High Percentages of Resistance to Tetracycline and Penicillin and Reduced Susceptibility to Azithromycin Characterize the Majority of Strain Types of Neisseria gonorrhoeae *Isolates in Cuba, 1995–1998*

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Background: **In many regions the susceptibility of *Neisseria gonorrhoeae* isolates to antimicrobial agents is rarely tested. The Gonococcal Antimicrobial Surveillance Program (GASP) in Cuba was established as part of a larger regional GASP program to facilitate the collection and reporting of antimicrobial susceptibility data for *N gonorrhoeae* isolates.**

Goal: **The goal was to retrospectively determine the antimicrobial susceptibility and molecular epidemiology of 91 isolates of *N gonorrhoeae* isolated from 11 centers in Cuba.**

Study Design: **Isolates of *N gonorrhoeae* were collected and presumptively identified from 11 Cuban provincial health centers. They were then forwarded to the National Laboratory of Pathogenic Neisseria Havana for confirmatory identification and were subsequently analyzed at the Center for GASP in Ottawa. Isolates were tested for susceptibility to penicillin, tetracycline, spectinomycin, ceftriaxone, ciprofloxacin, and azithromycin by the agar dilution method. To establish baseline data for molecular epidemiologic profiles, the auxotype (A), serovar (S), plasmid content (P), and TetM type of the isolates were determined. Certain A/S/P classes were further analyzed by pulsed field gel electrophoresis (PFGE).**

Results: **High percentages of the 91 *N gonorrhoeae* isolates were resistant to penicillin (68%) and tetracycline (83.5%), with 56% being penicillinase-producing (PPNG) and 64% carrying plasmid-mediated tetracycline resistance (TRNG; 50% were PP/TRNG). An additional 14% of the isolates carried chromosomal resistance (CMRNG) to either tetracycline or penicillin or both antibiotics. All isolates were susceptible to spectinomycin, ceftriaxone, and ciprofloxacin. However, nine isolates were resistant to azithromycin (MIC, ≥1.0 μg/ml), and 43 other isolates displayed reduced susceptibility to this antibiotic (MIC, 0.25–0.5 μg/ml). Although a total of 21 different A/S classes were identified, most of the isolates (61) belonged to three A/S classes: NR/IA-6 (35 isolates), NR/IB-1 (15 isolates), and P/IA-6 (11 isolates). Thirty-two of 45 PP/TRNG were A/S class NR/IA-6, and nine of the P/IA-6 isolates were TRNG. By contrast, most of A/S class NR/IB-1 (8) were CMRNG. PFGE analysis following digestion with *Nhe*I or *Spe*I further clustered the isolates into separate groups.**

Conclusions: **This study demonstrates high percentages of *N gonorrhoeae* isolates with penicillin and tetracycline resistance in Cuba. As has been noted in other studies in the Caribbean region and Latin America, resistance and reduced susceptibility to azithromycin are developing as emerging problems. Since penicillin and tetracycline continue to be widely used for the treatment of gonococcal infections in Cuba, this study indicates the importance of antimicrobial susceptibility surveillance so that effective antibiotics may be recommended for treatment of gonococcal infections.**

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THE ADEPTNESS OF ISOLATES of *Neisseria gonorrhoeae* in accruing resistance to antimicrobial agents is a formidable problem for the ongoing effective treatment and control of gonococcal disease.[1–4] In many countries, baseline data on the antimicrobial susceptibility of gonococcal isolates have not been collected. In the absence of such data, antibiotics such as penicillin or tetracycline may continue to be used despite reports from around the globe that high levels of resistance internationally preclude their use for the treatment of uncomplicated infections caused by *N gonorrhoeae*. Thus, the establishment of regional or national antimicrobial surveillance programs, such as the Gonococcal Antimicrobial Surveillance Program (GASP) in the Americas and the Caribbean, has become important in assisting health providers in making recommendations regarding effective antibiotics for treatment. The establishment of such baseline data is especially important when syndromic approaches are used to diagnose and treat gonococcal infections.

Antimicrobial resistance to various antibiotics is mediated in the gonococcus either through plasmid-mediated mechanisms (i.e., resistance to penicillin or tetracycline) or by mutation of various chromosomal genes (virtually all antibiotics).[1,2,4] In the Caribbean region, many countries have a high prevalence of *N gonorrhoeae* isolates that are resistant to penicillin. For example, in a report from Honduras, 83% of the gonococci isolated were pencillinase-

This study was partially funded by the Pan American Health Organization and the Canadian Bacterial Diseases Network Centers of Excellence. Work completed by J. S. at the University of Ottawa was in partial fulfillment of graduate degrees received from the University of Havana.

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Received September 4, 2002, revised December 5, 2002, and accepted December 6, 2002.

producing *N gonorrhoeae* (PPNG), as were 78% of the isolates reported in a study from Nicaragua.[5,6] Similarly, high percentages of PPNG were noted in Jamaica (62.9%) and Guyana (73%).[7,8] High percentages of *N gonorrhoeae* isolates with plasmid-mediated resistance to tetracycline (TRNG) have also been reported from many Caribbean countries, with values of 74.2% in Jamaica, 87.1% in Guyana, and 51.7% in St. Vincent.[7,8]

As in other parts of the world, an additional burden of resistance is presented by gonococcal isolates that carry chromosomal resistance to penicillin and tetracycline as well as to other antibiotics such as the macrolides (e.g., azithromycin) and the fluoroquinolones (e.g., ciprofloxacin).[8–11]

Between 1960 and 1994, cases of gonorrhea reported in Cuba increased from 130 in 1960 to 34,224 in 1994 and 33,948 in 1997.[12] The annual incidence of gonorrhea per 100,000 population was 310.5 and 307.6 for 1994 and 1997, respectively. Although sporadic investigations of gonococcal antimicrobial susceptibility have been conducted, such studies have been limited in frequency and scope due to economic constraints. The current study was undertaken to characterize the antimicrobial susceptibility profile and molecular epidemiology (auxotype/serovar/plasmid content [A/S/P] class, TetM type, and pulsed field gel electrophoresis [PFGE] type) of 91 *N gonorrhoeae* isolates from 11 Cuban cities in order to establish a baseline of susceptibilities and strain types circulating between 1995 to 1998.

Methods

Bacterial Strains and Identification

Between 1995 and 1998, urogenital, rectal, and conjunctival cultures (n = 435) were forwarded from 11 Cuban Provincial Centers of Epidemiology and Hygiene to the National Reference Laboratory of Pathogenic Neisserias (NRLPN) in Havana in transport medium that was formulated and prepared in Cuba (Cuban patent CU22547A1). Before their shipment, the specimens were inoculated locally onto chocolate agar and tested by Gram stain and oxidase reaction.

After shipment to the NRLPN, 110 isolates (81 from male patients and 29 from female patients) were recovered after subculture on Thayer Martin medium and incubation in a humid candle jar at 36° C. The identity of the isolates was confirmed by Gram stain and oxidase, catalase, and carbohydrate degradation tests (CTA base with sugars [1%]).[13] Isolates were then subcultured onto GC medium base agar (Difco, Detroit, MI) containing 1% Kellogg's supplement (GCMBK), tested for β-lactamase production with nitrocefin (Oxoid, Hampshire, England), and subsequently stored at −70° C in 20% (vol/vol) glycerol in brain–heart infusion (Difco).[8,13]

Finally, the isolates were lyophilized in 1% (vol/vol) inositol (Sigma, Oakville, ON) in horse serum and transported to the Center for the Gonococcal Antimicrobial Surveillance Program (GASP) in the Americas and the Caribbean, in Ottawa. Ninety-one isolates were recovered after subculture on GCMBK (51 from 1995, 21 from 1996, 15 from 1997, and 4 from January and February 1998).

Antimicrobial Susceptibility Determination

The minimum inhibitory concentration (MICs) of the *N gonorrhoeae* isolates to penicillin (Wyeth-Ayer Canada, St. Laurent, Quebec, Canada), tetracycline (Pfizer Canada, Pte. Claire, Quebec, Canada), spectinomycin (Upjohn Co. of Canada, Don Mills, Ontario, Canada), ceftriaxone (Hoffman-LaRoche, Mississauga, Ontario, Canada), azithromycin (Pfizer, Groton, CT), and ciprofloxacin (Bayer, Etobicoke, Ontario, Canada) were determined by the agar dilution method on GCMBK agar, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[14] Tests were completed in duplicate and included reference strains WHO III, WHO V, WHO VII, and ATCC 29465.[9,14]

Isolates were presumptively defined as PPNG if they were β-lactamase-positive, as determined by testing with nitrocefin; TRNG if the MICs of tetracycline were $\geq$16.0 μg/ml; and PP/TRNG if they were β-lactamase-positive with a tetracycline MIC $\geq$16.0 μg/ml. All isolates with tetracycline MICs $\geq$2.0 μg/ml were tested for the presence of a *tetM* determinant (see below). Chromosomally resistant (CMR) isolates were defined as having a penicillin MIC and/or tetracycline MIC $\geq$2.0 μg/ml (classified as CMTR for tetracycline resistance, CMPR for penicillin resistance, and CMRNG for resistance to both antibiotics), providing that they were neither PPNG nor TRNG.

Isolates with a ciprofloxacin MIC of $\geq$1.0 μg/ml or spectinomycin MIC of $\geq$128.0 μg/ml were also considered chromosomally resistant to these antibiotics.[14] Isolates were considered as being susceptible to ceftriaxone if the MIC was $\leq$0.25 μg/ml.[14] Although breakpoints defining azithromycin resistance have not been determined, we have used a consensus concentration of $\geq$1.0 μg/ml to delineate resistance to azithromycin, while MICs of 0.25–0.5 μg/ml were considered to show decreased susceptibility to this antibiotic.

Auxotype, Serovar, Plasmid, and TetM Typing

Nutritional growth requirements (auxotypes) were determined by the modified method of Hendry and Stewart.[15] Serological classification was carried out as described by Knapp et al.[16] Monoclonal antibodies for serovar determination were provided by Syva (Palo Alto, CA). Isolates were analyzed for plasmid content as described previously.[8,9,17] All isolates with a tetracycline MIC $\geq$2.0 μg/ml were characterized for TetM type with use of the PCR method, modified from that of Xia et al.[8,9,18]

Pulsed Field Gel Electrophoresis

Samples from the three major A/S groups (NR/IA-6, NR/IB-1, P/IA-6) of *N gonorrhoeae* isolated in Cuba were further analyzed by PFGE as described previously.[19] In brief, agarose plugs with the lysed cell suspensions were digested at 37° C with *Spe*I or *Nhe*I in a final volume of 100 μl, according to the manufacturer's instructions (New England BioLabs, Mississauga, Ontario, Canada). Electrophoresis was then performed in a contour-clamped homogeneous electrical field system (CHEF-DRII; BioRad, Mississauga, Ontario, Canada), at 5 V/cm and 14° C in 0.5 mol/l Tris:borate:EDTA buffer.

The pulse times for *Nhe*I were increased from 1 to 50 seconds during the first 13 hours and from 2 to 50 seconds in the next 10 hours; for *Spe*I the times were increased from 0.2 to 54.2 seconds over 24 hours. The gels were then stained with ethidium bromide, destained in double distilled water, and photographed under UV illumination with a digital imaging system (Bio Photonics Gel Print 2000i; BIO/CAN Scientific; Mississauga, Ontario, Canada). Phage lambda concatemers were used as size markers (BioRad). Patterns having differences of 1 to 3 bands were considered to indicate subtypes of the same strain, while digestion patterns having differences of more than three bands were classified as belonging to different strains.[20,21]

Results

Antimicrobial Susceptibility

The susceptibilities to penicillin, tetracycline, spectinomycin, ceftriaxone, ciprofloxacin, and azithromycin of 91 gonococcal

TABLE 1. Antimicrobial Susceptibility of 91 Cuban Isolates

	Cumulative % with an MIC (μg/ml) of:*																	
Antibiotic	≤0.001	0.002	0.004	0.008	0.016	0.032	0.063	0.125	0.25	0.50	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	>128.0
Penicillin							2.2	3.3	8.8	24.2	31.9	36.6	42.9	44	48.4	51.6	57.1	76.9	100
Tetracycline											16.5	24.2	33	36.3	52.7	97.8	100		
Spectinomycin														38.5	100				
Ceftriaxone	2		7.7	54.9	75.8	83.5	91.2	100											
Ciprofloxacin				48.4	78	91.2	95.6	100											
Azithromycin								19.8	42.9	70.3	90.1	94.5		100					

*Number tested = 91.

isolates from 11 Cuban cities are summarized in Table 1. Sixty-two isolates (68.1%) were resistant to penicillin (Tables 1 and 2), and 51 of these isolates (56%) were PPNG (45 were PP/TRNG, 5 were PPNG/CMTR, and 1 had decreased susceptibility to tetracycline, with an MIC of 1.0 μg/ml; Table 2). Twenty-five of the PP/TRNG isolates originated in Havana, while seven came from Camagüey. The 11 other penicillin-resistant isolates (12%) carried chromosomal resistance (10 CMRNG and 1 CMPR; Table 2).

Resistance to tetracycline was observed in 76 of 91 isolates (83.5%; Table 1). Fifty-eight isolates (63.7%) were TRNG (45 PP/TRNG and 13 TRNG; Table 2), and 18 (19.8%) demonstrated chromosomally mediated resistance to tetracycline. The majority of these strains were isolated in Havana (25 PP/TRNG and 8 TRNG). Ten of the CMTR isolates were also chromosomally resistant to penicillin (i.e., CMRNG), and 5 were also PPNG (Table 2). Overall, 27 isolates were non-PPNG and non-TRNG (Table 2). With the exception of two isolates for which the MIC of penicillin was 0.063 μg/ml (Table 1), all such isolates displayed either resistance or reduced susceptibility to penicillin and tetracycline (reduced susceptibility to penicillin: MIC ≥0.125–1.0 μg/ml; reduced susceptibility to tetracycline: ≥0.5–1.0 μg/ml).[14]

All isolates were susceptible to spectinomycin, ceftriaxone, and ciprofloxacin (Table 1). However, nine isolates were resistant to azithromycin (MIC, ≥1.0 μg/ml; Table 1); five of these isolates were also PP/TRNG, two were PPNG and also CMTR, one was a TRNG, and one was a CMRNG. Forty-three other isolates' azithromycin MICs were either 0.25 μg/ml or 0.5 μg/ml (13 of these isolates were PP/TRNG, 3 were PPNG, 4 were TRNG, 2 were CMTR, and 21 were CMRNG). Thus, isolates displaying resistance or reduced susceptibility to azithromycin also were resistant to other antibiotics.

Plasmid Content

Forty-eight of the 58 TRNG isolates carried the American type TetM determinant, and 10 carried the Dutch type.[22] Forty-four of the 48 American type TRNGs also carried a 3.2-MDa (i.e., Africa type) β-lactamase-producing plasmid. Of the 51 PPNG isolates (Table 2), 48 carried an Africa type plasmid (45 were PP/TRNG; Table 2) and 3 carried the Asia type (4.5 MDa) β-lactamase-producing plasmid and a 24.5-MDa transfer plasmid. All PPNG also carried the 2.6-MDa cryptic plasmid. The plasmid profile observed most frequently (49.5%) was 2.6, 3.2, and 25.2 MDa (32 of them were of A/S class NR/IA-6; see below). Ten isolates carried only the 2.6-MDa cryptic plasmid, while two other isolates of A/S class NR/IB-3 were plasmid-free.

Although these last 12 isolates did not carry antibiotic resistance plasmids, only two of them were susceptible to penicillin, and all of them had reduced susceptibility to tetracycline and azithromycin.

Auxotype/Serovar (A/S) Class

A total of 21 different A/S classes was identified (Table 2), with 61 of the 91 *N gonorrhoeae* isolates (67%) belonging to three

TABLE 2. Susceptibility to Penicillin and/or Tetracycline of the Different Auxotype/Serovar (A/S) Classes

	Susceptibility Class: No. (% of Total)							
				Non-PPNG or non-TRNG				
A/S Class*	PP/TRNG†	TRNG	PPNG	CMTR	CMPR	CMRNG	Reduced susceptibility‡	Total
NR/IA-6	32 (35.2)	3 (3.3)	0	0	0	0	0	35 (38.5)
NR/IB-1	1 (1.1)	0	3 (3.3)	1 (1.1)	0	8 (80)	2	15 (16.5)
P/IA-6	1 (1.1)	8 (8.8)§	2 (2.2)	0	0	0	0	11 (12.1)
NR/1A-3	4 (4.4)	0	0	0	0	0	0	4 (4.4)
Other (17)‖	7 (7.7)	2 (2.2)	1 (1.1)	2 (2.2)	1 (1.1)	2 (2.2)	11 (12.1)	26 (28.6)
Total	45 (49.5)	13 (14.3)	6 (6.6)¶	3 (3.3)	1 (1.1)	10 (11)	13 (14.3)	91 (100)

*NR = nonrequiring or wild type; P = proline-requiring; C = citrulline-requiring; U = uracil-requiring.
†All but one (NR/1A-3) PP/TRNG carried 3.2-MDa African type plasmid and American type TetM determinant.
‡Non-PPNG or TRNG with reduced susceptibility to penicillin and tetracycline (see text). Only two of these isolates were susceptible to penicillin.
§Seven were Dutch type.
‖NR/IA-8 = 2, NR/IA-21 = 1, NR/IB-2 = 3, NR/IB-3 = 2, NR/IB-22 = 1, NR/IB-23 = 1, CU/IB-1 = 2, CU/IB-3 = 3, H/IB-3 = 2, M/IA-6 = 1, O/IA-6 = 2, O/IB-3 = 1, P/IA-8 = 1, P/IB-3 = 2, PO/IB-1 = 1, ND/IB-22 = 1, P/IA-18 = 1;
¶Five isolates were also CMTR and one (P/IA-6) had a tetracycline MIC of 1.0 μg/ml.

TABLE 3. PFGE types of the three predominant A/S classes of N. gonorrhoeae isolated in Cuba

A/S class (total no.)	Plasmid Content	No. of Isolates	NheI	SpeI	NheI/SpeI Type
NR/IA-6 (35)*	2.6 + 3.2 + 25.2 (MDa)†§	17	I	I	I
	2.6 + 3.2 + 25.2 (MDa)‡§	5	II	I	II
	2.6 + 3.2 + 25.2 (MDa)§	6	II	II	III
	2.6 + 3.2 + 25.2 (MDa)§	2	I	II	IV
	2.6 + 3.2 + 25.2 (MDa)§	1	III	II	V
	2.6 + 3.2 + 25.2 (MDa)§	1	IV	I	VI
	2.6 + 3.2 + 25.2 (MDa)§	1	V	I	VII
	2.6 + 3.2 + 25.2 (MDa)§	1	VI	III	VIII
P/IA-6 (11)§	2.6 + 25.2§	3	VII	IV	XI
	2.6 + 25.2§	3	VIII	IV	X
	2.6 + 25.2§	1	IX	IV	IX
NR/IB-1 (15)‖	2.6 + 24.5	2	XIII	VI	XV
	2.6 + 24.5	5	X	V	XII
	2.6 + 4.5 + 24.5	1	XV	VIII	XVII
	2.6	1	XIV	VII	XVI
	2.6 + 24.5	1	XI	VI	XIII
	2.6 + 24.5	1	XII	V	XIV

*One isolate not tested.
†One isolate did not carry the 3.2-MDa plasmid.
‡Two isolates did not carry the 3.2-MDa plasmid.
§Four isolates not tested.
‖Four isolates not tested.

classes: NR/IA-6 (35 isolates), NR/IB-1 (15 isolates), and P/IA-6 (11 isolates). Most (32) of the 35 isolates of A/S class NR/IA-6 (Table 2) were PP/TRNG (American TetM determinant), with a plasmid profile of 2.6 MDa, 3.2 MDa, or 25.2 MDa. Sixteen of these isolates originated in Havana (isolated between 1995 and 1997) and six in Camagüey (between 1995 and 1998). Five NR/IB-1, strains isolated in Santiago de Cuba in 1995 and 1996, carried 2.6- and 24.5-MDa plasmids, and all were CMRNG. Finally, 8 of the 11 P/IA-6 isolates were TRNG (8.8%): 7 carried a Dutch-type TetM determinant and 1 the American type TetM determinant (Table 2). Six of these strains were isolated in Havana during 1995. It is interesting that 15 NR/IB-1 isolates (Table 2) displayed reduced susceptibility to azithromycin (MIC, 0.25–1 μg/ml; Table 1).

Pulsed Field Gel Electrophoresis

Fifty-two *N gonorrhoeae* isolates belonging to A/S classes NR/IA-6, P/IA-6, and NR/IB-1 were digested with *Nhe*I or *Spe*I to determine the genetic heterogeneity of the isolates within each A/S class. After PFGE analysis, 15 different digestion patterns were identified with *Nhe*I (Table 3), 8 patterns were observed after digestion with *Spe*I (Table 3), and 17 patterns were obtained with both enzymes combined (Table 3).

The 34 NR/IA-6 isolates that were typed were all TRNG, and 33 of these were also PPNG. They were grouped into eight PFGE patterns with *Nhe*I and *Spe*I. However, most (17) of the NR/IA-6 isolates were classified as type I (Table 3). They were recovered between 1995 and 1997, mainly from Havana (9 of 17 isolates), Camagüey (3 isolates), and three other cities (5 isolates). Five NR/IA-6 strains isolates were classified as PFGE type II: three were isolated in Havana (1995, 1996, and 1997) and the others in two different cities. Similarly, six isolates were typed as PFGE type III and five were isolated in Havana between 1995 to 1997.

The two predominant PFGE types for P/IA-6 isolates were X and XI (three isolates each). All of the type X isolates came from Havana, and two of three of the type XI isolates were from the same city. These isolates were recovered over a 3-year period.

The predominant PFGE type for NR/IB-1 isolates was type XII (Table 3). Four of these isolates came from Santiago de Cuba (1995 and 1996) and one was isolated in Havana (1998).

Discussion

Between 1995 and February 1998, a high percentage of the *N gonorrhoeae* isolates collected in Cuba displayed resistance to penicillin (68%) and tetracycline (83.5%). In most cases, this resistance was plasmid-mediated (56% PPNG and 63.5% TRNG). Only two isolates had MIC values within the ranges considered as showing susceptibility to penicillin,[14] and none of the isolates had MICs that could be interpreted as showing susceptibility to tetracycline. These percentages reflect the high levels of resistance to penicillin and tetracycline previously reported from other areas of Latin America and the Caribbean.[8,9,22–25]

Previous investigators in Cuba during the 1980s[26] also reported the presence of gonococcal isolates with decreased susceptibility to penicillin. In 1989, 37.5% of 24 *N gonorrhoeae* isolates from Cuba were PPNG, and 29.2% had chromosomally-mediated resistance to tetracycline (Dillon et al., unpublished data). In 1995, Sosa et al.[27] reported an outbreak of conjunctivitis caused by PPNG isolates in Camagüey. These isolates carried 2.6-, 3.2-, and 24.4-MDa plasmids, the predominant plasmid content pattern observed in the current study.

The results of the present and other studies confirm that the frequency of isolation of *N gonorrhoeae* resistant to penicillin and/or tetracycline (either plasmid mediated or chromosomally mediated) is increasing in Cuba and that resistant isolates are distributed across the country.[28] In contrast to the rest of the island, only three cities in Cuba—Havana, Camagüey, and Santiago de Cuba—recommend the use of antibiotics other than penicillin and tetracycline (i.e., ciprofloxacin) for the treatment of gonorrhea (unpublished data, Cuban Ministry of Public Health). A recent case report demonstrated the isolation of a gonococcal strain

resistant to ciprofloxacin in Holguín.[29] Similarly, four strains isolated in the city of Morón showed reduced susceptibility to ciprofloxacin.[30] Therefore, this study underscores the urgency for recommending effective antimicrobials for treatment against antibiotic-resistant *N gonorrhoeae*.

The presence of isolates carrying predominantly American-type TetM determinants is an interesting contrast to other countries in Latin America and the Caribbean, from which investigators have reported predominantly Dutch type TRNG.[8,9] Most TRNG in Canada also carry Dutch type plasmids (Dillon et al., unpublished). The A/S classes of the TRNG from Cuba were dissimilar to the predominant classes reported elsewhere in Latin America.[9,25]

All the isolates tested in this study were susceptible to spectinomycin, ceftriaxone, and ciprofloxacin, drugs currently recommended in many areas for the treatment of *N gonorrhoeae*.[31,32] The inclusion of ciprofloxacin as a frontline antibiotic for treating uncomplicated gonococcal infections should be closely monitored. Some regions report that over 30% of isolates tested are resistant to ciprofloxacin, especially in the Asian-Pacific region.[2,31,32]

Since ciprofloxacin resistance is expected to increase in most countries, surveillance of gonococcal antimicrobial susceptibility has become important for continued treatment efficacy. The recent emergence of *N gonorrhoeae* resistant to ciprofloxacin in Cuba underscores the importance of maintaining a gonococcal antimicrobial surveillance program (GASP) to monitor trends in antimicrobial susceptibility and to enable the modification of treatment guidelines accordingly.[29,30]

Although all isolates tested from Cuba in the current study were susceptible to ceftriaxone, surveillance of antimicrobial susceptibility to this antibiotic should be on-going since reduced susceptibility or resistance has been recently reported.[31,33] Azithromycin is an antibiotic sometimes used in Latin America either for simultaneous treatment of uncomplicated gonorrhea and chlamydia or as an additional antibiotic for treatment of uncomplicated gonorrhea.[9] Either resistance (MIC, $\geq$2 μg/ml) or reduced susceptibility (MIC, 0.25–1 μg/ml) to azithromycin was noted in 52 of the 91 isolates tested from Cuba, a trend reported in other Latin American countries.[8,9,34,35]

For example, 23 of the 81 isolates from Brazil and 17 of 68 isolates from St. Vincent were reported to be resistant or to have reduced susceptibility to azithromycin.[8,9] Azithromycin-resistant isolates have been reported from both Guyana and Uruguay.[8,34] The increased resistance to azithromycin in Cuba might reflect the use of erythromycin to treat gonorrhea in patients allergic to penicillins or to treat other conditions. Cross-resistance between azithromycin and erythromycin has been reported elsewhere.[34,36,37]

Although 3 clusters of isolates were identified on the basis of A/S class, further analysis of these strains by PFGE showed that they belonged to 17 different groups. PFGE is one of the most discriminatory methods for distinguishing strains of *N gonorrhoeae*.[38,39] Despite this high level of discrimination, in this study it was possible to identify similar strains from a retrospective population study that were isolated over a 3-year period in different cities. For example, although 8 different PFGE patterns were found within the major cluster of NR/IA-6 isolates, 17 of 34 typed isolates belonged to only 1 PFGE group. This indicates that common strains circulated in different Cuban cities and were maintained over a period of several years.

The core group(s) carrying these strains were not identified but were coupled with epidemiologic data. PFGE analysis would permit the identification of such groups.

This study highlights the importance of establishing surveillance of the antimicrobial susceptibility of *N gonorrhoeae* isolates circulating in Cuba, which would be the basis for ongoing rationalization of treatment regimens for gonococcal infections.

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