National Surveillance of Antimicrobial Susceptibilities of *Neisseria gonorrhoeae*

Annual Summary 2010

Streptococcus and STI Unit
Bacteriology and Enteric Diseases Program
National Microbiology Laboratory
Public Health Agency of Canada

Professional Guidelines and Public Health Practice Division
Surveillance and Epidemiology Division
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Public Health Agency of Canada

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Figures and Tables

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Executive Summary

- This report compares laboratory surveillance data for *Neisseria gonorrhoeae* isolates submitted by provincial microbiology laboratories to the National Microbiology Laboratory (NML) from 2006-2010.
- The Canadian reported rate of gonorrhea is on the rise and has almost doubled from 14.9 per 100,000 in 1997 to a projected rate of 33.4 per 100,000 in 2010.
- Antimicrobial resistance in *N. gonorrhoeae* is of foremost concern for effective treatment of gonococcal infections. Over time, *N. gonorrhoeae* has acquired resistance to many antibiotics such as penicillin, tetracycline, erythromycin and ciprofloxacin.
- In Canada, the MICs of the 3rd generation cephalosporins have been increasing over time. There has been a shift in the modal MICs of ceftriaxone from 0.016/0.032 mg/L in 2006 to 0.063 mg/L in 2010. There was also a shift in the modal MICs of cefixime from 0.016 mg/L in 2006 to 0.125 mg/L in 2010. Two isolates (one each in 2007 and 2008) were identified with a cefixime MIC equal to 0.5 mg/L, and classified as non-susceptible.
- The number of azithromycin resistant (MIC >=2 mg/L) *N. gonorrhoeae* isolates out of all isolates tested increased from 8 in 2006 (0.19%) to 37 in 2010 (1.25%). There has been a shift in the modal MICs of azithromycin from 0.25 mg/L in 2006 to 0.5 mg/L in 2010.
- In Canada, ciprofloxacin resistance in *N. gonorrhoeae* has increased to a level where ciprofloxacin is no longer an option for first-line treatment at the national level. The number of ciprofloxacin resistant *N. gonorrhoeae* isolates out of all isolates tested increased from 59 in 2000 (1.3%) to 1068 in 2010 (35.9%).
- Between 2006 and 2010, there was an increasing proportion of isolates that were classified as Chromosomal Mediated Resistant *Neisseria gonorrhoeae*, while the plasmid-mediated resistant strains occurred at relatively low rates.
- One of the challenges faced by the laboratories that perform surveillance of antimicrobial resistance of *N. gonorrhoeae* is the shift from the use of cultures (required for antimicrobial susceptibility testing) to the Nucleic Acid Amplification Test (NAAT) for the diagnosis of gonorrhea
- In 2012, a sentinel public health practice and surveillance pilot study for *N. gonorrhoeae* is being proposed in Canada to collect integrated practice, epidemiological and laboratory information. The objectives of the pilot study are to determine the trends and characteristics of antimicrobial resistance in *N. gonorrhoeae* and the treatment failure rate associated with regimens recommended by the Canadian STI Guidelines. Both antimicrobial susceptible and resistant strains of gonorrhea will be characterized in order to understand the pattern of spread of strains in various populations in Canada to inform Canadian STI management guidelines.

Introduction

Neisseria gonorrhoeae, the causative agent of gonorrhea is the second most commonly reported bacterial sexually transmitted infection in Canada with approximately 11,000 cases reported in 2010 (1). Reported rates of gonorrhea have almost doubled from 14.9 cases per 100,000 population in 1997 to 33.4 cases per 100,000 population (projected) in 2010 (1). Although reported cases continue to increase, the number of available cultures, required for antimicrobial susceptibility testing, is on the decline due to the shift from the use of culture to Nucleic Acid Amplification Test (NAAT) for the diagnosis of gonorrhea (Figure 1). The treatment and control of gonorrhea is complicated by the ability of *N. gonorrhoeae* to evolve and develop resistance to many of the antibiotics used to treat it including penicillins, tetracyclines, macrolides and quinolones (2,3). The National Microbiology Laboratory (NML) has been monitoring the antimicrobial susceptibilities of *N. gonorrhoeae* since 1985 and these results contribute to the Canadian Guidelines on Sexually Transmitted Infections for the treatment of gonorrhea.

Methods

N. gonorrhoeae strains detected via culture were submitted to the NML from sexually transmitted infection clinics and provincial public health laboratories for antimicrobial susceptibility testing (Table 1). These data are provided to indicate the overall submission rate for resistance testing from the different provinces across Canada and the overall percentage of isolates resistant to at least one antibiotic.

Isolates are submitted to NML when the provincial laboratories identify resistance to at least one antibiotic or if the provincial laboratories do not do any antimicrobial susceptibility testing. Submission of isolates is voluntary and not standardized across the country. The overall interpretation of the results is difficult due to the limitations related to the isolates available for testing. Therefore, the total number of isolates cultured in all provinces was used as the denominator to calculate resistance proportion. To standardize the susceptibility testing between laboratories, proficiency surveys were conducted twice annually. Minimum inhibitory concentration, MIC (the minimum concentration of antibiotic which will inhibit the growth of the organism) was performed using agar dilution and interpretations were based on the criteria of the Clinical Laboratory Standards Institute (Table 2). Resistance characterization definitions are provided in Table 3. All isolates were also characterized by auxotyping, plasmid profiles analysis, production of β-lactamase and presence of *tetM* determinant.

N. gonorrhoeae isolates were also analyzed by molecular genotyping using the *N. gonorrhoeae* multiantigen sequence type (NG-MAST) method (4) that incorporates the amplification of the porin gene (*por*) and the transferrin-binding protein gene (*tbpB*). DNA sequences of both strands were edited, assembled and compared using DNAStar, Inc software. The resulting sequences were submitted to the NG-MAST website (http://www.ng-mast.net/) to determine the sequence types (ST).

Table 1. Number of *Neisseria gonorrhoeae* culture isolates tested at the NML from each province, 2006-2010

Province	2006	2007	2008	2009	2010	Total
British Columbia	86	95	104	183	256	724
Alberta	96	189	55	91	166	597
Saskatchewan	39	34	1	30	11	115
Manitoba	53	2	3	3	11	72
Ontario	855	705	539	383	383	2865
Québec	392	391	230	216	338	1567
New Brunswick	2	8	5	4	9	28
Nova Scotia	0	0	0	2	69	71
Newfoundland	9	14	10	2	7	42
Total isolates tested at NML	1532	1438	947	914	1250	6081
Total viable isolates available for testing	1528	1432	947	913	1233	6053
Total isolates resistant to at least one antibiotic	1472	1395	929	873	1138	5807
Total number of isolates tested in all provinces*	4201	4275	3907	3106	2970	18459
Percentage of isolates resistant to at least one antibiotic	35.0%	32.6%	23.8%	28.1%	38.3%	31.4%
Percentage of total cases tested	37.1%	36.0%	30.7%	27.8%	27.0%**	31.7%
Total cases reported in Canada	**11334**	**11873**	**12723**	**11178**	**11000****	**58108**

* Total number of isolates tested by the provincial laboratories is used as the denominator in all % resistance calculations

** Number of cases reported in 2010 and percentage of total cases tested is considered preliminary data

Table 2. *Neisseria gonorrhoeae* Antimicrobial Resistance Criteria

MIC Interpretative standards used to determine the ranges of the antibiotic concentrations in media for testing *N. gonorrhoeae* as recommended by the Clinical and Laboratory Standards Institute (5) except for erythromycin (6) and azithromycin (7, 8).

Antibiotic	Recommended Testing Concentration Ranges (mg/L)	MIC Interpretive Standard (mg/L)*				Sources of Antibiotics
		S	NS	I	R	
Penicillin	0.004 – 128.0	≤ 0.06		0.12- 1.0	≥ 2.0	Sigma #P 7794
Tetracycline	0.064 – 64.0	≤ 0.25		0.5 - 1.0	≥ 2.0	Sigma #T 3383
Erythromycin	0.032 – 32.0	≤ 1.0			≥ 2.0	Sigma #E 5389
Spectinomycin	4.0 – 256.0	≤ 32.0		64	≥ 128.0	Sigma #S 9007
Ciprofloxacin	0.001 – 64.0	≤ 0.06		0.12 - 0.5	≥ 1.0	Bayer Health Care
Ceftriaxone	0.000125 – 2.0	≤ 0.25	≥ 0.5			Sigma #C 5793
Cefixime	0.00025 – 2.0	≤ 0.25	≥ 0.5			Wyeth - Ayerst
Azithromycin	0.016 – 32.0	≤ 1.0			≥ 2.0	Pfizer

*S= Susceptible, I=Intermediate, R= Resistant, NS= Non-susceptible

Table 3. *Neisseria gonorrhoeae* Antimicrobial Resistance Characterization Definitions

Characterization		Definition
PPNG	Penicillinase Producing *Neisseria gonorrhoeae*	Pen MIC ≥ 2.0 mg/L, β-lactamase positive, β-lactamase plasmid (3.05, 3.2 or 4.5 Mdal plasmid)
TRNG	Tetracycline Resistant *Neisseria gonorrhoeae*	Tet MIC ≥ 16.0 mg/L, 25.2 Mdal plasmid, TetM PCR positive
CMRNG	Chromosomal Mediated Resistant *Neisseria gonorrhoeae*	Pen MIC ≥ 2.0 mg/L, Tet MIC ≥ 2.0 mg/L but ≤ 8.0 mg/L, and Ery MIC ≥ 2.0 mg/L
Probable CMRNG	Probable Chromosomal Mediated Resistant *Neisseria gonorrhoeae*	One of the MIC values of Pen, Tet, Ery = 1 mg/L, the other two ≥ 2.0 mg/L
PenR	Penicillin Resistant *Neisseria gonorrhoeae*	Pen MIC ≥ 2.0 mg/L, β-lactamase negative
TetR	Tetracycline Resistant *Neisseria gonorrhoeae*	Tet MIC ≥ 2.0 mg/L but ≤ 8.0 mg/L
EryR	Erythromycin Resistant *Neisseria gonorrhoeae*	Ery MIC ≥ 2.0 mg/L
CipR	Ciprofloxacin Resistant *Neisseria gonorrhoeae*	Cip MIC ≥ 1.0 mg/L
AzR	Azithromycin Resistant *Neisseria gonorrhoeae*	Az MIC ≥ 2.0 mg/L
SpecR	Spectinomycin Resistant *Neisseria gonorrhoeae*	Spec R ≥ 128 mg/L
CxNS	Ceftriaxone Non-susceptible *Neisseria gonorrhoeae*	Cx MIC ≥ 0.5 mg/L
CeNS	Cefixime Non-susceptible *Neisseria gonorrhoeae*	Ce MIC ≥ 0.5 mg/L

Results and Discussion

Of the 6,053 viable isolates tested at the NML between 2006 and 2010, 5,807 isolates (95.9%) were found to be resistant to at least one of the following antibiotics: penicillin, tetracycline, ciprofloxacin, azithromycin, and erythromycin. A total of 246 (4.1%) isolates were found to be susceptible to all of these antibiotics. In 2010, 38.3% (1,138 of 2,970) of all *N. gonorrhoeae* isolates tested in each jurisdiction across Canada were found to be resistant to at least one antibiotic (Figure 1). The characterization of each resistant *N. gonorrhoeae* isolate is provided in Table 4.

Figure 1. *Neisseria gonorrhoeae* Isolates in Canada, 2006 to 2010

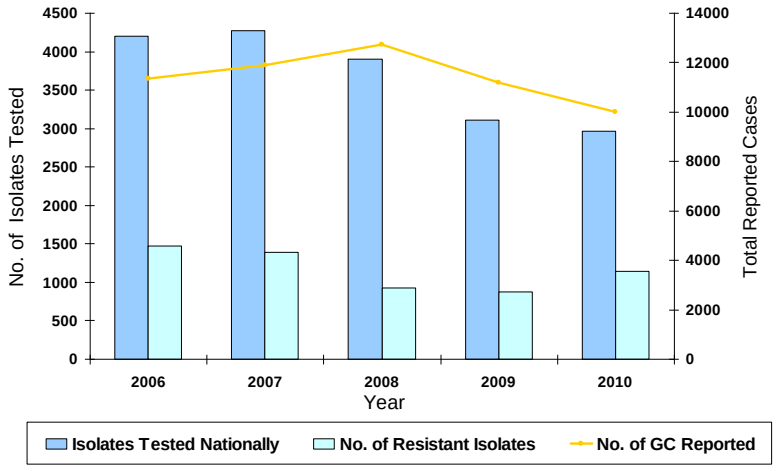
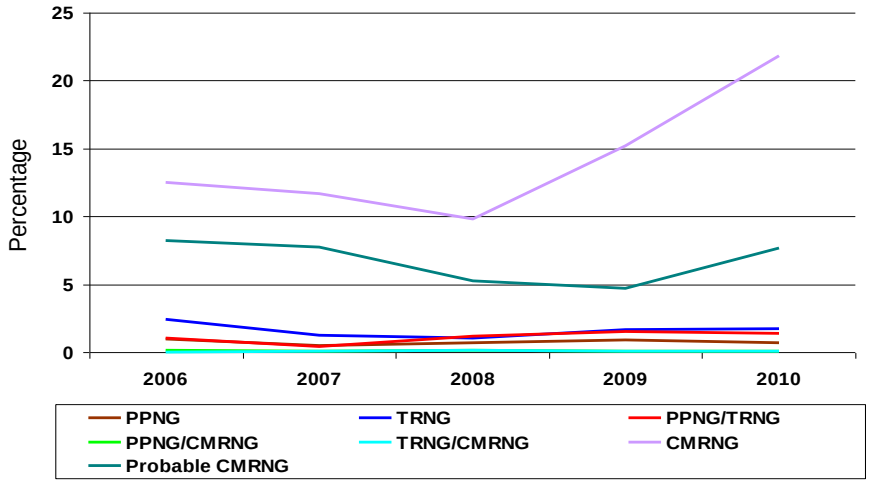


Table 4. Characterization of all *Neisseria gonorrhoeae* isolates submitted to National Microbiology Laboratory, 2006-2010

Characterization		2006	2007	2008	2009	2010	Totals
Plasmid Mediated Resistances	PPNG	26	12	10	8	7	**63**
	PPNG/CipR	3	3	13	11	10	**40**
	PPNG/EryR	0	1	3	2	1	**7**
	PPNG/EryR/CipR	0	0	0	0	1	**1**
	PPNG/TetR	10	6	1	3	1	**21**
	PPNG/TetR/CipR	2	0	0	2	1	**5**
	PPNG/CMRNG	5	3	0	0	0	**8**
	PPNG/CMRNG/CipR	0	0	5	3	3	**11**
	PPNG/TRNG	10	4	10	11	0	**35**
	PPNG/TRNG/CipR	31	9	31	33	32	**136**
	PPNG/TRNG/EryR	0	0	0	1	0	**1**
	PPNG/TRNG/CipR/EryR	4	4	4	4	9	**25**
	PPNG/TRNG/AziR/CipR/EryR	0	1	0	2	1	**4**
	TRNG	84	37	29	28	12	**190**
	TRNG/CipR	12	11	8	12	22	**65**
	TRNG/CipR/EryR	1	3	3	4	11	**22**
	TRNG/EryR	5	0	0	5	6	**16**
	TRNG/CipR/PenR	0	1	1	0	0	**2**
	TRNG/CMRNG/CipR	0	2	5	2	2	**11**
	Total	**193**	**97**	**123**	**131**	**119**	**663**
Chromosomal Mediated Resistances	AziR/EryR	3	0	0	0	21	**24**
	AziR/EryR/TetR	0	2	0	1	2	**5**
	AziR/CipR/EryR	0	1	0	0	0	**1**
	CeNS/CipR	0	1	0	0	0	**1**
	CeNS/CipR/EryR	0	0	1	0	0	**1**
	CipR	163	161	87	40	55	**506**
	CipR/EryR	54	214	62	37	11	**378**
	CipR/EryR/TetR	22	28	8	8	15	**81**
	CipR/PenR	6	0	2	0	0	**8**
	CipR/TetR	100	34	48	26	27	**235**
	EryR	7	2	0	1	0	**10**
	EryR/TetR	16	0	0	0	3	**19**
	PenR	2	0	1	1	0	**4**
	PenR/TetR	0	3	2	0	0	**5**
	TetR	37	23	9	9	9	**87**
	CMRNG	6	3	3	3	3	**18**
	CMRNG/AziR/CipR	2	2	4	8	10	**26**
	CMRNG/CipR	514	494	375	462	634	**2,479**
	CMRNG/AziR	3	0	0	0	1	**4**
	Probable CMRNG	24	11	5	6	4	**50**
	Probable CMRNG/AziR/CipR	0	1	1	0	2	**4**
	Probable CMRNG/CipR	320	318	198	140	221	**1,197**
	Susceptible Strain	56	37	18	40	96	**246**
	Total	**1,335**	**1,335**	**824**	**782**	**1,114**	**5,390**
	Contaminated or No Growth	**4**	**6**	**0**	**1**	**12**	**23**
Total		**1,532**	**1,438**	**947**	**914**	**1,245**	**6,076**

Between 2006 and 2010, there was an increasing proportion of *N. gonorrhoeae* isolates that were classified as CMRNG, while the plasmid-mediated resistance strains (PPNG, TRNG, and PPNG/TRNG) occurred at relatively low rates, shown in Figure 2. The rate of CMRNG increased from 12.5% in 2006 (525 of 4,201 isolates) to 21.8% by 2010 (648 of 2,970 isolates) and 7.6% of isolates were characterized as Probable CMRNG. During the same time period, the rate of PPNG isolates remained stable at 0.9% (41 of 4,201 isolates) to 0.7% (21 of 2,970 isolates). The TRNG isolates slightly decreased from 2.4% (102 of 4,201 isolates) in 2006 to 1.7% (51 of 2,970 isolates) in 2010 (Figure 2).

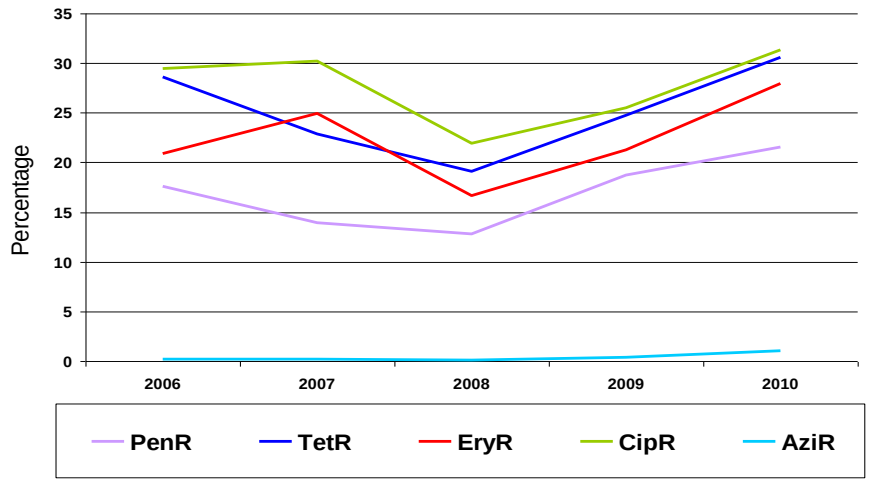
Figure 2. Trends of PPNG, TRNG, CMRNG, Probable CMRNG *Neisseria gonorrhoeae* in Canada from 2006 to 2010



Percentage based on total number of isolates tested nationally: 2006=4201; 2007=4275; 2008=3907; 2009=3106; 2010=2970

Erythromycin resistance in *N. gonorrhoeae* continues to rise. In 2006, only 20.9% (879 of 3,619 isolates) were found to be erythromycin resistant increasing to 31.5% (936 of 2,970 isolates) by 2010. Of the 936 erythromycin resistant isolates identified during in 2009, all were also resistant to at least one other antibiotic. Strains with higher MICs to erythromycin also have higher MICs to azithromycin. Penicillin resistance experienced an increase in levels between 2006 and 2010 from 17.6% (739 of 4,201 isolates) to 25.1% (744 of 2,970 isolates). Tetracycline resistance increased between 2006 and 2010 from 28.6% (1,201 of 4,201 isolates) to 34.6% (1,028 of 2,970 isolates) (Figure 3).

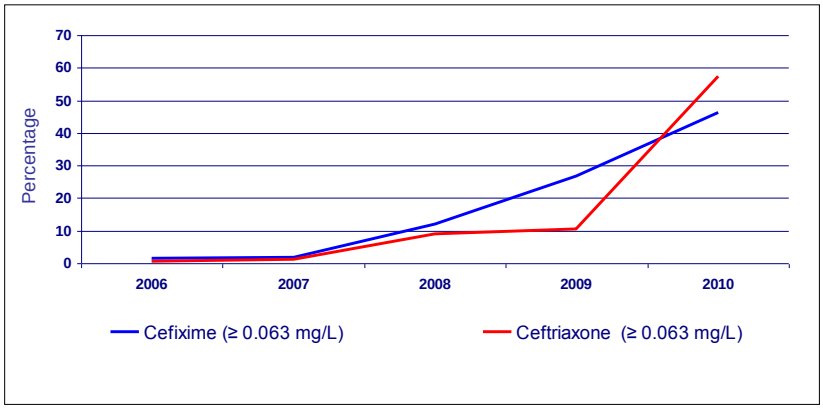
Figure 3. Antimicrobial Susceptibility of *Neisseria gonorrhoeae* Isolates Tested in Canada Between 2006-2010



Percentage based on total number of isolates tested nationally: 2006=4201; 2007=4275; 2008=3907; 2009=3106; 2010=2970

Of the 6,053 viable isolates tested at NML between 2006 and 2010, none were identified with resistance to spectinomycin or ceftriaxone. Two isolates (one each in 2007 and 2008) were identified with a cefixime MIC of 0.5 mg/L, classified as non-susceptible by CLSI guidelines. By 2010, 708 isolates with MICs approaching non-susceptibility to ceftriaxone (includes MICs ≥ 0.063 mg/L, three dilutions within the CLSI Interpretive Standard Non-Susceptibility ≥ 0.5 mg/L) and 570 to cefixime were identified (Figure 4).

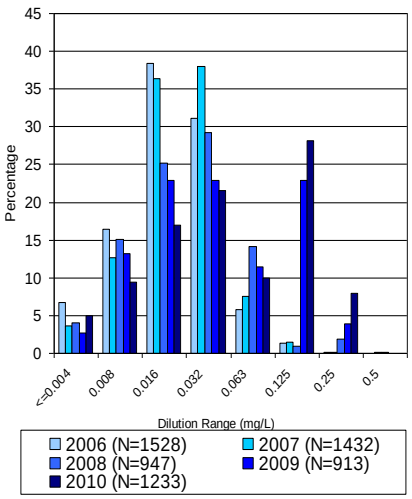
Figure 4. *Neisseria gonorrhoeae* Isolates Received by NML between 2006 and 2010 with MICs Approaching Non-Susceptibility to Cefixime and Ceftriaxone

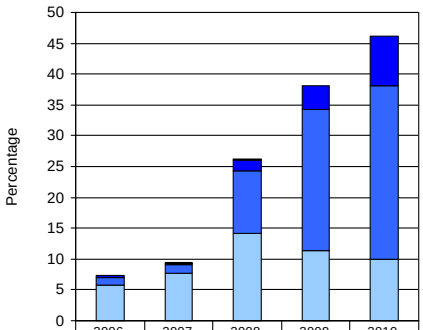


Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N). 2006, N=1525; 2007, N=1432; 2008, N=947; 2009, N=913; 2010, N=1233

The MICs of the 3rd generation cephalosporins have been increasing over time. There has been a 'right' shift in the modal MICs of cefixime from 0.016 mg/L in 2006 to 0.125 mg/L in 2010. There was also a shift in the modal MICs of ceftriaxone from 0.016/0.032 mg/L in 2006 to 0.063 mg/L in 2010 (Figures 5 and 6). Preliminary data suggest the trend towards a 'right' shift in MICs continued during 2011 (data not shown). These results indicate that the MICs of these 3rd generation cephalosporins are increasing over time.

Figure 5. Trends of Cefixime Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2006 to 2010

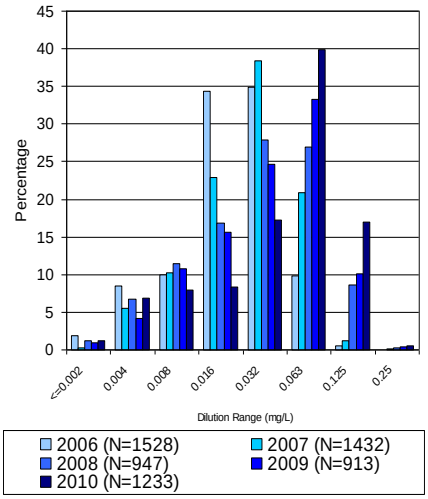


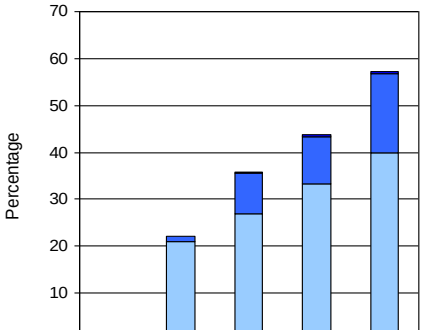


	2006	2007	2008	2009	2010
0.5 mg/L	0.00	0.07	0.11	0.00	0.00
0.25 mg/L	0.20	0.21	1.90	3.83	7.95
0.125 mg/L	1.31	1.47	10.03	22.89	28.14
0.063 mg/L	5.76	7.61	14.20	11.39	9.98
Total Isolates Tested at NML	1528	1432	947	912	1233

Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N).

Figure 6. Trends of Ceftriaxone Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2006 to 2010



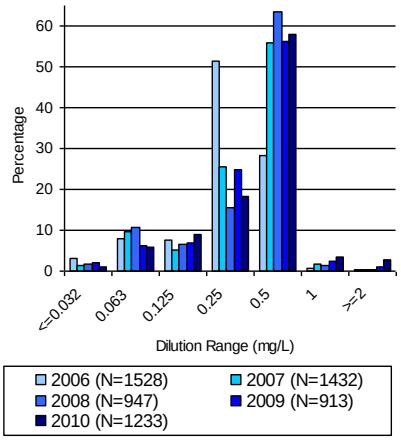


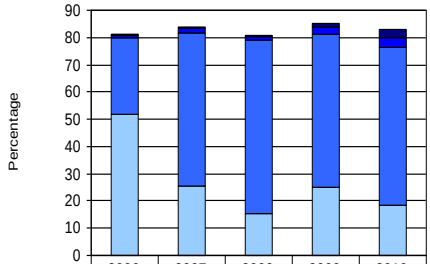
	2006	2007	2008	2009	2010
0.25 mg/L	0.00	0.07	0.30	0.44	0.57
0.125 mg/L	0.52	1.19	8.60	10.08	16.95
0.063 mg/L	0.98	20.88	26.90	33.3	39.82
Total Isolates Tested at NML	1528	1432	947	913	1233

Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N).

The mode of MICs of azithromycin has shifted from 0.25 mg/L in 2006 to 0.5 mg/L in 2010 (Figure 7). The number of azithromycin resistant *N. gonorrhoeae* isolates out of all isolates tested increased from 8 in 2006 (0.19%) to 37 in 2010 (1.25%). Overall, azithromycin resistant *N. gonorrhoeae* accounted for 0.37% (68 of 18,459) of all strains isolated between 2006 and 2010. Each of the 68 azithromycin resistant isolates is associated with resistance to at least one other antibiotic. The distribution of azithromycin resistant isolates across Canada is represented in Figure 8.

Figure 7. Trends of Azithromycin Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2006 to 2010

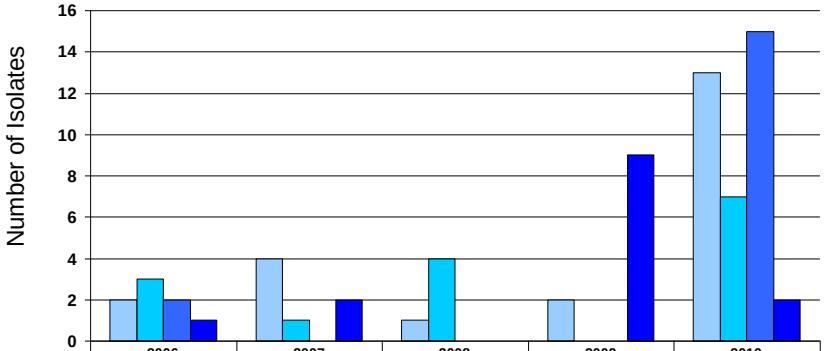




	2006	2007	2008	2009	2010
>=2 mg/L	0.52	0.49	0.53	1.20	3.00
1 mg/L	0.72	1.75	1.37	2.41	3.57
0.5 mg/L	28.42	56.15	63.57	56.52	58.15
0.25 mg/L	51.60	25.49	15.52	24.86	18.25
Total Isolates Tested at NML	1528	1432	947	913	1233

Percentages were calculated using the total number of viable strains (both resistant and susceptible isolates) tested by NML as the denominator (N).

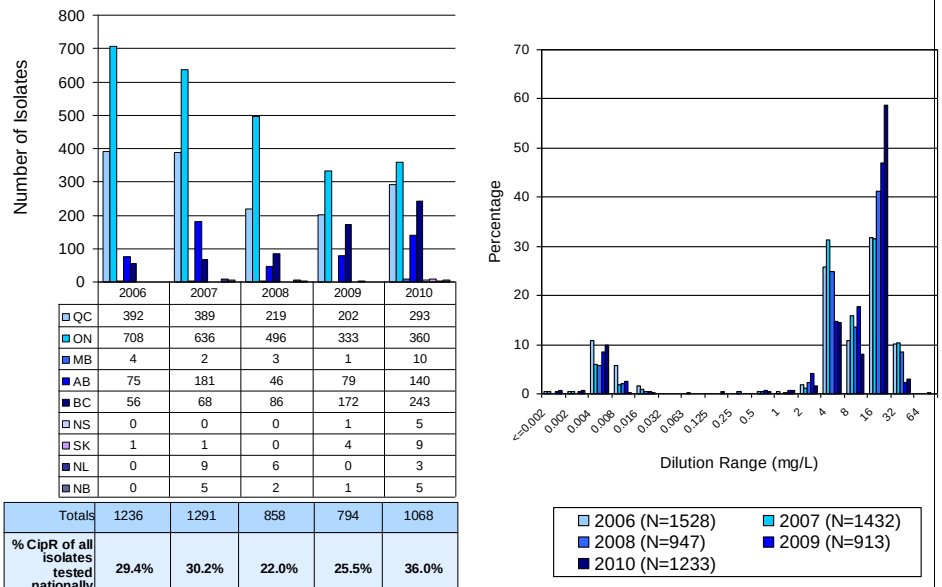
Figure 8. Geographical Distribution of Azithromycin Resistant *Neisseria gonorrhoeae* Isolates, 2006 to 2010



	2006	2007	2008	2009	2010
QC	2	4	1	2	13
ON	3	1	4	0	7
AB	2	0	0	0	15
BC	1	2	0	9	2
Total	8	7	5	11	37
% AzR of all isolates tested nationally	0.19%	0.16%	0.12%	0.35%	1.25%

Ciprofloxacin resistant *N. gonorrhoeae* accounted for 28.4% (5,247 of 18,459) of all strains isolated between 2006 and 2010. The number of isolates increased from 59 in 2000 (1.3%) to 1068 in 2010 (36.0%) and percentage rates for each province are represented in Figure 9. The mode of MICs of ciprofloxacin has shifted dramatically from 0.008 mg/L in 2004 to 4 mg/L in 2006 and now to 16.0 mg/L in 2010. Of the 1068 ciprofloxacin resistant isolates identified in 2010, 94.9% (n=1013) were also resistant to at least one other antibiotic; 704 (65.9%) were characterized as CMRNG.

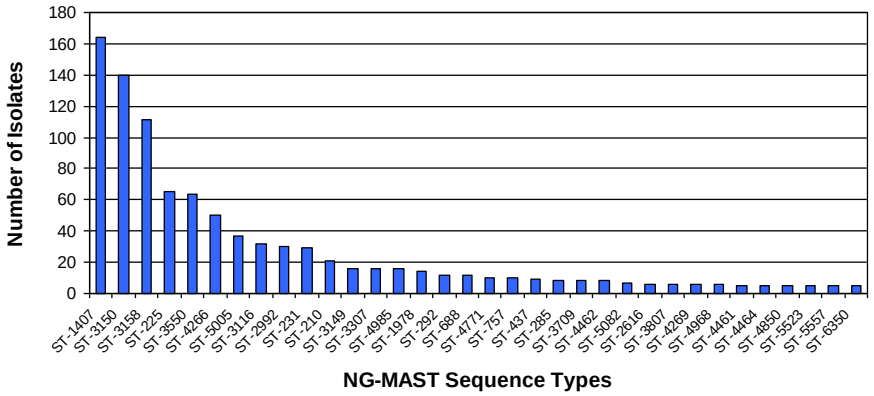
Figure 9. Geographical Distribution of Ciprofloxacin Resistant *Neisseria gonorrhoeae* Isolates, 2006 to 2010



	2006	2007	2008	2009	2010
QC	392	389	219	202	293
ON	708	636	496	333	360
MB	4	2	3	1	10
AB	75	181	46	79	140
BC	56	68	86	172	243
NS	0	0	0	1	5
SK	1	1	0	4	9
NL	0	9	6	0	3
NB	0	5	2	1	5
Totals	1236	1291	858	794	1068
% CipR of all isolates tested nationally	**29.4%**	**30.2%**	**22.0%**	**25.5%**	**36.0%**

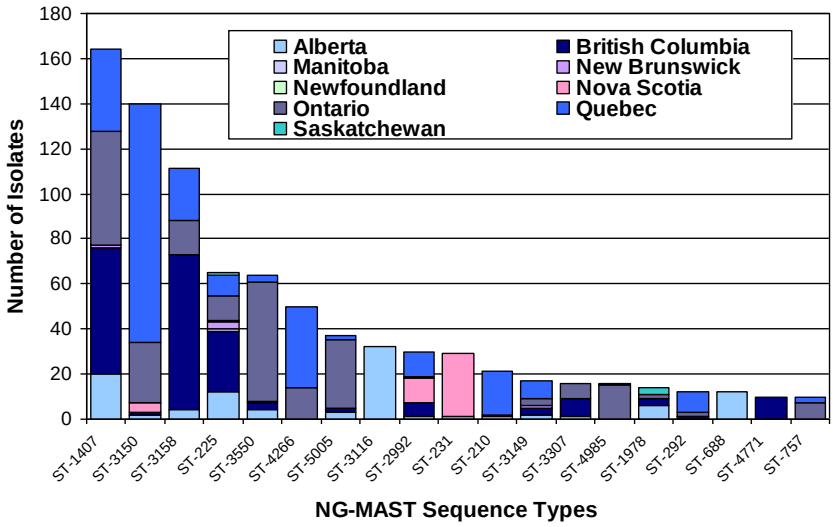
NG-MAST molecular-based sequence typing provides a substantial level of discrimination between isolates. In 2010, the most common STs were ST-1407, ST-3150 and ST-3158 at 13.3%, 11.3% and 9.0% respectively (Figure 10). These STs were also seen in previous years in Canada and are of particular interest since these STs have been identified in other countries among isolates that have decreased susceptibility MICs to 3rd generation cephalosporins. ST-1407 has been reported in England (9, 10), the USA (11), Australia (12) and Sweden (13). ST-3158, which is very closely related to ST-1407, has been reported in Australia (12) and Sweden (13). Distribution of STs within provinces is represented in Figures 11 and 12. ST-1407 was identified in 5 provinces including BC (34.1%), ON (31.1%), QC (22.0%), AB (12.2%), and NB (0.6%). ST-3150, the next most prevalent ST type, was identified primarily in QC (75.0%) followed by ON (19.3%), NS (2.8%), AB (1.4%) and BC (0.7%). The majority of ST-3158 were identified in BC (62.2%) followed by ON (13.5%), QC (20.7%) and AB (3.6%).

Figure 10. Prevalent NG-MAST Sequence Type Distribution of *Neisseria gonorrhoeae* Isolates Received by NML, 2010; N=1233



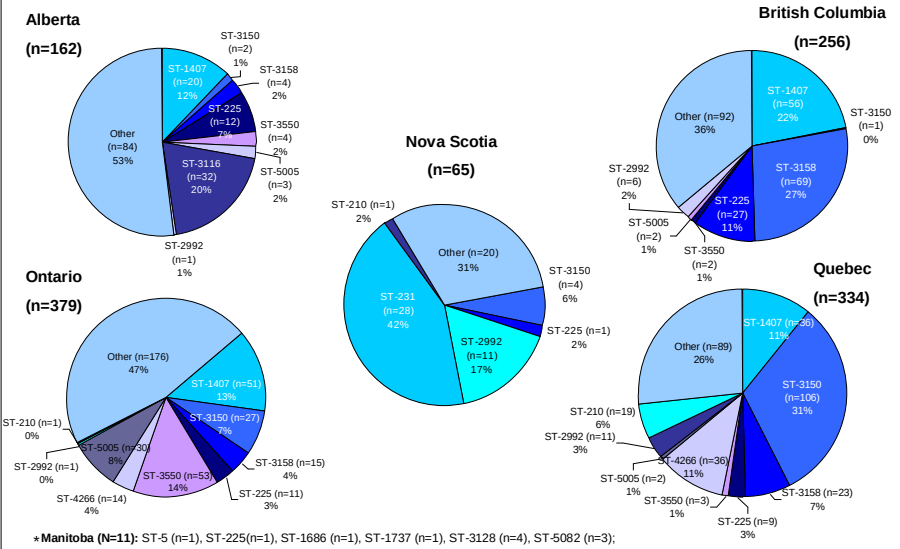
The remaining isolates (n=290) are dispersed among 215 sequence types (STs) containing 1 to 4 isolates each.

Figure 11. Distribution of *Neisseria gonorrhoeae* NG-MAST Sequence Types within Provinces, 2010; N=1233



The remaining isolates (n=384) are dispersed among 230 sequence types (STs) containing 1 to 9 isolates each.

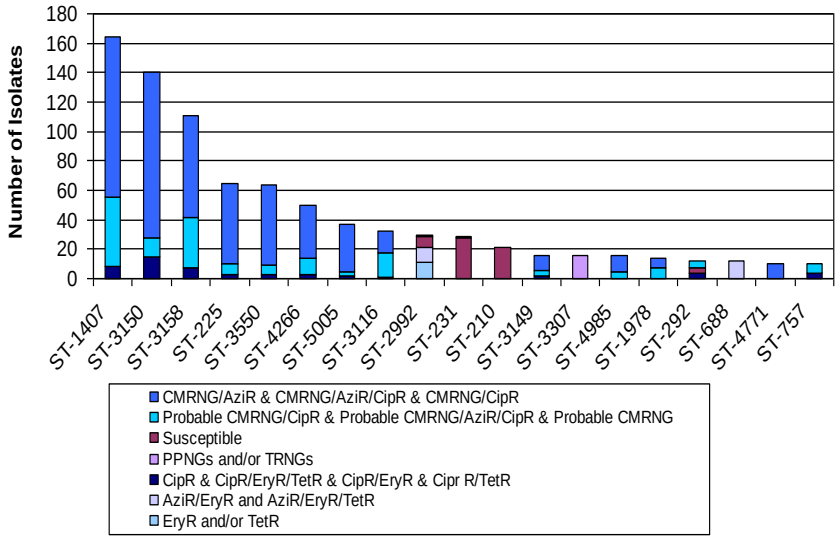
Figure 12. Distribution of *Neisseria gonorrhoeae* NG-MAST Sequence Types within Provinces, 2010; N=1233*



⋆**Manitoba (N=11):** ST-5 (n=1), ST-225(n=1), ST-1686 (n=1), ST-1737 (n=1), ST-3128 (n=4), ST-5082 (n=3);
New Brunswick (N=8): ST-225 (n=3), ST-231 (n=1), ST-1407 (n=1), ST-3556 (n=1), ST-4966 (n=2)
Newfoundland (N=7): ST-25 (n=1), ST-437 (n=2), ST-1387 (n=1), ST-3550 (n=1), ST-4987 (n=1), ST-6298 (n=1)
Saskatchewan (N=11): ST-225 (n=1), ST-1978 (n=3), ST-4637 (n=1), ST-4709 (n=3), ST-4837 (n=1), ST-4841 (n=2)

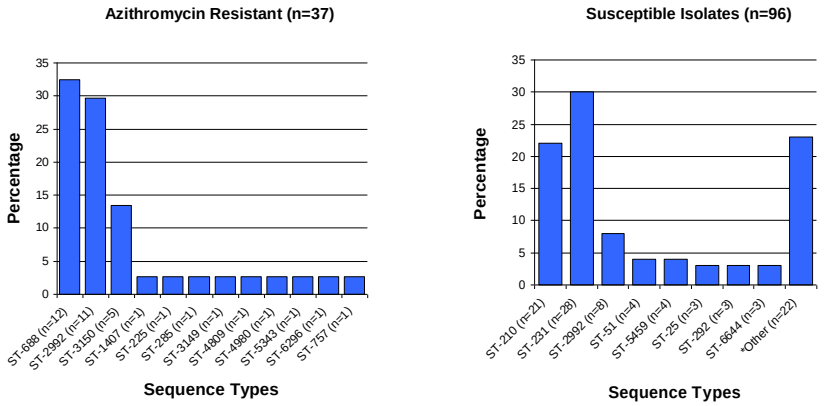
Figure 13 provides a distribution of resistance characterizations among specific ST types. The most prevalent ST types were comprised of multi-drug resistant profiles including CMRNG/Probable CMRNG along with CipR or AziR. The ST types identified among the AziR isolates are displayed in Figure 14. ST-688 is the most prevalent followed by ST-2992 and then ST-3150 at 32.4%, 29.7% and 13.5%, respectively. Of all the susceptible isolates available for testing, ST-231 (29.8%) was the most prevalent followed by ST-210 (22.3%) and then ST-2992 (8.5%) (Figure 14). The most prevalent STs of isolates with MICs approaching non-susceptibility to cefixime and ceftriaxone were ST-1407 (27.4% and 22.0%, respectively) and ST-3158 (19.1% and 14.4%, respectively) (Figure 15).

Figure 13. Distribution of Resistance Characterizations within *Neisseria gonorrhoeae* NG-MAST Sequence Types, 2010; N=849*



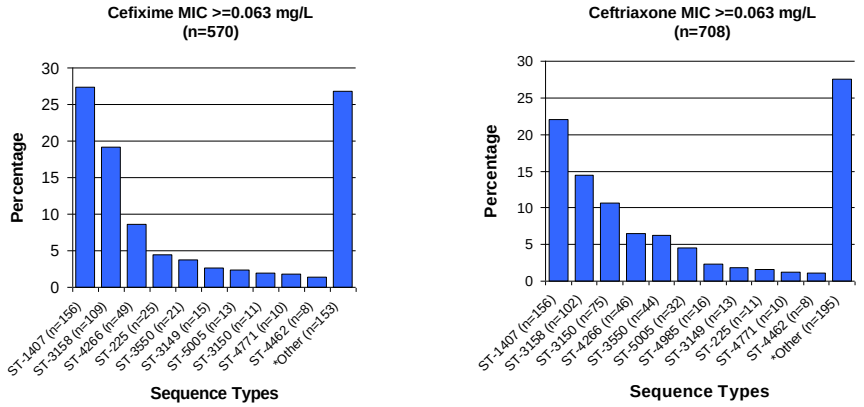
*The remaining isolates (n=384) are dispersed among 230 sequence types (STs) containing 1 to 9 isolates each and exhibit a variety of resistance/susceptibility patterns.

Figure 14. NG-MAST Sequence Types of Azithromycin Resistant Isolates (N=37) and Susceptible Isolates (N=96)



*"Other" sequence types contain 1 or 2 isolates each.

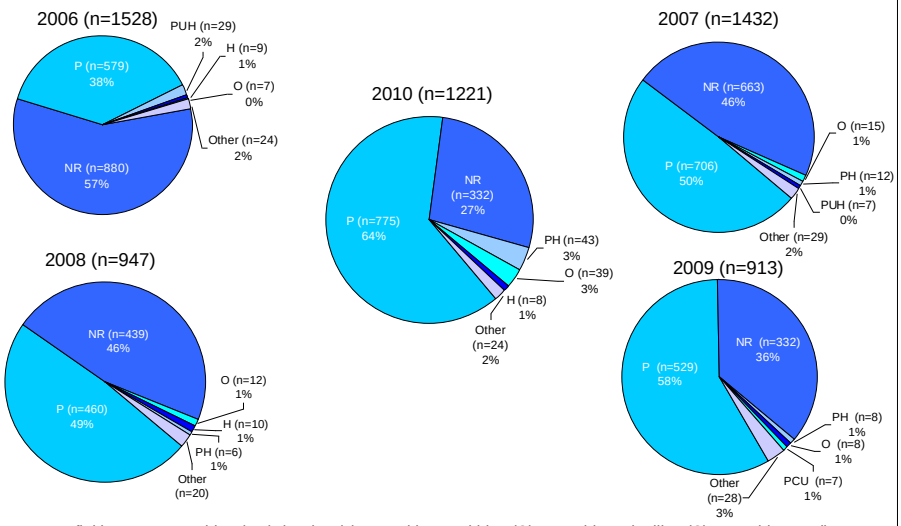
Figure 15. NG-MAST Sequence Types of *Neisseria gonorrhoeae* Isolates with MICs Approaching Non-Susceptibility (>=0.063 mg/L) to Cefixime (N=570) and Ceftriaxone (N=708)



*"Other" sequence types contain 8 isolates or less each.

Auxotypes for all isolates were also determined. Proline-requiring (P) was the most common auxotype in 2007, 2008 and 2009 at 49.3%, 48.6% and 58.1% respectively. By 2010 P-requiring isolates increased to 63.4% followed by non-requiring (NR) at 27.3%. In 2010 a variety of other auxotypes were also identified at a low frequency including proline, hypoxanthine-requiring (PH) at 3.5%, ornithine-requiring (O) at 3.2% and hypoxanthine-requiring (H) at 0.7% (Figure 16).

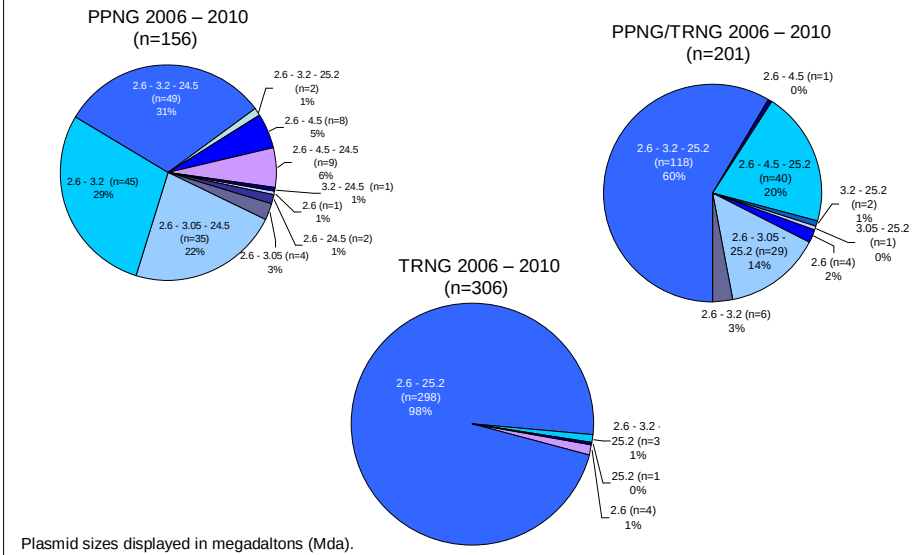
Figure 16. Auxotype Distribution of *Neisseria gonorrhoeae* Isolates Received by NML, 2006 – 2010



Auxotype Definitions: Non-requiring (NR); leucine (L) – requiring; ornithine (O) – requiring; citrulline (C) – requiring; proline (P) – requiring; arginine (A) – requiring; hypoxanthine (H) – requiring; uracil (U) - requiring and methionine (M) - requiring

Plasmid profiles for PPNG, TRNG and PPNG/TRNG isolates are shown in Figure 17. The β-lactamase gene was encoded in three different types of plasmids of sizes 3.05 megadaltons (Mda), 3.2 Mda and 4.5 Mda. The 3.2 Mda plasmid was the most common type amongst the 156 PPNG strains isolated between 2006 and 2010 at 62.2%, followed by the 3.05 Mda plasmid at 25.0% and then the 4.5 Mda plasmid at 10.9%. These plasmids co-existed with the 2.6 Mda cryptic plasmid and sometimes with the 24.5 Mda conjugal plasmid. The 3.2 Mda plasmid is also the most common β-lactamase encoding plasmid in PPNG/TRNG strains at 62.7%. The 25.2 Mda plasmid that encodes tetracycline resistance (Tet M) co-existed with the cryptic plasmids in most TRNG and PPNG/TRNG strains. Among the TRNG isolates tested between 2006 and 2010, 97.4% had the 2.6 and 25.2 Mda plasmids. TRNG isolates accounted for 76.5% of all the plasmid mediated resistance in *N. gonorrhoeae* between 2006 and 2010 (507 of 663 PPNG, PPNG/TRNG and TRNG strains).

Figure 17. Plasmid Distribution within Antimicrobial Classifications of *Neisseria gonorrhoeae* Isolates Received by NML, 2006 - 2010



Conclusion

Although penicillin and tetracycline have not been used in gonorrhea treatment for many years, the identification of the penicillin resistance phenotypes assists in monitoring the susceptibilities to the 3rd generation cephalosporins since these isolates also exhibit higher MICs of ceftriaxone and cefixime. Despite the difficulties noted in the Methods section regarding data representativeness and interpretation, continued surveillance of the antimicrobial susceptibilities and sequence types of *N. gonorrhoeae* is important to monitor changes in the characteristics and prevalence of the resistant isolate populations and their spread across the country in order to guide therapeutic recommendations. These surveillance data will be utilized in the current and future iterations of the Canadian treatment guidelines to provide the most effective information for treatment of *N. gonorrhoeae* and to reduce the spread and rate of resistance of these organisms. This surveillance is particularly important as molecular testing becomes the most commonly used method for the diagnosis of *N. gonorrhoeae* in Canada, for which susceptibility data are not available.

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