

# **National Surveillance of Antimicrobial Susceptibilities of *Neisseria gonorrhoeae***

Annual Summary 2011

**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  
Centre for Communicable Diseases and Infection Control  
Public Health Agency of Canada**

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To obtain additional copies, please contact:  
Streptococcus and STI Unit  
Bacteriology and Enteric Diseases Program  
National Microbiology Laboratory  
Canadian Science Centre for Human and Animal Health  
Public Health Agency of Canada  
1015 Arlington Street, Room H2600  
Winnipeg, Manitoba R3E 3R2  
Tel: (204) 789-6063 Fax: (204) 789-5012  
NML.StrepSTI@phac-aspc.gc.ca

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### Authorship and Lead Contributors:

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

Pam Sawatzky, Gary Liu, Michelle Boyd, Irene Martin (Unit Head)  
 Dr. Michael Mulvey (A/Director)

### Other Contributors:

**Surveillance and Epidemiology Division**  
**Centre for Communicable Diseases and Infection Control**  
**Public Health Agency of Canada**

Dr. Chris Archibald, Jane Njihia, Stephanie Totten

**Professional Guidelines and Public Health Practice Division**  
**Centre for Communicable Diseases and Infection Control**  
**Public Health Agency of Canada**

Dr. Tom Wong, Dr. Margaret Gale-Rowe

The results presented in this report represent *Neisseria gonorrhoeae* isolates kindly submitted from the following hospitals or provincial public health laboratories:

**British Columbia Centre for Disease Control**, Vancouver, British Columbia:

Dr. Linda Hoang, Ingrid Pocock, Ana Paccagnella

**Provincial Laboratory of Public Health Alberta**, Edmonton, Alberta:

Dr. Graham Tipples, Marguerite Lovgren

**Saskatchewan Disease Control Laboratory**, Regina, Saskatchewan:

Dr. Greg Horsman, Rosanne Kitkul

**Cadham Provincial Laboratory**, Winnipeg, Manitoba:

Dr. Paul Van Caessele, Sandra Giercke, Denise Sitter

**Public Health Laboratories, Public Health Ontario**, Etobicoke, Ontario:

Dr. Vanessa Allen, Prasad Rawte, Lynn Towns, Dayle Noda

**Laboratoire de santé publique du Québec**, Ste-Anne-de-Bellevue, Québec:

Dr. Michel Couillard, Dr. Brigitte Lefebvre

**Queen Elizabeth II Health Science Centre**, Halifax, Nova Scotia

Dr. David Haldane

**New Brunswick Regional Hospitals:**

Dr. Lewis Abbott, Dr. Richard Garceau

**Newfoundland Public Health Laboratory**, St. John's, Newfoundland:

Dr. L. Robberts

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## TABLE OF CONTENTS

Acknowledgements .....	3
Executive Summary.....	6
Introduction .....	7
Methods .....	7
Results and Discussion .....	10
Conclusion .....	25
Appendix .....	26
References.....	27

## FIGURES AND TABLES

Table 1. Number of <i>Neisseria gonorrhoeae</i> culture isolates tested at the NML from each province, 2007-2011 .....	8
Table 2. Demographic data for <i>Neisseria gonorrhoeae</i> isolates tested at NML, 2011 (N=1158).....	8
Table 3. Clinical isolation sites for <i>Neisseria gonorrhoeae</i> isolates tested at NML, 2011 (N=1158).....	8
Table 4. <i>Neisseria gonorrhoeae</i> Antimicrobial Resistance Criteria .....	9
Table 5. <i>Neisseria gonorrhoeae</i> Antimicrobial Resistance Characterization Definitions.....	9
Figure 1. <i>Neisseria gonorrhoeae</i> Isolates in Canada, 2007 to 2011 .....	10
Table 6. Characterization of all <i>Neisseria gonorrhoeae</i> Isolates Submitted to the NML, 2007-2011 .....	11
Figure 2. Trends of PPNG, TRNG, CMRNG, Probable CMRNG <i>Neisseria gonorrhoeae</i> in Canada from 2007-2011.....	13
Figure 3. Antimicrobial Susceptibility of <i>Neisseria gonorrhoeae</i> Isolates Tested in	

Canada between 2007-2011 .....	14
Figure 4. <i>Neisseria gonorrhoeae</i> Isolates Received by NML between 2007 and 2011 with Decreased Susceptibility to Cefixime and Ceftriaxone .....	15
Figure 5. Trends of Cefixime Susceptibilities of <i>Neisseria gonorrhoeae</i> Isolates Received by NML from 2007 to 2011 .....	16
Figure 6. Geographical Distribution of <i>Neisseria gonorrhoeae</i> Isolates with Decreased Susceptibility to Cefixime, 2007 to 2011 .....	16
Figure 7. Trends of Ceftriaxone Susceptibilities of <i>Neisseria gonorrhoeae</i> Isolates Received by NML from 2007 to 2011 .....	17
Figure 8. Geographical Distribution of <i>Neisseria gonorrhoeae</i> Isolates with Decreased Susceptibility to Ceftriaxone, 2007 to 2011 .....	17
Figure 9. Trends of Azithromycin Susceptibilities of <i>Neisseria gonorrhoeae</i> Isolates Received by NML from 2007 to 2011 .....	18
Figure 10. Geographical Distribution of Azithromycin Resistant <i>Neisseria gonorrhoeae</i> Isolates, 2007 to 2011 .....	18
Figure 11. Geographical Distribution of Ciprofloxacin Resistant <i>Neisseria gonorrhoeae</i> Isolates, 2007 to 2011 .....	19
Figure 12. Prevalent NG-MAST Sequence Type Distribution of <i>Neisseria gonorrhoeae</i> Isolates, 2011; N=1158 .....	20
Figure 13. Provincial Distribution within <i>Neisseria gonorrhoeae</i> NG-MAST Sequence Types, 2011; N=1158 .....	20
Figure 14. Distribution of <i>Neisseria gonorrhoeae</i> NG-MAST Sequence Types within Provinces, 2011; N=1158 .....	21
Figure 15. Distribution of Resistance Characterizations within <i>Neisseria gonorrhoeae</i> NG-MAST Sequence Types, 2011; N=768 .....	22
Figure 16. NG-MAST Sequence Types of Susceptible Isolates (N=13) and Azithromycin Resistant Isolates (N=83) .....	22
Figure 17. NG-MAST Sequence Types of <i>Neisseria gonorrhoeae</i> Isolates with Decreased Susceptibility to Cefixime ( $\geq 0.25$ mg/L), N=140 and Ceftriaxone ( $\geq 0.125$ mg/L), N=209 .....	23
Figure 18. Auxotype Distribution of <i>Neisseria gonorrhoeae</i> Isolates Received by NML, 2007 – 2011 .....	24
Figure 19. Plasmid Distribution within Antimicrobial Classifications of <i>Neisseria gonorrhoeae</i> Isolates Received by NML, 2007 - 2011 .....	25
Appendix Table A. <i>Neisseria gonorrhoeae</i> culture isolates in Canada, 2009 –2011 .....	26

## EXECUTIVE SUMMARY

- This report compares laboratory surveillance data for *Neisseria gonorrhoeae* isolates submitted by provincial microbiology laboratories to the National Microbiology Laboratory (NML) from 2007-2011.
- The Canadian reported rate of gonorrhea is on the rise and has more than doubled from 14.9 per 100,000 in 1997 to a projected rate of 33.1 per 100,000 in 2011.
- Antimicrobial resistance in *N. gonorrhoeae* is of foremost concern for effective treatment of gonococcal infections in the context of the global public health threat of untreatable multi-drug resistant gonorrhea. Over time, *N. gonorrhoeae* has acquired resistance to many antibiotics such as penicillin, tetracycline, erythromycin and ciprofloxacin.
- In Canada, the minimum inhibitory concentrations (MICs) of the 3rd generation cephalosporins have been increasing over time. There has been a shift in the modal MICs of ceftriaxone from 0.032 mg/L in 2007 to 0.063 mg/L in 2011. There was also a shift in the modal MICs of cefixime from 0.032 mg/L in 2007 to 0.125 mg/L in 2011.
- In 2011, 140 (4.2%) isolates were identified as having decreased susceptibility to cefixime and 209 (6.2%) isolates were identified as having decreased susceptibility to ceftriaxone according to WHO Guidelines (decreased susceptibility MICs  $\geq 0.25$  mg/L for cefixime and  $\geq 0.125$  mg/L for ceftriaxone). Two isolates (one each in 2007 and 2008) were identified with a cefixime MIC equal to 0.5 mg/L.
- The number of azithromycin resistant (MIC  $\geq 2$  mg/L) *N. gonorrhoeae* isolates increased from 7 in 2007 (0.16%) to 13 in 2011 (0.39%). In 2010 there were 37 (1.25%) azithromycin resistant isolates and 2 (0.07%) with high level resistance (MIC  $\geq 256$  mg/L) identified in Canada.
- In Canada, ciprofloxacin resistance in *N. gonorrhoeae* has increased to a level where ciprofloxacin is no longer recommended at the national level. Ciprofloxacin resistant *N. gonorrhoeae* isolates increased from 59 in 2000 (1.3%) to 1068 in 2010 (35.9%). In 2011 there were 985 (29.3%) ciprofloxacin resistant isolates.
- Between 2007 and 2011, 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.
- 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*, antimicrobial use 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; preliminary data for 2011 indicate that 11,397 cases were reported in that year (1). Preliminary rates of reported cases of gonorrhea have more than doubled from 14.9 cases per 100,000 population in 1997 to 33.1 cases per 100,000 population in 2011 (1). Although reported cases have levelled off in the last few years, 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

In 2011 a total of 1158 viable *N. gonorrhoeae* isolates were submitted from provincial public health laboratories to the NML for antimicrobial susceptibility testing as part of the passive National *Neisseria gonorrhoeae* Surveillance Program (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. In addition to the isolates, information on age and gender of the patient and anatomical site of infection were also submitted to NML (Table 2).

*N. gonorrhoeae* isolates are submitted to NML when the provincial laboratories identify resistance to at least one antibiotic or if the provincial laboratories do not perform 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 and the World Health Organization (WHO) criteria for decreased susceptibility to cephalosporins (5-8, 14) (Table 3). Resistance characterization definitions are provided in Tables 4 and 5. All isolates were also characterized by auxotyping, plasmid profiles analysis, production of  $\beta$ -lactamase and presence of *tetM* determinant.

*N. gonorrhoeae* isolates were also analyzed by molecular genotyping using the *N. gonorrhoeae* multi-antigen 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, 2007-2011**

Province	2007	2008	2009	2010	2011	Total
British Columbia	95	104	183	256	176	814
Alberta	189	55	91	166	156	657
Saskatchewan	34	1	30	11	35	111
Manitoba	2	3	3	11	12	31
Ontario	705	539	383	383	428	2438
Québec	391	230	216	338	282	1457
New Brunswick	8	5	4	9	6	32
Nova Scotia	0	0	2	69	77	148
Newfoundland	14	10	2	7	0	33
Total isolates received at NML	1438	947	914	1250	1172	5721
Total viable isolates available for testing for antimicrobial resistance	1432	947	913	1233	1158	5683
Total isolates resistant to at least one antibiotic	1395	929	873	1138	1075	5410
Total number of isolates tested in all provinces*	4275	3907	3106	2970	3360	17618
Percentage of isolates resistant to at least one antibiotic	32.6%	23.8%	28.1%	38.3%	32.0%	30.7%
Percentage of total cases tested	36.0%	30.7%	27.8%	27.0%	29.5%**	30.1%
<b>Total cases reported in Canada</b>	<b>11873</b>	<b>12723</b>	<b>11178</b>	<b>11397</b>	<b>11394**</b>	<b>58565</b>

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

\*\* Number of cases reported in 2011 and percentage of total cases tested is considered preliminary data

**Table 2. Demographic Data for the *Neisseria gonorrhoeae* isolates Tested at NML, 2011 (N=1158)**

Age	Male	Female	Not Specified	Totals
Under 15	1	6	0	7
15 - 20	85	70	0	155
21 - 25	203	83	1	287
26 - 35	284	89	1	374
36 - 45	151	33	0	184
45 - 55	88	18	1	107
56 - 65	23	7	2	32
65 +	5	1	0	6
Not Specified	2	1	3	6
<b>Total</b>	<b>842</b>	<b>308</b>	<b>8</b>	<b>1158</b>

**Table 3. Clinical Isolation Sites for the *Neisseria gonorrhoeae* Tested at NML, 2011 (N=1158)**

Isolation Site	Male	Female	Totals
Penis/Urethra	261	0	261
Rectum	78	2	80
Throat	63	17	80
Cervix	0	77	77
Vagina	0	14	14
Other	2	4	6
Not Specified	438	194	640*
<b>Total</b>	<b>842</b>	<b>308</b>	<b>1158</b>

\*gender not available for 8 isolates



**Table 4. *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) and ceftriaxone and cefixime (14).

Antibiotic	Recommended Testing Concentration Ranges (mg/L)	MIC Interpretive Standard (mg/L)*				Sources of Antibiotics
		S	DS	I	R	
<b>Penicillin</b>	0.032 – 128.0	≤ 0.06		0.12- 1.0	≥ 2.0	Sigma #P 7794
<b>Tetracycline</b>	0.064 – 64.0	≤ 0.25		0.5 - 1.0	≥ 2.0	Sigma #T 3383
<b>Erythromycin</b>	0.032 – 32.0	≤ 1.0			≥ 2.0	Sigma #E 5389
<b>Spectinomycin</b>	4.0 – 256.0	≤ 32.0		64	≥ 128.0	Sigma #S 9007
<b>Ciprofloxacin</b>	0.001 – 64.0	≤ 0.06		0.12 - 0.5	≥ 1.0	Bayer Health Care
<b>Ceftriaxone</b>	0.001 – 2.0		≥ 0.125			Sigma #C 5793
<b>Cefixime</b>	0.002 – 2.0		≥ 0.25			Wyeth - Ayerst
<b>Azithromycin</b>	0.016 – 32.0	≤ 1.0			≥ 2.0	Pfizer

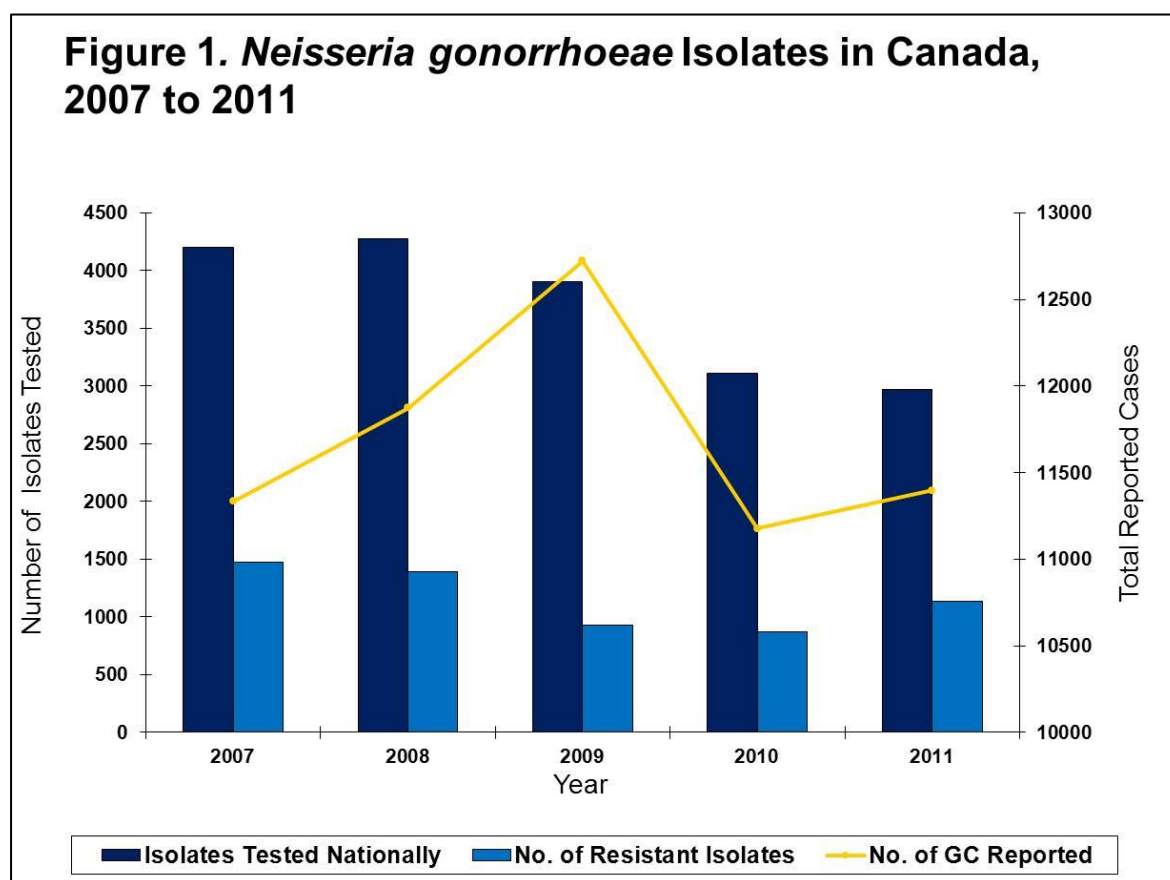
\*S= Susceptible, I=Intermediate, R= Resistant, DS= Decreased-susceptibility

**Table 5. *Neisseria gonorrhoeae* Antimicrobial Resistance Characterization Definitions**

Characterization		Definition
PPNG	Penicillinase Producing <i>Neisseria gonorrhoeae</i>	Pen MIC ≥ 2.0 mg/L, β-lactamase positive, β-lactamase plasmid (3.05, 3.2 or 4.5 Mdal plasmid)
TRNG	Tetracycline Resistant <i>Neisseria gonorrhoeae</i>	Tet MIC ≥ 16.0 mg/L, 25.2 Mdal plasmid, TetM PCR positive
CMRNG	Chromosomal Mediated Resistant <i>Neisseria gonorrhoeae</i>	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 <i>Neisseria gonorrhoeae</i>	One of the MIC values of Pen, Tet, Ery = 1 mg/L, the other two ≥ 2.0 mg/L
PenR	Penicillin Resistant <i>Neisseria gonorrhoeae</i>	Pen MIC ≥ 2.0 mg/L, β-lactamase negative
TetR	Tetracycline Resistant <i>Neisseria gonorrhoeae</i>	Tet MIC ≥ 2.0 mg/L but ≤ 8.0 mg/L
EryR	Erythromycin Resistant <i>Neisseria gonorrhoeae</i>	Ery MIC ≥ 2.0 mg/L
CipR	Ciprofloxacin Resistant <i>Neisseria gonorrhoeae</i>	Cip MIC ≥ 1.0 mg/L
AzR	Azithromycin Resistant <i>Neisseria gonorrhoeae</i>	Az MIC ≥ 2.0 mg/L
SpecR	Spectinomycin Resistant <i>Neisseria gonorrhoeae</i>	Spec R ≥ 128 mg/L
CxDS	<i>Neisseria gonorrhoeae</i> with Decreased Susceptibility to Ceftriaxone	Cx MIC ≥ 0.125 mg/L
CeDS	<i>Neisseria gonorrhoeae</i> with Decreased Susceptibility to Cefixime	Ce MIC ≥ 0.25 mg/L

## RESULTS AND DISCUSSION

Of the 5,683 viable isolates tested at the NML between 2007 and 2011, 5,410 isolates (95.2%) were found to be resistant to at least one of the following antibiotics: penicillin, tetracycline, ciprofloxacin, azithromycin, and erythromycin. A total of 274 (4.8%) isolates were found to be susceptible to all of these antibiotics. In 2011, 32.0% (1,075 of 3,360) of all *N. gonorrhoeae* isolates tested in all jurisdictions 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 6.



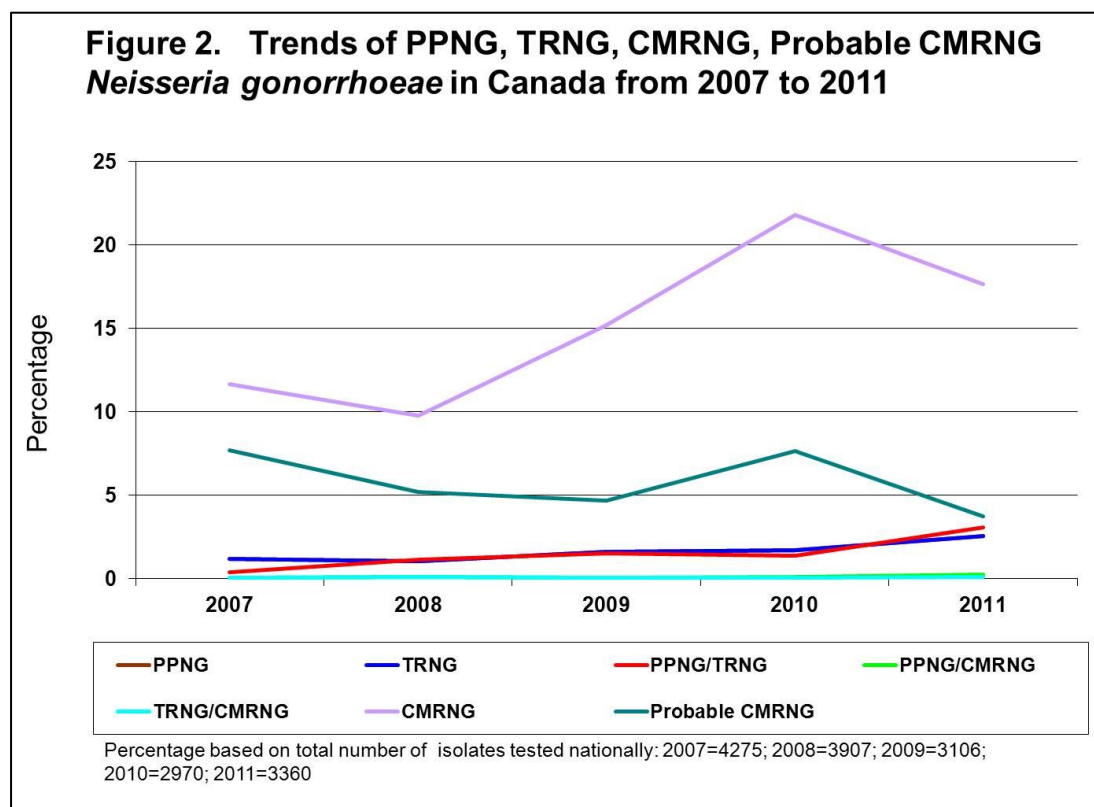
**Table 6. Characterization of all *Neisseria gonorrhoeae* Isolates Submitted to the NML, 2007-2011**

	Characterization	2007	2008	2009	2010	2011	Totals
Plasmid Mediated Resistances	PPNG	12	10	8	7	2	39
	PPNG/CipR	3	13	11	10	9	46
	PPNG/EryR	1	3	2	1	6	13
	PPNG/EryR/CipR	0	0	0	1	0	1
	PPNG/TetR	6	1	3	1	0	11
	PPNG/TetR/CipR	0	0	2	1	1	4
	PPNG/CMRNG	3	0	0	0	2	5
	PPNG/CMRNG/CipR	0	5	3	3	6	17
	PPNG/CMRNG/CxDS/CipR	0	0	0	0	1	1
	PPNG/TRNG	4	10	11	0	2	27
	PPNG/TRNG/CeDS/CipR	0	0	0	0	2	2
	PPNG/TRNG/CeDS/CipR/EryR	0	0	0	0	6	6
	PPNG/TRNG/CeDS/CxDS/CipR/EryR	0	0	0	0	3	3
	PPNG/TRNG/CipR	9	31	33	32	43	148
	PPNG/TRNG/EryR	0	0	1	0	0	1
	PPNG/TRNG/CipR/EryR	4	4	4	7	34	53
	PPNG/TRNG/CxDS/CipR/EryR	0	0	0	2	14	16
	PPNG/TRNG/AziR/CipR/EryR	1	0	1	0	0	2
	PPNG/TRNG/AziR/CipR/CxDS/EryR	0	0	1	1	0	2
	TRNG	37	29	28	12	21	127
	TRNG/CipR	11	8	12	22	15	68
	TRNG/CipR/EryR	2	2	4	11	36	55
	TRNG/CipR/EryR/CeDS	0	0	0	0	1	1
	TRNG/CipR/EryR/CeDS/CxDS	0	0	0	0	1	1
	TRNG/CipR/EryR/CxDS	1	1	0	0	8	10
	TRNG/EryR	0	0	5	6	1	12
	TRNG/CipR/PenR	1	1	0	0	1	3
	TRNG/CxDS/CipR	0	0	0	0	1	1
	TRNG/CMRNG/CipR	2	5	2	1	2	12
	TRNG/CMRNG/CxDS/CipR	0	0	0	1	2	3
	TRNG/PenR	0	0	0	0	1	1
	<b>Total 1</b>	<b>97</b>	<b>123</b>	<b>131</b>	<b>119</b>	<b>221</b>	<b>691</b>

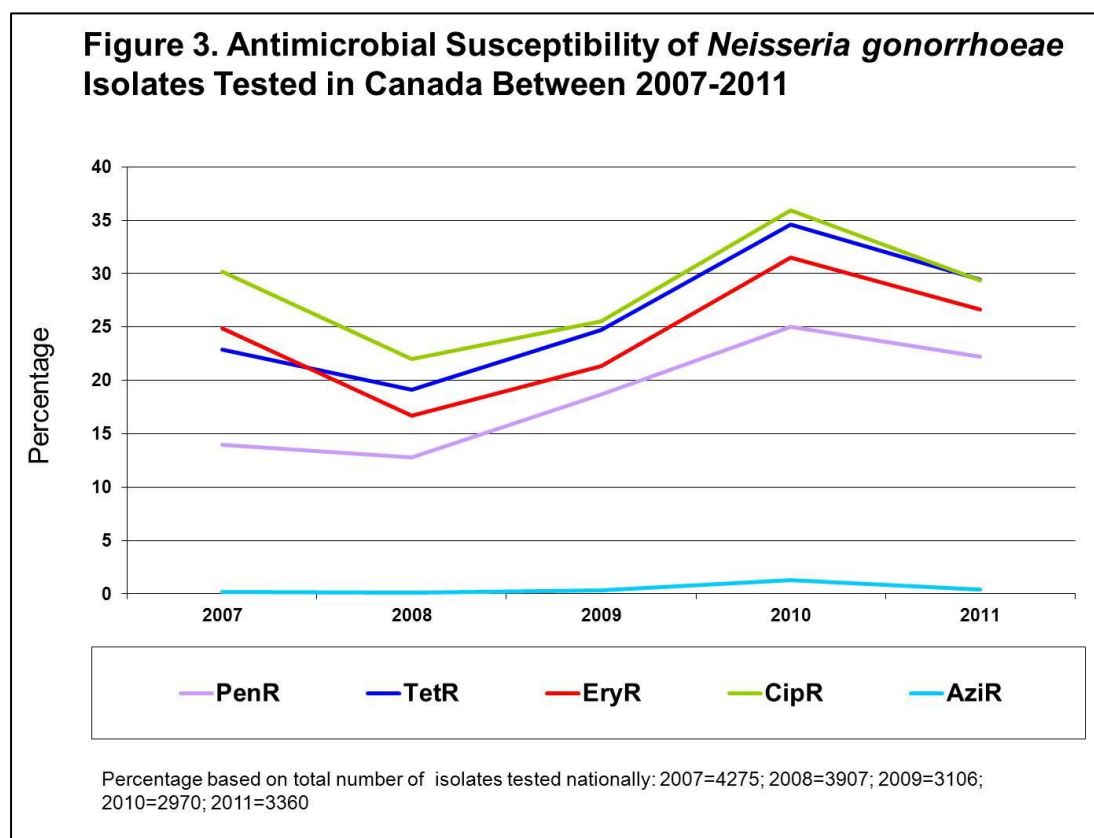
**Table 6. Characterization of all *Neisseria gonorrhoeae* isolates submitted to the NML, 2007-2011 (continued)**

	Characterization	2007	2008	2009	2010	2011	Totals
<b>Chromosomal Mediated Resistances</b>	AziR/EryR	0	0	0	21	5	26
	AziR/EryR/TetR	2	0	1	2	2	7
	AziR/CipR/EryR	1	0	0	0	0	1
	AziR/EryR/PenR	0	0	0	0	1	1
	CeDS/CipR/EryR	0	0	0	1	0	1
	CeDS/CxDS/CipR/EryR	0	1	0	0	0	1
	CeDS/CipR/PenR/TetR	0	0	0	0	1	1
	CxDS/CipR/EryR/TetR	0	0	0	0	1	1
	CipR	157	84	40	54	34	369
	CipR/CeDS	1	0	0	0	0	1
	CipR/CxDS	4	3	0	1	0	8
	CipR/EryR	214	62	37	10	16	339
	CipR/EryR/TetR	28	8	8	15	9	68
	CipR/PenR	0	2	0	0	0	2
	CipR/TetR	34	48	26	26	23	157
	CipR/TetR/CxDS	0	0	0	1	0	1
	EryR	2	0	1	0	9	12
	EryR/TetR	0	0	0	3	16	19
	PenR	0	1	1	0	0	2
	PenR/TetR	3	2	0	0	0	5
	TetR	20	9	9	9	17	64
	TetR/CxDS	1	0	0	0	0	1
	TetR/CeDS	2	0	0	0	0	2
	CMRNG	3	3	3	3	2	14
	CMRNG/AziR/CipR	2	4	8	8	5	27
	CMRNG/AziR/CipR/CxDS	0	0	0	2	0	2
	CMRNG/CipR	483	302	367	443	387	1,982
	CMRNG/AziR	0	0	0	1	0	1
	CMRNG/CeDS/CipR	0	2	3	8	32	45
	CMRNG/CeDS/CxDS/CipR	1	11	32	79	88	211
	CMRNG/CxDS/CipR	10	60	60	104	80	314
	Probable CMRNG	11	5	6	4	3	29
	Probable CMRNG/AziR/CipR	1	1	0	2	0	4
	Probable CMRNG/CipR	317	188	137	194	110	946
	Probable CMRNG/CeDS/CipR	0	2	0	0	5	7
	Probable CMRNG/CeDS/CxDS/CipR	0	3	0	10	1	14
	Probable CMRNG/CxDS/CipR	1	5	3	17	7	33
	Susceptible Strain	37	18	40	96	83	274
	<b>Total 2</b>	<b>1,335</b>	<b>824</b>	<b>782</b>	<b>1,114</b>	<b>937</b>	<b>4,992</b>
	<b>Contaminated or No Growth</b>	<b>6</b>	<b>0</b>	<b>1</b>	<b>12</b>	<b>14</b>	<b>33</b>
<b>Final Total</b>		<b>1,438</b>	<b>947</b>	<b>914</b>	<b>1,245</b>	<b>1,172</b>	<b>5,716</b>

Between 2007 and 2011, 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 11.7% in 2007 (499 of 4,275 isolates) to 17.7% by 2011 (594 of 3,360 isolates) and 3.8% of isolates were characterized as Probable CMRNG. During the same time period, the rate of PPNG isolates remained stable at 0.5% (22 of 4,275 isolates) in 2007 and 0.5% (18 of 3,360 isolates) in 2011. The TRNG isolates slightly increased from 1.2% (52 of 4,275 isolates) in 2007 to 2.6% (86 of 3,360 isolates) in 2011 (Figure 2).



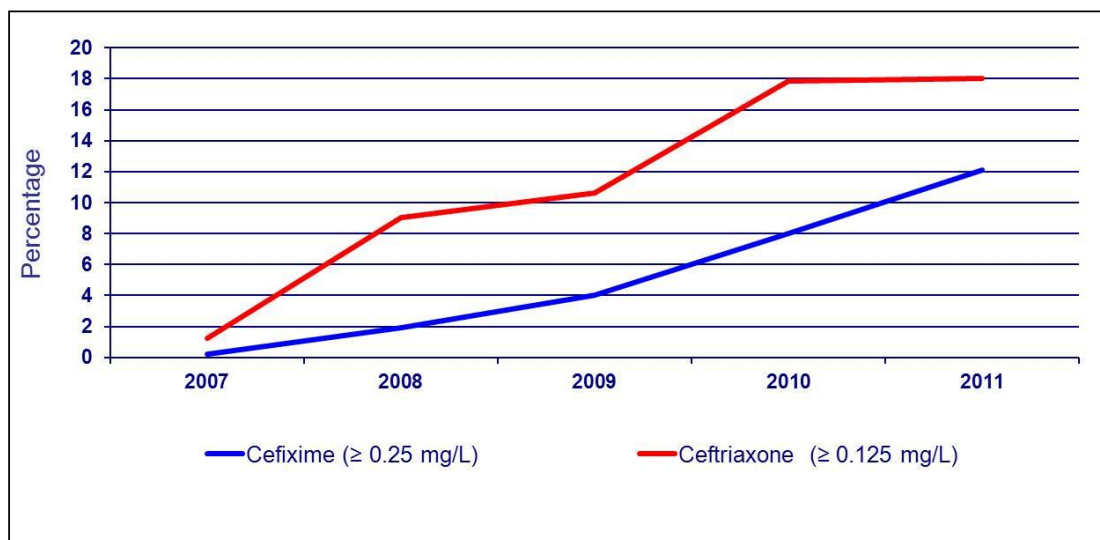
Erythromycin resistance in *N. gonorrhoeae* continues to rise. In 2007, only 24.9% (1,064 of 4,275 isolates) were found to be erythromycin resistant increasing to 26.6% (893 of 3,360 isolates) by 2011. Of the 893 erythromycin resistant isolates identified in 2011, 99.0% (884/893) were also resistant to at least one other antibiotic. Strains with higher MICs to erythromycin also have higher MICs to azithromycin. Penicillin resistance increased between 2007 and 2011 from 13.9% (596 of 4,275 isolates) to 22.2% (746 of 3,360 isolates), respectively. Tetracycline resistance increased between 2007 and 2011 from 22.9% (978 of 4,275 isolates) to 29.4% (987 of 3,360 isolates), respectively (Figure 3). Of the 5,683 viable isolates tested at NML between 2007 and 2011, none were identified with resistance to spectinomycin.



In 2011, 140 isolates were identified as having decreased susceptibility to cefixime and 209 isolates were identified as having decreased susceptibility to ceftriaxone according to WHO Guidelines (MICs  $\geq 0.25$  mg/L for cefixime and  $\geq 0.125$  mg/L for ceftriaxone) (Figure 4). Two isolates (one each in 2007 and 2008) were identified with a cefixime MIC of 0.5 mg/L, the highest MIC recorded among all Canadian isolates.



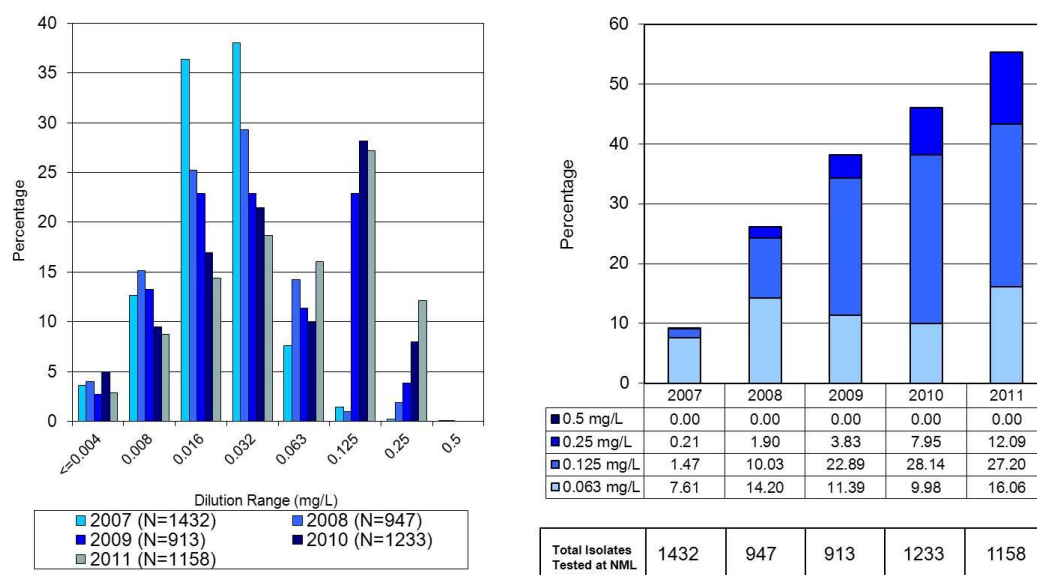
**Figure 4. *Neisseria gonorrhoeae* Isolates Received by NML between 2007 and 2011 with Decreased Susceptibility to Cefixime and Ceftriaxone**



Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N). 2007, N=1432; 2008, N=947; 2009, N=913; 2010, N=1233; 2011, N=1158

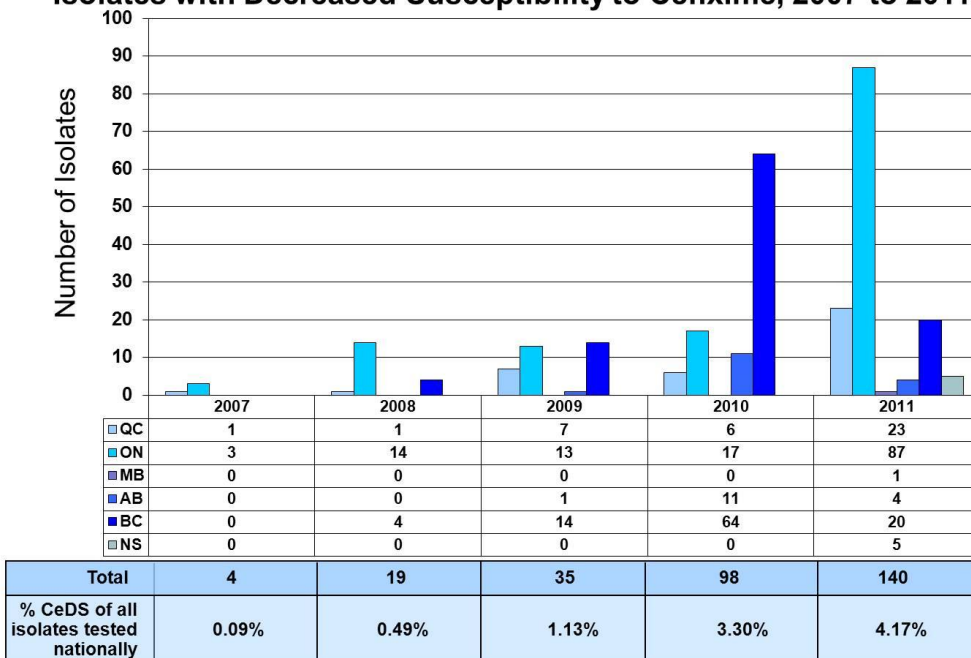
The MICs of the 3rd generation cephalosporins have been increasing over time. There has been a 'right' shift in the modal MICs of cefixime from 0.063 mg/L in 2007 to 0.125 mg/L in 2011. There was also a shift in the modal MICs of ceftriaxone from 0.032 mg/L in 2007 to 0.063 mg/L in 2011 (Figures 5 to 8).

**Figure 5. Trends of Cefixime Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2007 to 2011**



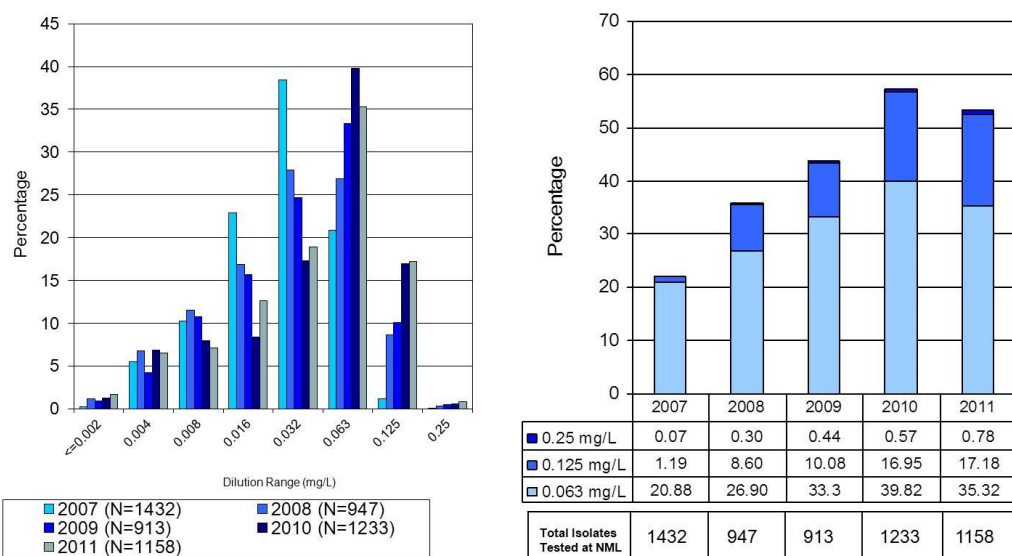
Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N).

**Figure 6. Geographical Distribution of *Neisseria gonorrhoeae* Isolates with Decreased Susceptibility to Cefixime, 2007 to 2011**

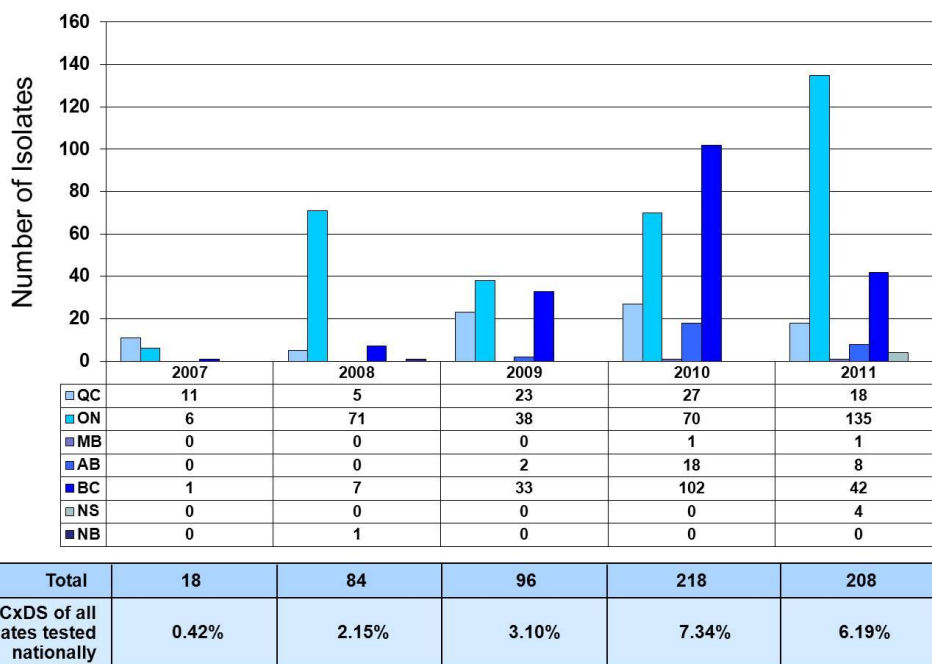


Percentage based on total number of isolates tested nationally: 2007=4275; 2008=3907; 2009=3106; 2010=2970; 2011=3360

**Figure 7. Trends of Ceftriaxone Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2007 to 2011**



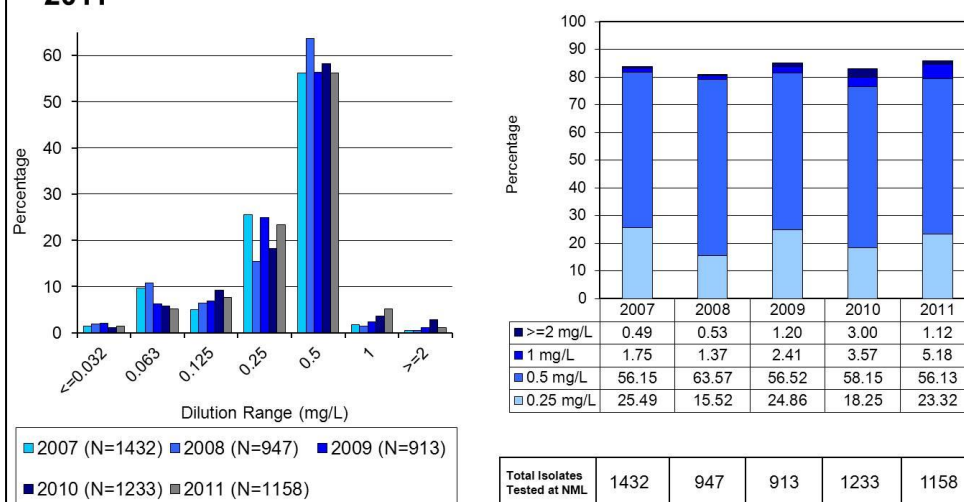
**Figure 8. Geographical Distribution of *Neisseria gonorrhoeae* Isolates with Decreased Susceptibility to Ceftriaxone, 2007 to 2011**



Percentage based on total number of isolates tested nationally: 2007=4275; 2008=3907; 2009=3106; 2010=2970; 2011=3360

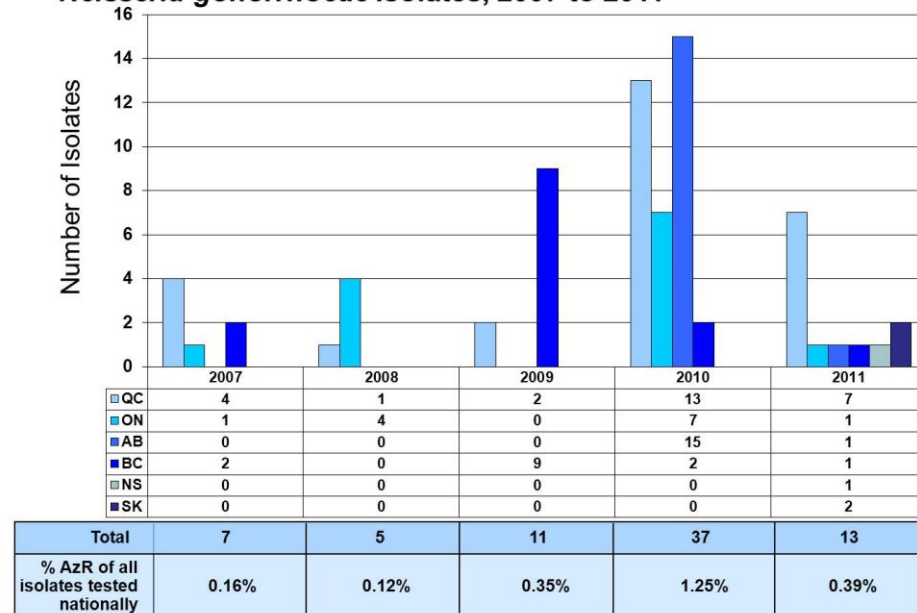
The number of azithromycin resistant *N. gonorrhoeae* isolates out of all isolates tested nationally increased from 7 in 2007 (0.16%) to 13 in 2011 (0.39%). Overall, azithromycin resistant *N. gonorrhoeae* accounted for 0.41% (73 of 17,618) of all strains isolated between 2007 and 2011 (Figure 9). Each of the 73 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 10.

**Figure 9. Trends of Azithromycin Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2007 to 2011**



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

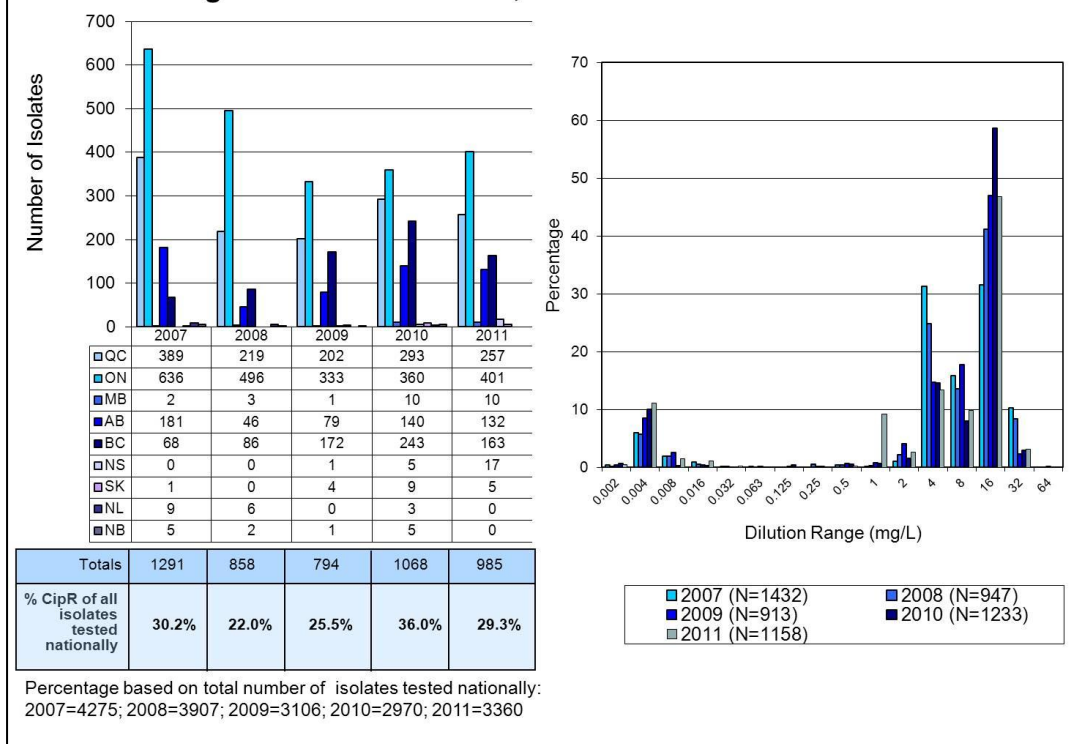
**Figure 10. Geographical Distribution of Azithromycin Resistant *Neisseria gonorrhoeae* Isolates, 2007 to 2011**



Percentage based on total number of isolates tested nationally: 2007=4275; 2008=3907; 2009=3106; 2010=2970; 2011=3360

Ciprofloxacin resistant *N. gonorrhoeae* accounted for 28.4% (4,996 of 17,618) of all strains isolated nationally between 2007 and 2011. The number of isolates increased from 59 in 2000 (1.3%) to 985 in 2011 (29.3%). Percentage rates for each province are represented in Figure 11. The mode of MICs of ciprofloxacin has shifted dramatically from 0.008 mg/L in 2004 to 4 mg/L in 2007 and now to 16.0 mg/L in 2011. Of the 985 ciprofloxacin resistant isolates identified in 2011, 96.5% (n=951) were also resistant to at least one other antibiotic; 592 (60.1%) were characterized as CMRNG.

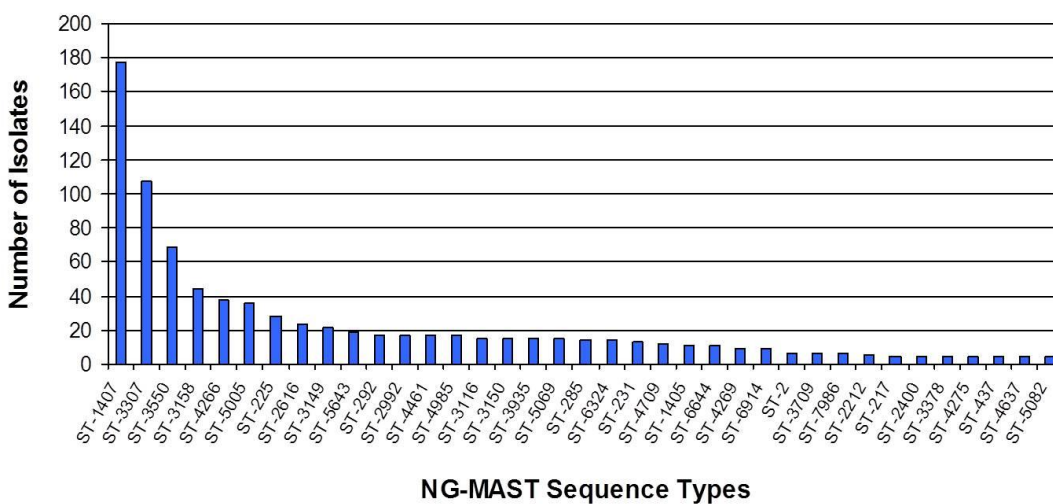
**Figure 11. Geographical Distribution of Ciprofloxacin Resistant *Neisseria gonorrhoeae* Isolates, 2007 to 2011**



NG-MAST molecular-based sequence typing provides a substantial level of discrimination between isolates. In 2011, the most common STs were ST-1407, ST-3307 and ST-3550 at 15.3%, 9.3% and 6.0%, respectively (Figure 12). These STs were also seen in previous years in Canada. ST-1407 is of particular interest since it has been reported in England among isolates that have decreased susceptibility MICs to 3<sup>rd</sup> generation cephalosporins. Distribution of STs within provinces is represented in Figures 13 and 14. ST-1407 was identified in 4 provinces including ON (39.0%), BC (32.8%), QC (15.3%) and AB (13.0%). ST-3307, the next most prevalent ST type, was identified primarily in ON (86.1%) followed by QC (10.2%), AB, BC, NS and SK (0.9% each). ST-3550 isolates were identified in QC (49.3%), ON (34.8%), AB (10.1%) and BC (5.8%).

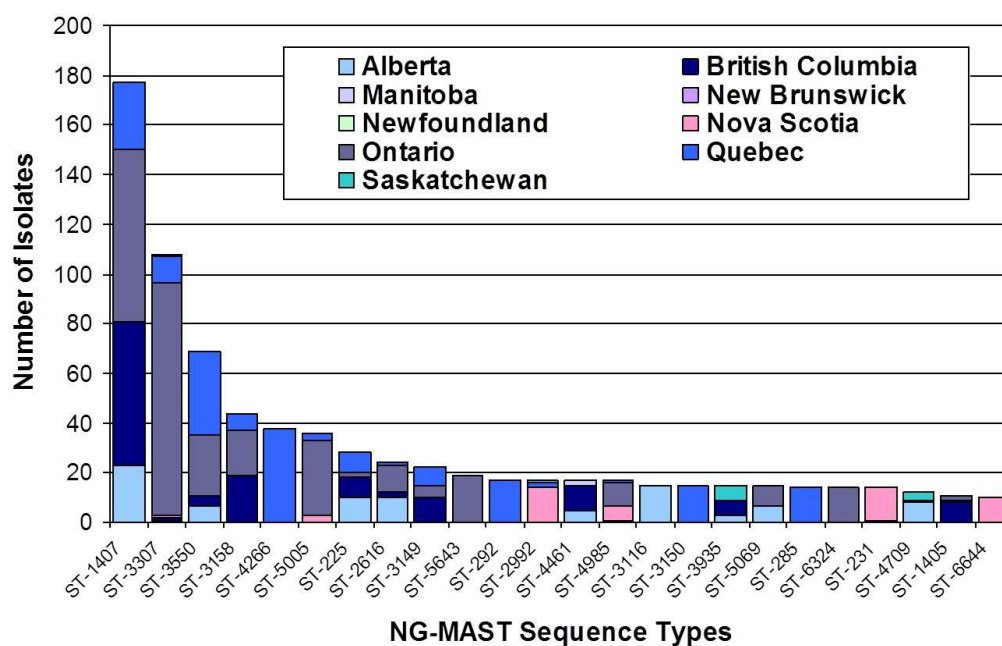


**Figure 12. Prevalent NG-MAST Sequence Type Distribution of *Neisseria gonorrhoeae* Isolates Received by NML, 2011; N=1158**



The remaining isolates (n=310) are dispersed among 212 sequence types (STs) containing 1 to 4 isolates each.

**Figure 13. Provincial Distribution within *Neisseria gonorrhoeae* NG-MAST Sequence Types, 2011; N=1158**



The remaining isolates (n=391) are dispersed among 225 sequence types (STs) containing 1 to 9 isolates each.



**Figure 14. Distribution of *Neisseria gonorrhoeae* NG-MAST Sequence Types within Provinces, 2011; N=1158\***

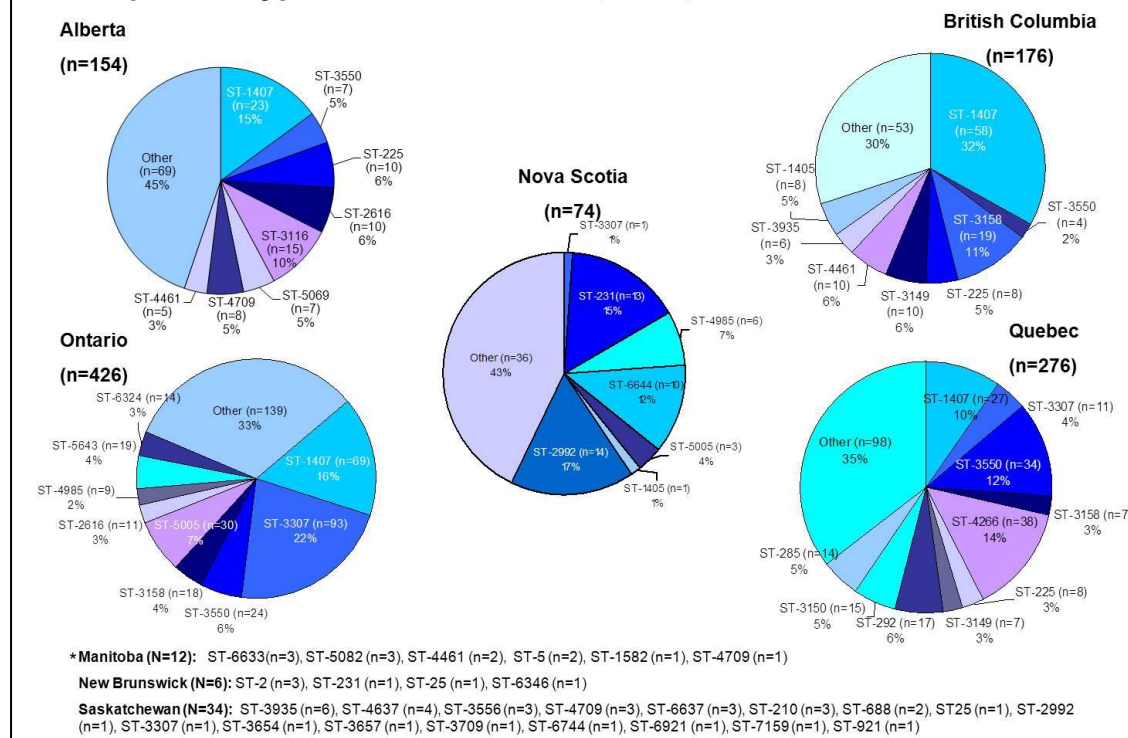
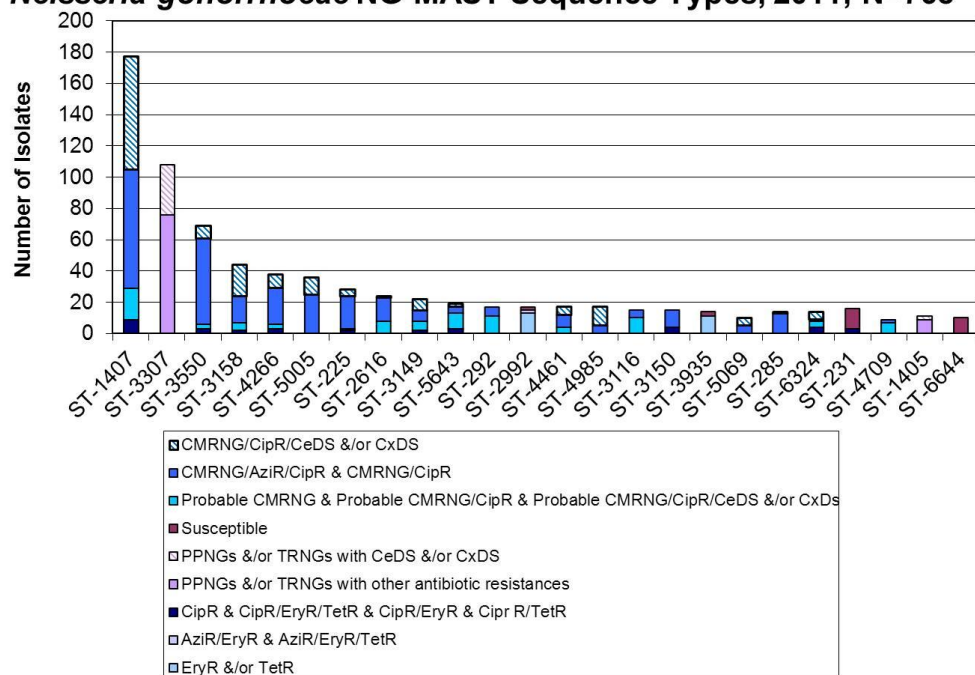


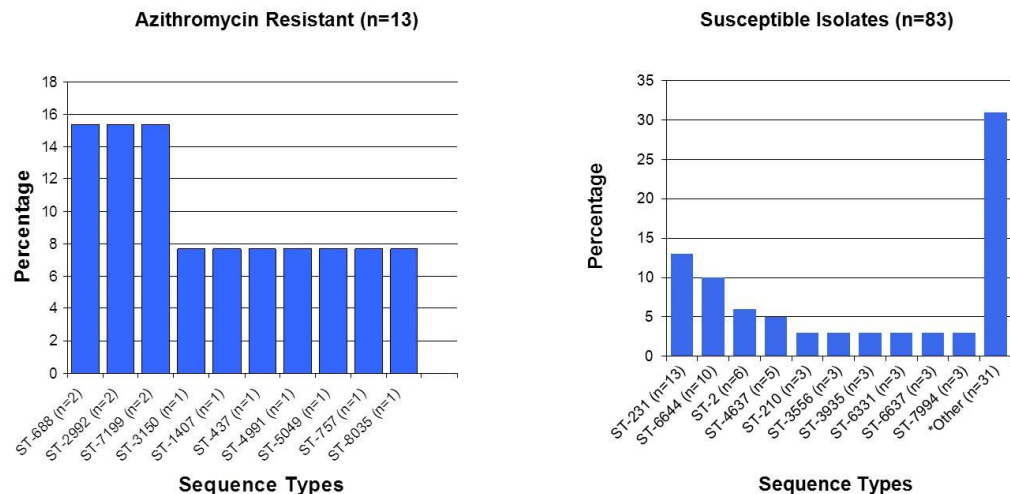
Figure 15 provides a distribution of resistance characterizations among specific ST types. ST-1407 and ST-3550 were predominantly comprised of multi-drug resistant profiles including CMRNG/Probable CMRNG along with CipR and/or CeDS and/or CxDS. ST-3307, however, consisted of only PPNGs and/or TRNGs with other antibiotic resistances including CeDS and/or CxDS. The ST types identified among the AziR isolates are displayed in Figure 16. ST-688, ST-2992 and ST-7199 (15.4% each) are the most prevalent. Of all the susceptible isolates available for testing, ST-231 (15.7%) was the most prevalent followed by ST-6644 (12.0%) and then ST-2 (7.2%), (Figure 16). The most prevalent STs of isolates with decreased susceptibility to cefixime and ceftriaxone were ST-1407 (31.4% and 30.1%, respectively) followed by ST-3158 (10.0%) and ST-3307 (8.6%) for cefixime and ST-3307 (11.5%) and ST-3158 (8.1%) for ceftriaxone (Figure 17).

**Figure 15. Distribution of Resistance Characterizations within *Neisseria gonorrhoeae* NG-MAST Sequence Types, 2011; N=768\***



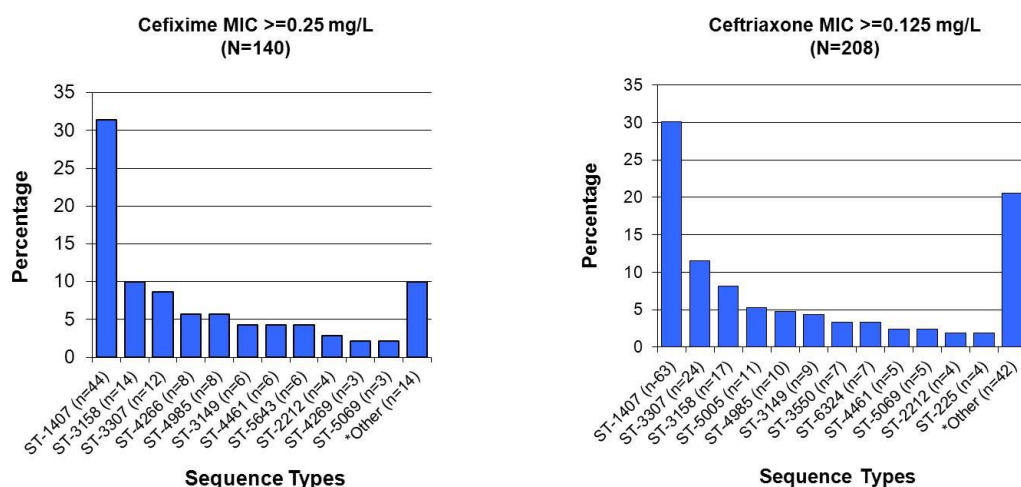
\*The remaining isolates (n=390) are dispersed among 225 sequence types (STs) containing 1 to 9 isolates each and exhibit a variety of resistance/susceptibility patterns.

**Figure 16. NG-MAST Sequence Types of Azithromycin Resistant Isolates (N=13) and Susceptible Isolates (N=83)**



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

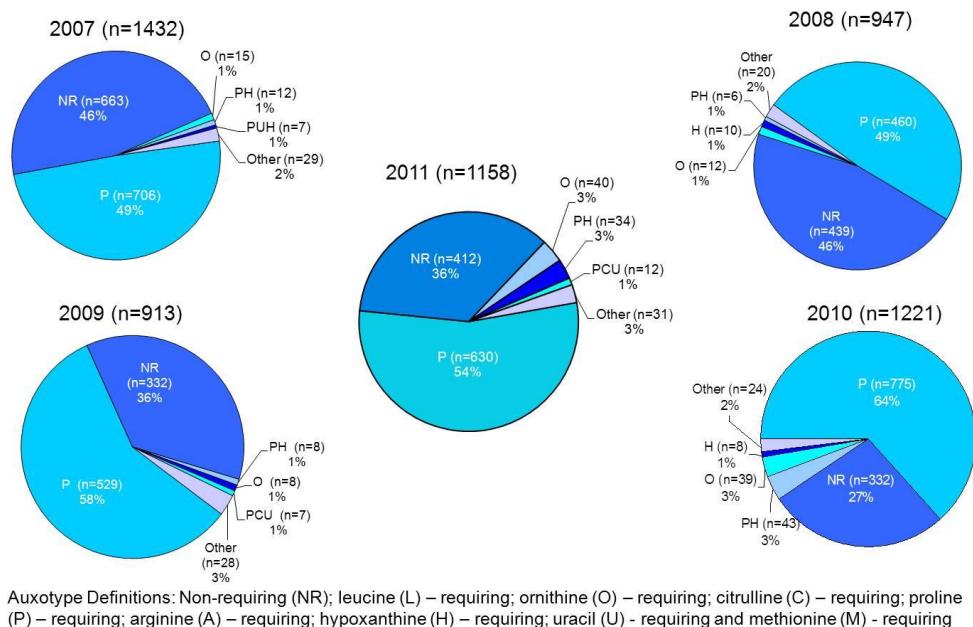
**Figure 17. NG-MAST Sequence Types of 2011 *Neisseria gonorrhoeae* Isolates with Decreased Susceptibility to Cefixime ( $\geq 0.25$  mg/L), N=140 and Ceftriaxone ( $\geq 0.125$  mg/L), N=209**



\*"Other" sequence types contain 1 to 3 isolates each.

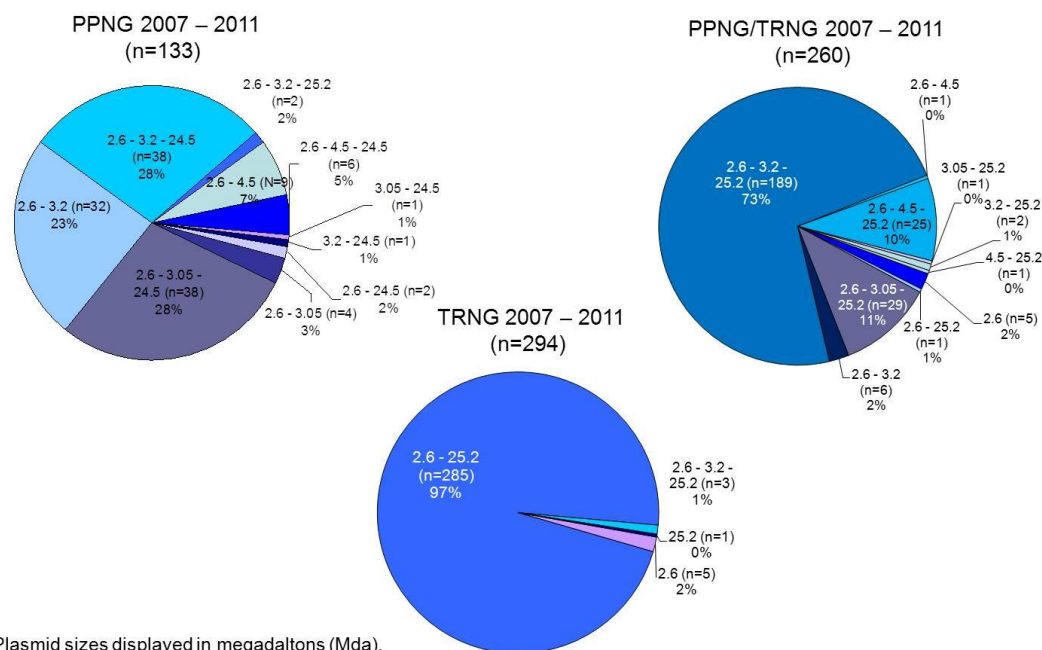
Auxotypes for all isolates were also determined. Proline-requiring (P) remains the most common auxotype in 2011 at 54.4% followed by non-requiring (NR) at 35.6%. A variety of other auxotypes were also identified at a low frequency including ornithine-requiring (O) at 3.4%, proline-hypoxanthine-requiring (PH) at 2.9%, and proline-citrulline-uracil-requiring (PCU) at 1.0% (Figure 18).

**Figure 18. Auxotype Distribution of *Neisseria gonorrhoeae* Isolates Received by NML, 2007 – 2011**



Plasmid profiles for PPNG, TRNG and PPNG/TRNG isolates are shown in Figure 19. The beta-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 133 PPNG strains isolated between 2007 and 2011 at 54.9%, followed by the 3.05 Mda plasmid at 32.3% and then the 4.5 Mda plasmid at 11.3%. 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  $\beta$ -lactamase encoding plasmid in PPNG/TRNG strains at 75.8%. 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 2007 and 2011, 96.9% had the 2.6 and 25.2 Mda plasmids. TRNG isolates accounted for 80.6% of all the plasmid mediated resistance in *N. gonorrhoeae* between 2007 and 2011 (554 of 687 PPNG, PPNG/TRNG and TRNG strains).

**Figure 19. Plasmid Distribution within Antimicrobial Classifications of *Neisseria gonorrhoeae* Isolates Received by NML, 2007 - 2011**



## CONCLUSION

Although penicillin and tetracycline have not been recommended in gonorrhea treatment for many years in Canada, 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 STI treatment guidelines to provide information on the most effective treatment of *N. gonorrhoeae* and to reduce the prevalence and spread of drug resistant gonorrhea. 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.



## APPENDIX

Table A. *Neisseria gonorrhoeae* culture isolates in Canada, 2009 – 2011\*

Province	2009 GC Cultures			2010 GC Cultures			2011 GC Cultures		
	Tested in each province	Received at NML	% Sent to NML for Testing	Tested in each province	Received at NML	% Sent to NML for Testing	Tested in each province	Received at NML	% Sent to NML for Testing
British Columbia	296	183	61.82%	342	256	74.85%	490	176	35.92%
Alberta	383	91	23.76%	283	166	58.66%	457	156	34.14%
Saskatchewan	36	30	83.33%	35	11	31.43%	35	35	100.00%
Manitoba	34	3	8.82%	24	11	45.83%	31	12	38.71%
Ontario	1383	383	27.69%	1101	383	34.79%	1212	428	35.31%
Québec	959	216	22.52%	1100	338	30.73%	1045	282	26.99%
New Brunswick	11	4	36.36%	9	9	100.00%	12	6	50.00%
Nova Scotia	103	2	1.94%	69	69	100.00%	77	77	100.00%
Newfoundland	2	2	100.00%	7	7	100.00%	1	0	0.00%
<b>Totals</b>	<b>3207</b>	<b>914</b>	<b>28.50%</b>	<b>2970</b>	<b>1250</b>	<b>42.09%</b>	<b>3360</b>	<b>1172</b>	<b>34.88%</b>

\*no *Neisseria gonorrhoeae* cultures were reported to NML or received from P.E.I., Northwest Territories, Nunavut or the Yukon from 2009 to 2011.

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