The objective of this study was to determine if ciprofloxacin, a previously recommended antimicrobial, could be used for precision treatment in the STI clinic setting and to identify correlates associated with ciprofloxacin-resistant NG infections.

METHODS

NG isolates, collected from men at the Baltimore City Health Department (BCHD) between January 2014 and October 2016 were recovered from freezing media, and confirmed as NG by PCR.

In order to determine ciprofloxacin susceptibility at the molecular level, all isolates (viable and non-viable) were analyzed by real-time PCR for the presence/absence of mutation(s) in the *gyrA* gene using a previously described assay. Viable isolates were further analyzed by the E-test method to determine the minimum inhibitory concentration (MIC) of each isolate to ciprofloxacin and to confirm the susceptibility results obtained from the PCR assay. Briefly, NG suspensions (10^8 CFU/mL), were streaked over the entire surface of NG agar plates with 1% IsoVitalex and E-test strips individually placed on the plates. The E-test strips contain an exponential concentration of antimicrobials on one side and a MIC reading scale on the other side. Plates were incubated for 18 – 24 hours at 37°C in 5% CO_2. The MIC to ciprofloxacin was determined from the E-test readings and susceptibility reported as susceptible, intermediate, or resistant based on breakpoints from the Clinical Laboratory Standard Institute (CLSI) guidelines.

Clinical characteristics and demographics were evaluated by multivariate regression analysis to identify correlates of ciprofloxacin-resistant NG infections.

RESULTS

1. Characteristics of 510 men with *Neisseria gonorrhoeae* infection in Baltimore, 2014 – 2016

Characteristics	Categories	Number (%)
		N=510
Age (years)	15 – 19	60 (11.8)
	20 – 24	117 (22.9)
	25 – 29	109 (21.4)
	30 – 34	57 (11.2)
	35 – 44	85 (16.7)
	≥ 45	82 (16.1)
Race/Ethnicity	African American	492 (96.5)
	Non-Hispanic White	11 (2.2)
	Hispanic	3 (0.6)
	Other	4 (0.8)
Sexual orientation	Heterosexual	437 (85.7)
	Bisexual	17 (3.3)
	Gay	50 (9.8)
	Unknown/Unspecified	6 (1.2)
Calendar year	2014	170 (33.3)
	2015	185 (36.3)
	2016	155 (30.4)
Symptoms	Discharge	459 (90.0)
	Dysuria	253 (49.6)
	Itch in urogenital area	19 (3.7)
	Lesion in urogenital area	16 (3.1)
	Irritation or tingling feeling	13 (2.5)
	Burning sensation	7 (1.4)
	Rash	6 (1.2)
	Pain in urogenital area	5 (1.0)
	Other	2 (0.4)
	None	17 (3.3)
HIV infection	Yes	34 (6.7)
	No	472 (92.5)
	Unknown	4 (0.8)
Concurrent syphilis infection	Yes	22 (4.3)
	No	488 (95.7)
Syphilis diagnosis in the past	Yes	25 (4.9)
	No	485 (95.1)
GyrA genotype	Wild type	345 (67.7)
	Mutant	165 (32.4)

- The overall rate of *gyrA*-mutant (ciprofloxacin resistance) was 32.4% and increased from 24.7% in 2014 to 45.2% in 2016.
- All viable isolates with *gyrA* mutation(s) were phenotypically confirmed as ciprofloxacin resistant.
- Individuals older than 35 years of age were more likely to have a *gyrA*-mutant NG infection.

2. Bivariate analysis of association between demographic and clinical characteristics and *gyrA* genotype

		gyrA Genotype		p-value
Characteristics	Categories	Wildtype (%)	Mutant (%)	
		N=345	N=165	
Age (years)*	15 – 24	135 (39.1)	42 (25.5)	0.002
	25 – 34	113 (32.8)	53 (32.1)	
	≥ 35	97 (28.1)	70 (42.4)	
Race/Ethnicity	African American	334 (96.8)	158 (95.8)	0.546
Sexual orientation	Bisexual or Gay	51 (14.8)	16 (9.7)	0.112
Calendar year†	2014	128 (37.1)	42 (25.5)	<0.001
	2015	132 (38.3)	53 (32.1)	
	2016	85 (24.6)	70 (42.4)	
Symptom - Discharge	Discharge	312 (90.4)	147 (89.1)	0.636
Symptom - Dysuria	Dysuria	176 (51.0)	77 (46.7)	0.358
No symptoms	Yes	9 (2.6)	8 (4.9)	0.187
HIV infection	Yes	26 (7.5)	8 (4.9)	0.255
Concurrent syphilis infection	Yes	15 (4.4)	7 (4.2)	0.956
Syphilis diagnosis in the past	Yes	16 (4.6)	9 (5.5)	0.689

* p<0.001 for Cochran-Armitage Trend Test
† p<0.001 for Cochran-Armitage Trend Test

3. Multivariate regression analysis of factors association with presence of *gyrA* mutant genotype

Variables	Categories	Odds Ratio (95% CI)	p-value
Age group (years)	15 – 24	1.00	
	25 – 34	1.46 (0.90, 2.37)	0.123
	≥ 35	2.35 (1.47, 3.76)	<0.001
Calendar year	Increasing each year	1.61 (1.27, 2.05)	<0.001

CONCLUSIONS

- The levels of ciprofloxacin-resistant NG infections are low to moderate in Baltimore.
- Ciprofloxacin could be a suitable option for targeted treatment if antimicrobial susceptibility could be determined at the point-of-care.
- *GyrA*-mutant (ciprofloxacin resistant) NG infections are more common in older (>35 years) individuals.
- Enhanced surveillance practices are necessary to monitor the evolution of antimicrobial resistance in Maryland.

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