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# National Surveillance of Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Annual Summary 2009

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



Public Health  
Agency of Canada

Agence de la santé  
publique du Canada

Canada

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## Acknowledgements

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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. Marie Louie, Marguerite Lovgren

**Saskatchewan Disease Control Laboratory**, Regina, Saskatchewan:  
Dr. Greg Horsman, Evelyn Nagle, Rosanne Kitzul

**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, Sandra March

We would also like thank Dr. Tom Wong, Director, Professional Guidelines and Public Health Practice Division, Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada for his expert guidance and contribution to this report.

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## Executive Summary

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- This report compares laboratory surveillance data for *Neisseria gonorrhoeae* isolates submitted by provincial microbiology laboratories to the National Microbiology Laboratory (NML) from 2005-2009
- Effective antibiotic treatment is a vital part of gonococcal infection control and the impact of antimicrobial resistance in *N. gonorrhoeae* is of foremost concern. 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 mg/L in 2000 to 0.063 mg/L in 2009. There was also a shift in the MICs of cefixime, although a modal MIC can't be determined since isolates were evenly distributed over 3 MICs (0.016 mg/L, 0.032 mg/L, 0.125 mg/L). Two isolates (one each in 2007 and 2008) were identified with a cefixime MIC equal to 0.5 mg/L, classified as non-susceptible.
- 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 794 in 2009 (25.5%).
- Oral cephalosporin treatment failures and in vitro reduced susceptibilities have been reported from Asia and Australia. A WHO/CDC Global Consultation on the strategic response to the emergence of cephalosporin resistance in *N. gonorrhoeae* took place in April 2010.
- Between 2005 and 2009, 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. Epidemiologic data indicates that reported gonorrhea rates have been rising since 1997.
- In 2011, a sentinel public health practice and surveillance pilot study for *N. gonorrhoeae* is being initiated in Canada to collect integrated practice, epidemiological and laboratory information. The objectives of the pilot study would be to determine the treatment failure probability associated with regimens recommended by the Canadian STI Guidelines and the trends of antimicrobial resistance in *N. gonorrhoeae*. 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.

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## Introduction

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*Neisseria gonorrhoeae*, the causative agent of gonorrhoea is the second most commonly reported bacterial sexually transmitted infection in Canada with over 11000 cases reported in 2009 (1). Reported rates of gonorrhoea have more than doubled from 14.9 cases per 100,000 population in 1997 to 33.1 cases per 100,000 population in 2009 (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 gonorrhoea (Figure 1). The treatment and control of gonorrhoea 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 gonorrhoea.

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## Methods

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*N. gonorrhoeae* strains 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 AMR testing from the different regions across Canada.

Isolates were 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 each jurisdiction 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  $\beta$ -lactamase and presence of *tetM* determinant.

Selected *N. gonorrhoeae* isolates were 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* isolates tested at the NML from each province, 2005-2009**

Province	2005	2006	2007	2008	2009	Total
British Columbia	117	86	95	104	183	585
Alberta	50	96	189	55	90	480
Saskatchewan	49	39	34	1	30	153
Manitoba	54	53	2	3	3	115
Ontario	452	855	705	539	383	2934
Québec	179	392	391	230	216	1408
New Brunswick	2	2	8	5	4	21
Nova Scotia	1	0	0	0	2	3
Newfoundland	1	9	14	10	2	36
Total isolates tested at NML	905	1532	1438	947	914	5736
Total viable isolates available for testing	898	1528	1432	947	913	5718
Total isolates resistant to at least one antibiotic	850	1472	1395	929	873	5519
Total number of isolates tested by the provincial laboratories*	3619	4201	4275	3907	3106	19108
Percentage of isolates resistant to at least one antibiotic	23.5%	35.0%	32.6%	23.8%	28.1%	28.9%
Percentage of total cases tested	39.3%	37.1%	36.0%	30.7%	27.8%	42.2%
Total cases reported in Canada	9199	11334	11873	12723	11178	45307

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

**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	I	R	NS	
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	64.0	≥ 128.0		Sigma #S 9007
Ceftriaxone	0.000125 – 2.0	≤ 0.25			≥ 0.5	Sigma #C 5793
Ciprofloxacin	0.001 – 64.0	≤ 0.06	0.12 - 0.5	≥ 1.0		Bayer Health Care
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**

<b>Characterization</b>		<b>Definition</b>
PPNG	Penicillinase Producing <i>Neisseria gonorrhoeae</i>	Pen MIC $\geq$ 2.0 mg/L, $\beta$ -lactamase positive, $\beta$ -lactamase plasmid (3.05, 3.2 or 4.5 Mdal plasmid)
TRNG	Tetracycline Resistant <i>Neisseria gonorrhoeae</i>	Tet MIC $\geq$ 16.0 mg/L, 25.2 Mdal plasmid, TetM PCR positive
CMRNG	Chromosomal Mediated Resistant <i>Neisseria gonorrhoeae</i>	Pen MIC $\geq$ 2.0 mg/L, Tet MIC $\geq$ 2.0 mg/L but $\leq$ 8.0 mg/L, and Ery MIC $\geq$ 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 $\geq$ 2.0 mg/L
PenR	Penicillin Resistant <i>Neisseria gonorrhoeae</i>	Pen MIC $\geq$ 2.0 mg/L, $\beta$ -lactamase negative
TetR	Tetracycline Resistant <i>Neisseria gonorrhoeae</i>	Tet MIC $\geq$ 2.0 mg/L but $\leq$ 8.0 mg/L
EryR	Erythromycin Resistant <i>Neisseria gonorrhoeae</i>	Ery MIC $\geq$ 2.0 mg/L
CipR	Ciprofloxacin Resistant <i>Neisseria gonorrhoeae</i>	Cip MIC $\geq$ 1.0 mg/L
AzR	Azithromycin Resistant <i>Neisseria gonorrhoeae</i>	Az MIC $\geq$ 2.0 mg/L
SpecR	Spectinomycin Resistant <i>Neisseria gonorrhoeae</i>	Spec R $\geq$ 128 mg/L
CxNS	Ceftriaxone Non-susceptible <i>Neisseria gonorrhoeae</i>	Cx MIC $\geq$ 0.5 mg/L
CeNS	Cefixime Non-susceptible <i>Neisseria gonorrhoeae</i>	Ce MIC $\geq$ 0.5 mg/L

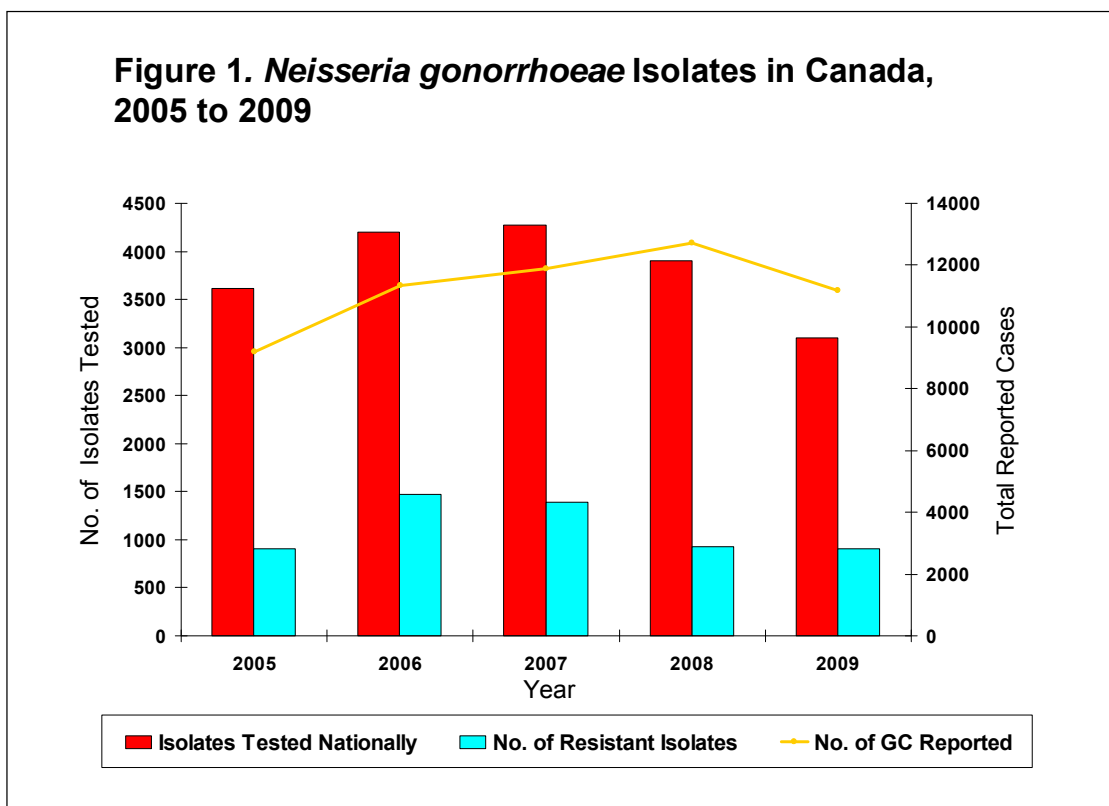


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## Results and Discussion

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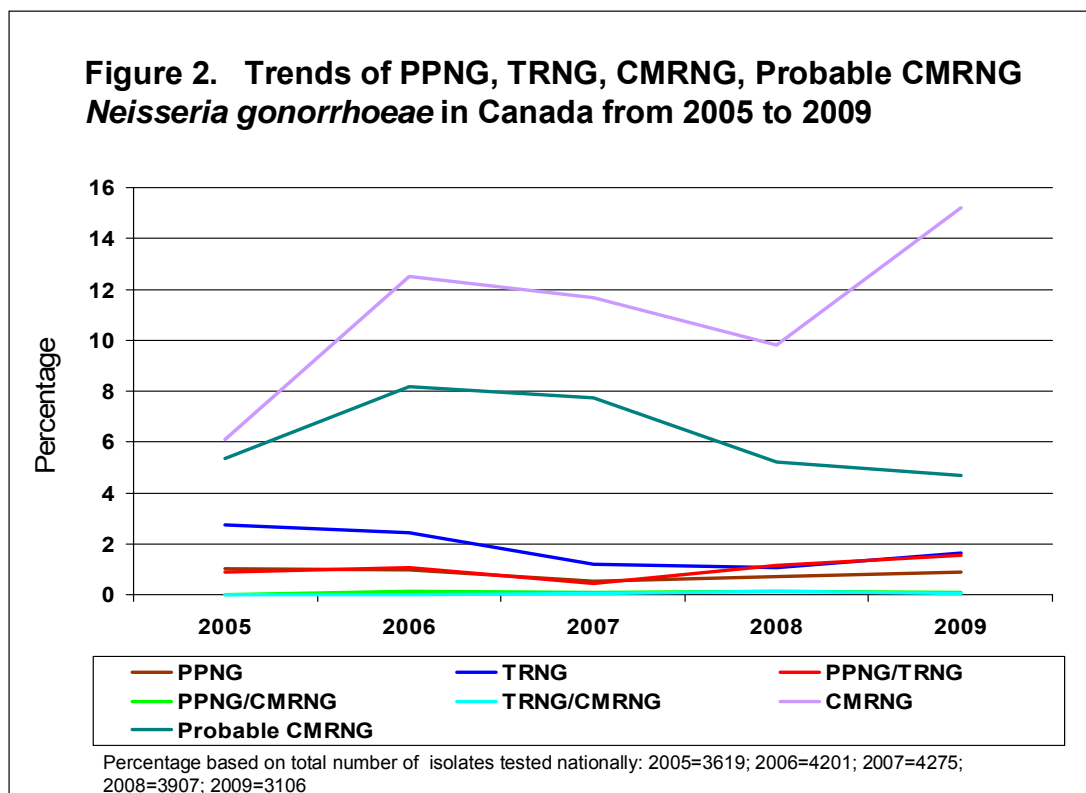
Of the 5,718 viable isolates tested at the NML between 2005 and 2009, 5,519 isolates (96.5%) were found to be resistant to at least one of the following antibiotics: penicillin, tetracycline, ciprofloxacin, azithromycin, and erythromycin. A total of 199 (3.5%) isolates were found to be susceptible to all of these antibiotics. In 2009, 28.1% (873 of 3,106) 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.



**Table 4. Characterization of all *Neisseria gonorrhoeae* isolates submitted to National Microbiology Laboratory, 2005-2009**

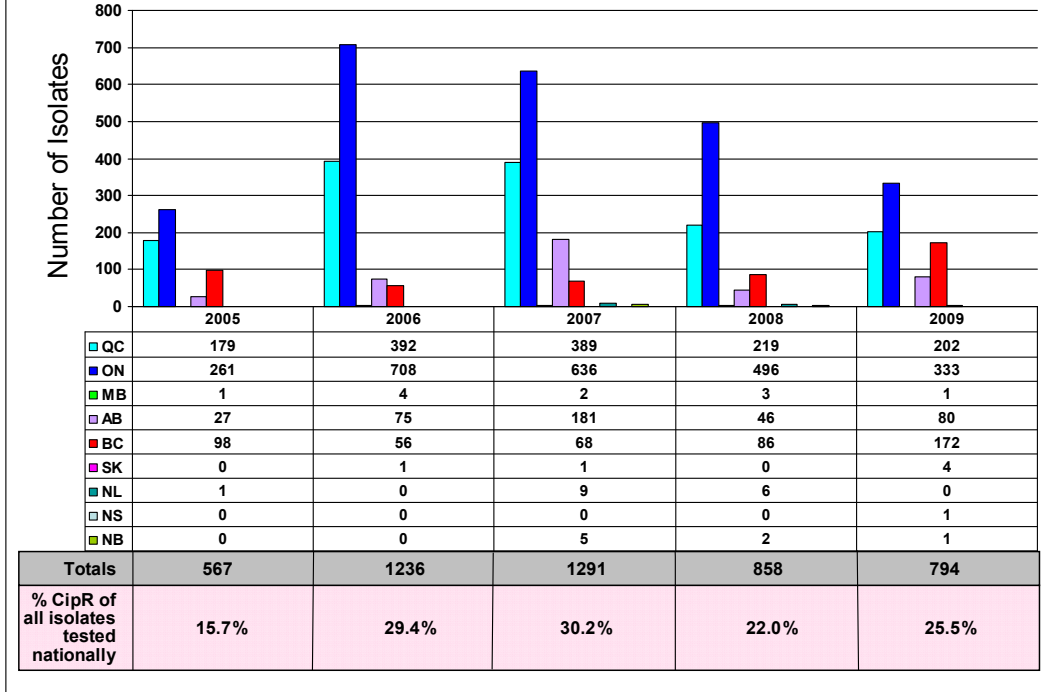
Characterization		2005	2006	2007	2008	2009	Totals
<b>Plasmid Mediated Resistances</b>	PPNG	17	26	12	10	8	73
	PPNG/CipR	10	3	3	13	11	40
	PPNG/CipR/EryR	1	0	0	0	0	1
	PPNG/CipR/TetR	3	2	0	0	0	5
	PPNG/TetR	6	10	6	1	3	26
	PPNG/EryR	0	0	1	3	2	6
	PPNG/TetR/CipR	0	0	0	0	2	2
	PPNG/CMRNG	0	5	3	0	0	8
	PPNG/CMRNG/CipR	0	0	0	5	3	8
	PPNG/TRNG	8	10	4	10	11	43
	PPNG/TRNG/CipR	23	31	9	31	33	127
	PPNG/TRNG/CipR/EryR	1	4	4	4	4	17
	PPNG/TRNG/AzR/CipR/EryR	0	0	1	0	2	3
	PPNG/TRNG/EryR	0	0	0	0	1	1
	TRNG	82	84	37	29	28	260
	TRNG/CipR	14	12	11	8	12	57
	TRNG/CipR/EryR	2	1	3	3	4	13
	TRNG/EryR	1	5	0	0	5	11
	TRNG/PenR	1	0	0	0	0	1
	TRNG/CipR/PenR	0	0	1	1	0	2
TRNG/CMRNG/CipR	0	0	2	5	2	9	
<b>Chromosomal Mediated Resistances</b>	AzR/CipR	1	0	0	0	0	1
	AzR/CipR/EryR/TetR	1	0	0	0	0	1
	AzR/EryR	3	3	0	0	0	6
	AzR/EryR/TetR	1	0	2	0	1	4
	AzR/CipR/EryR	0	0	1	0	0	1
	CeNS/CipR	0	0	1	0	0	1
	CeNS/CipR/EryR	0	0	0	1	0	1
	CipR	44	163	161	87	40	495
	CipR/EryR	8	54	214	62	37	375
	CipR/EryR/TetR	39	22	28	8	8	105
	CipR/PenR	1	6	0	2	0	9
	CipR/TetR	71	100	34	48	26	279
	EryR	8	7	2	0	1	18
	EryR/TetR	23	16	0	0	0	39
	PenR	0	2	0	1	1	4
	PenR/TetR	0	0	3	2	0	5
	TetR	67	37	23	9	9	145
	CMRNG	10	6	3	3	3	25
	CMRNG/AzR/CipR	2	2	2	4	8	18
	CMRNG/CipR	209	514	494	375	462	2,054
CMRNG/AzR	0	3	0	0	0	3	
Probable CMRNG	55	24	11	5	6	101	
Probable CMRNG/AzR/CipR	1	0	1	1	0	3	
Probable CMRNG/CipR	137	320	318	198	140	1,112	
Susceptible Strain	48	56	37	18	40	199	
Contaminated or No Growth	7	4	6	0	1	18	
<b>Total isolates tested at NML</b>	<b>905</b>	<b>1,532</b>	<b>1,438</b>	<b>947</b>	<b>914</b>	<b>5,736</b>	
<b>Total viable isolates available for testing</b>	<b>898</b>	<b>1,528</b>	<b>1,432</b>	<b>947</b>	<b>913</b>	<b>5,718</b>	

Between 2005 and 2009, 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 6.1% in 2005 (221 of 3,619 isolates) to 15.2% by 2009 (472 of 3,106 isolates) and 4.7% of isolates were characterized as Probable CMRNG. During the same time period, the rate of PPNG isolates remained stable at 1.0% (37 of 3,619 isolates) to 0.9% (28 of 3,106 isolates). The TRNG isolates slightly decreased from 2.8% (100 of 3,619 isolates) in 2005 to 1.6% (51 of 3,106 isolates) in 2009 (Figure 2).

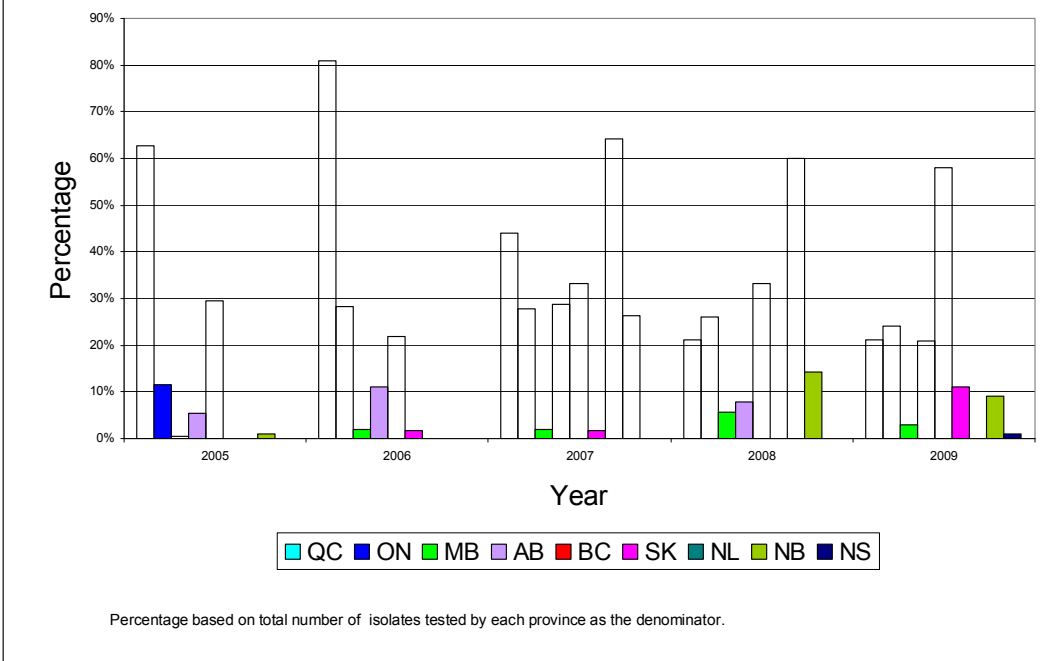


Ciprofloxacin resistant *N. gonorrhoeae* accounted for 24.8% (4,745 of 19,108) of all strains isolated between 2005 and 2009 (Figure 3a). The number of isolates increased from 59 in 2000 (1.3%) to 793 in 2009 (25.5%) and percentage rates for each province are shown in Figure 3b. The mode of MICs of ciprofloxacin has shifted from 0.008 mg/L in 2004 to 16.0 mg/L in 2009 (Figure 4). Of the 794 ciprofloxacin resistant isolates identified in 2009, 95.0% (n=754) were also resistant to at least one other antibiotic; 475 (59.9%) were characterized as CMRNG.

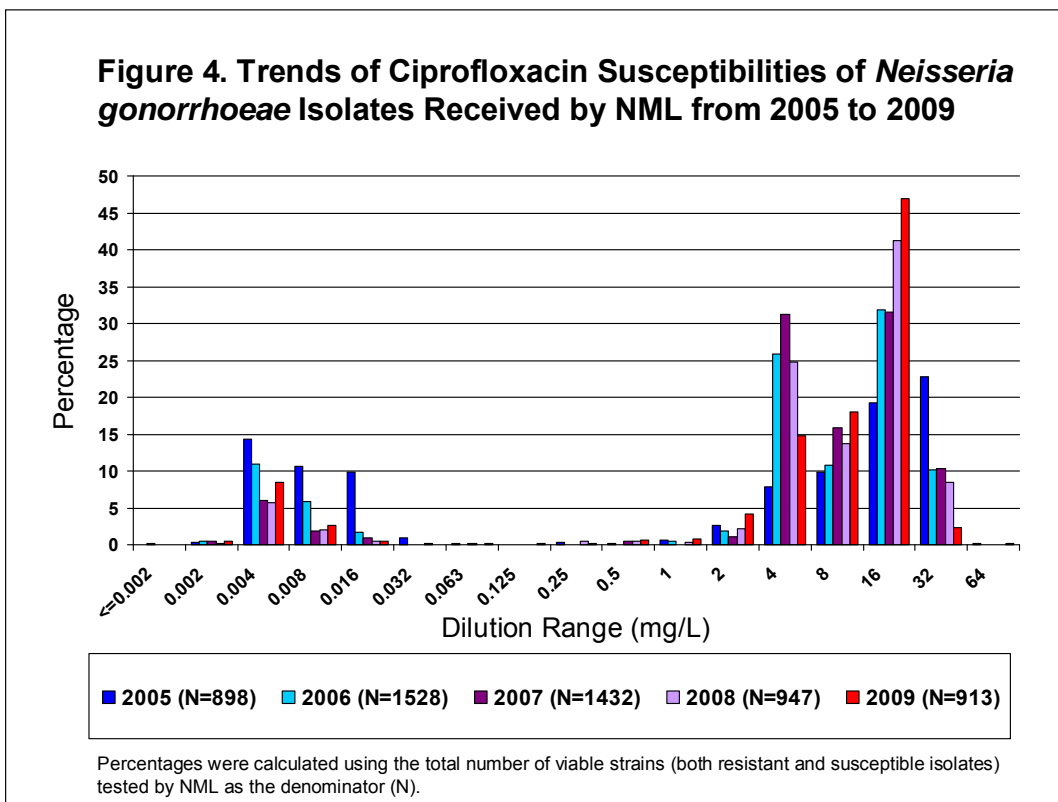
**Figure 3a. Geographical Distribution of Ciprofloxacin Resistant *Neisseria gonorrhoeae* Isolates, 2005 to 2009**



**Figure 3b. Geographical Distribution (%) of Ciprofloxacin Resistant *Neisseria gonorrhoeae* Isolates, 2005 to 2009**

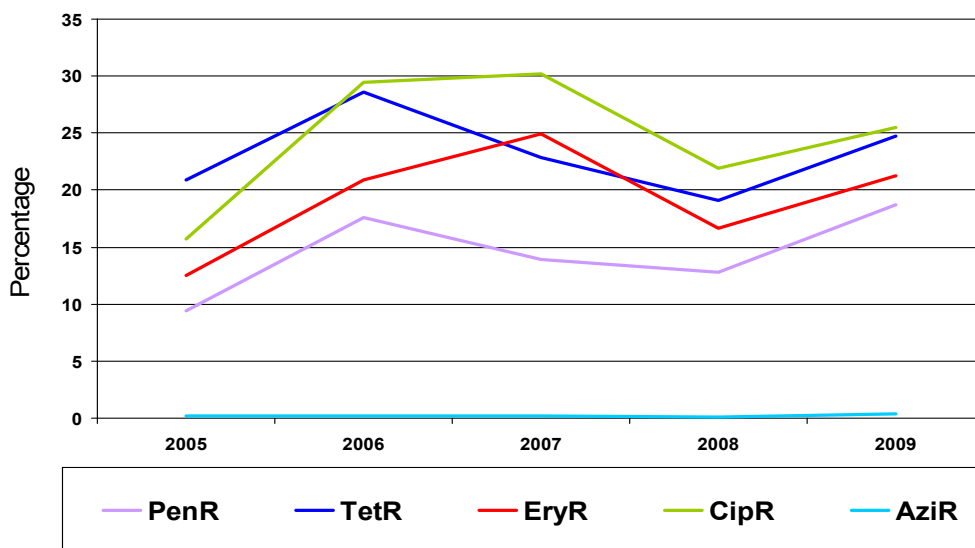


**Figure 4. Trends of Ciprofloxacin Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2005 to 2009**



Erythromycin resistance in *N. gonorrhoeae* continues to rise and accounted for 19.4% (3,712 of 19,108) of all strains isolated between 2005 and 2009. In 2005, only 12.5% (454 of 3,619 isolates) were found to be erythromycin resistant increasing to 21.3% (663 of 3,106 isolates) by 2009. Of the 663 erythromycin resistant isolates identified during in 2009, 99.8% were also resistant to at least one other antibiotic. A total of 478 (72.2%) of these were characterized as CMRNG. Strains with higher MICs to erythromycin also have higher MICs to azithromycin. Penicillin resistance experienced an increase in levels between 2005 and 2009 from 9.4% (341 of 3,619 isolates) to 18.7% (580 of 3,106 isolates). Tetracycline resistance increased between 2005 and 2009 from 20.9% (757 of 3,619 isolates) to 24.8% (771 of 3,106 isolates) (Figure 5).

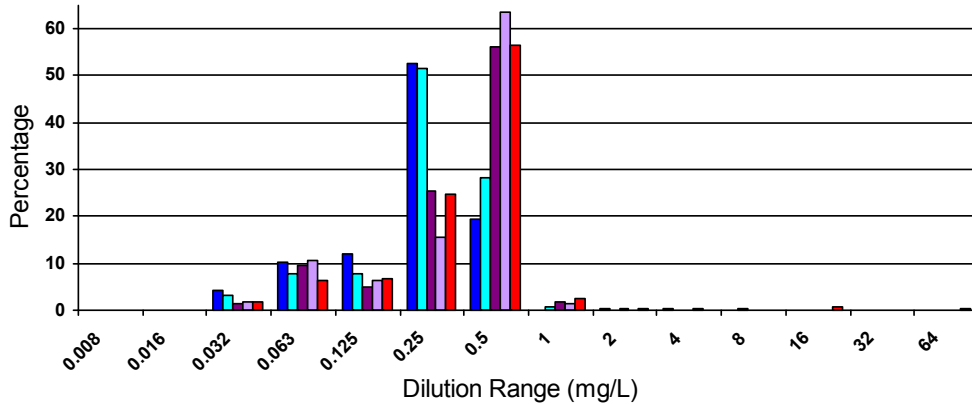
**Figure 5. Antimicrobial Susceptibility of *Neisseria gonorrhoeae* Isolates Tested in Canada Between 2005-2009**



Percentage based on total number of isolates tested nationally: 2005=3619; 2006=4201; 2007=4275; 2008=3907; 2009=3106

Azithromycin resistant *N. gonorrhoeae* accounted for 0.2% (39 of 19,108) of all strains isolated between 2005 and 2009. The mode of MICs of azithromycin has shifted from 0.25 mg/L in 2005 to 0.5 mg/L in 2007 and remained at 0.5 mg/L in 2008 and 2009 (Figure 6). Each of the 39 azithromycin resistant isolates is associated with resistance to at least one other antibiotic (Figure 7).

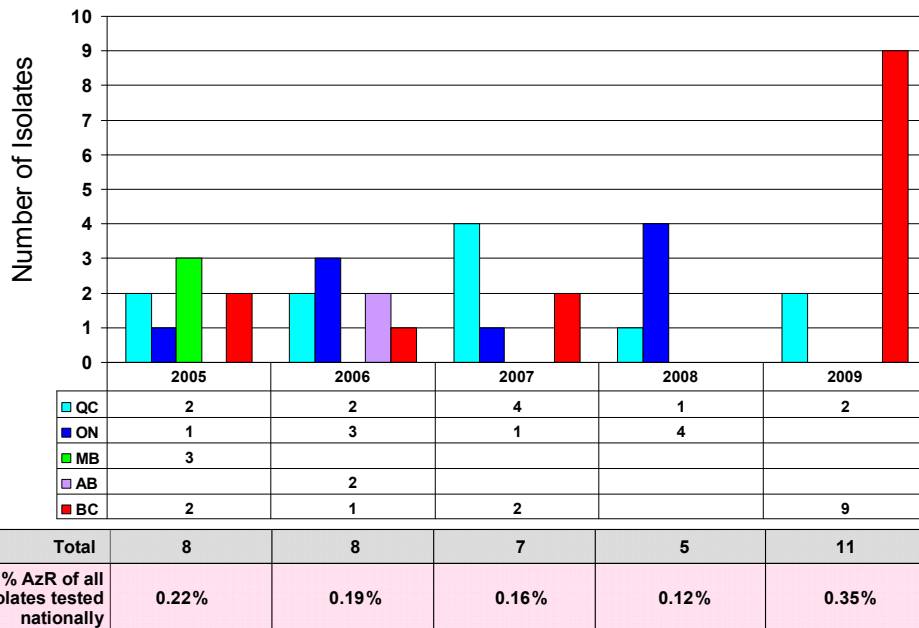
**Figure 6. Trends of Azithromycin Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2005 to 2009**



■ 2005 (N=898)   ■ 2006 (N=1528)   ■ 2007 (N=1432)   ■ 2008 (N=947)   ■ 2009 (N=913)

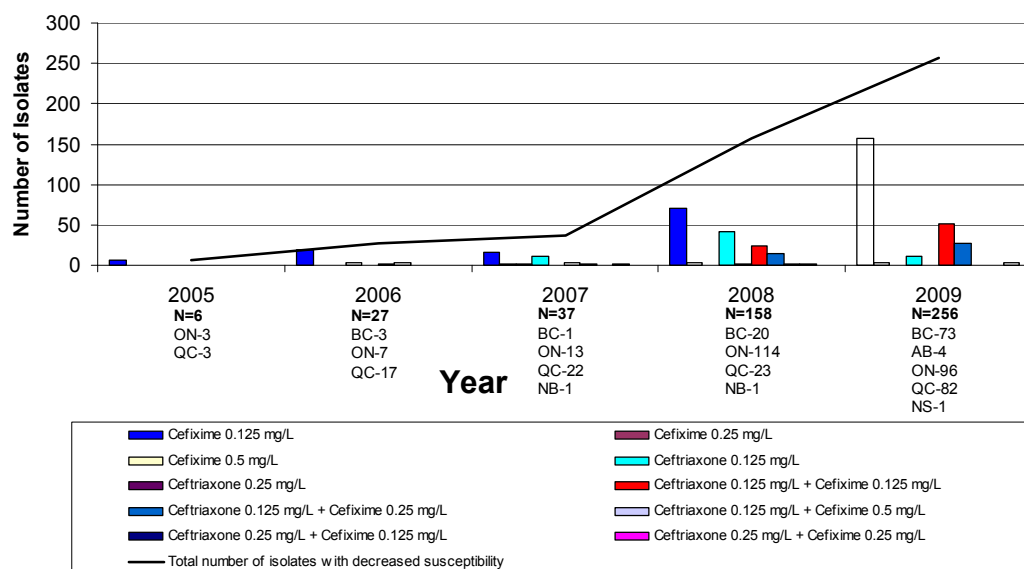
Percentages were calculated using the total number of viable strains (both resistant and susceptible isolates) tested by NML as the denominator (N).

**Figure 7. Geographical Distribution of Azithromycin Resistant *Neisseria gonorrhoeae* Isolates, 2005 to 2009**



**Figure 8. *Neisseria gonorrhoeae* Isolates with Decreased/Non-Susceptibility to Cefixime (0.125 mg/L, 0.25 mg/L and 0.5 mg/L) and Decreased Susceptibility to Ceftriaxone (0.125 mg/L and 0.25 mg/L) for Isolates Received Between 2005-2009 (N=484)**

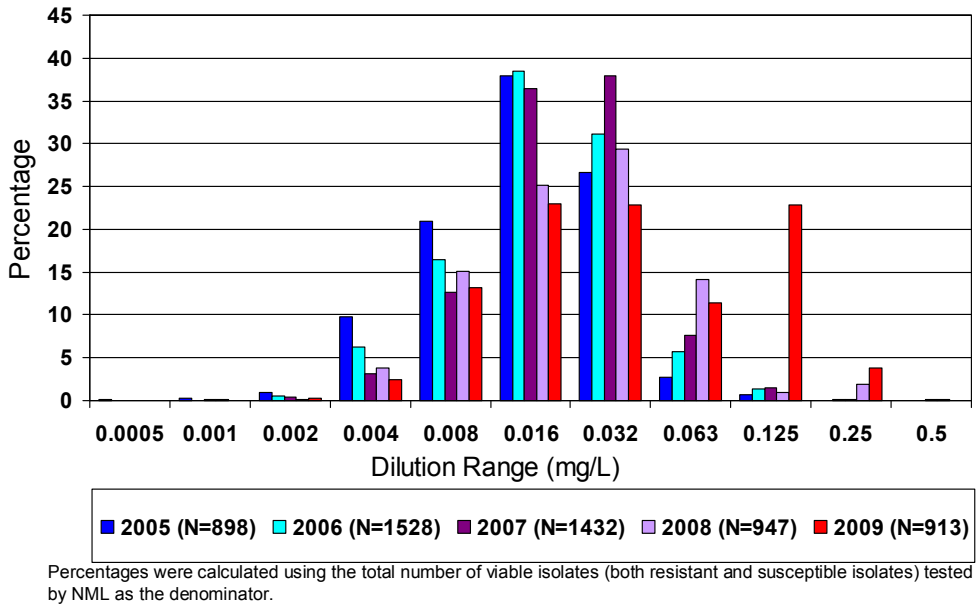
(Includes MICs within 2 dilutions of CLSI Interpretive Standard Non-Susceptibility  $\geq 0.5$  mg/L)



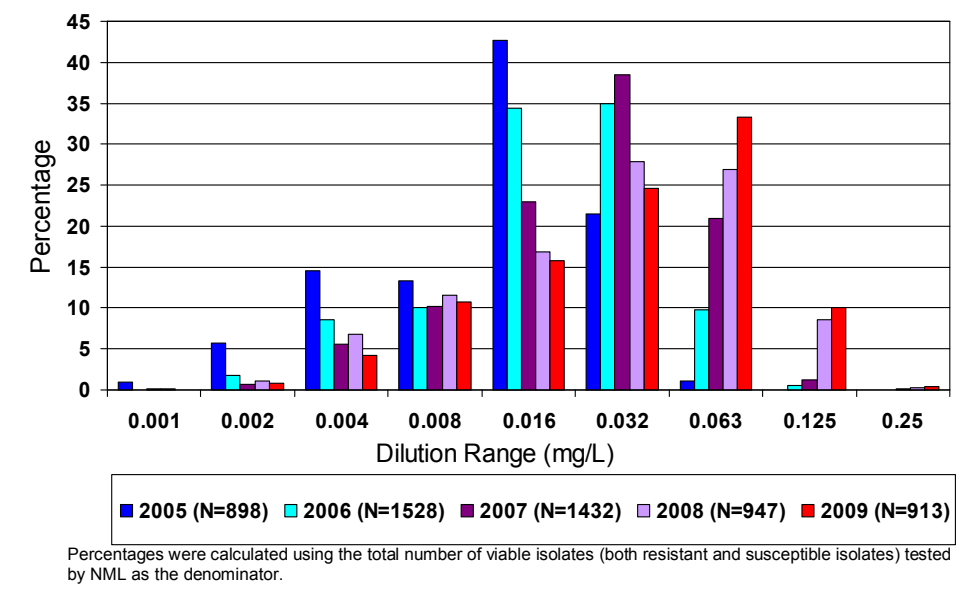
Of the 5,718 viable isolates tested at NML between 2005 and 2009, none were identified with resistance to spectinomycin or ceftriaxone. Two isolates (one in 2007 and one in 2008) were identified with a cefixime MIC of 0.5 mg/L, classified as non-susceptible by CLSI guidelines. A total of 484 *N. gonorrhoeae* isolates with decreased susceptibility MICs (includes MICs within 2 dilutions of CLSI Interpretive Standard Non-Susceptibility  $\geq 0.5$  mg/L) to ceftriaxone and cefixime were identified between 2005 and 2009 (Figure 8). The MICs of the 3rd generation cephalosporins have been increasing over time. There has been a 'right' shift in the modal MICs of ceftriaxone from 0.016 mg/L in 2000 to 0.063 mg/L in 2009. There was also a shift in the MICs of cefixime, although a modal MIC can't be determined since isolates were evenly distributed over 3 MICs (0.016 mg/L, 0.032 mg/L, 0.125 mg/L) (Figures 9 and 10). Preliminary data suggest the trend towards a 'right' shift in MICs continued during 2010 (data not shown). These results indicate that the MICs of these 3rd generation cephalosporins are increasing over time.



**Figure 9. Trends of Cefixime Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2005 to 2009**

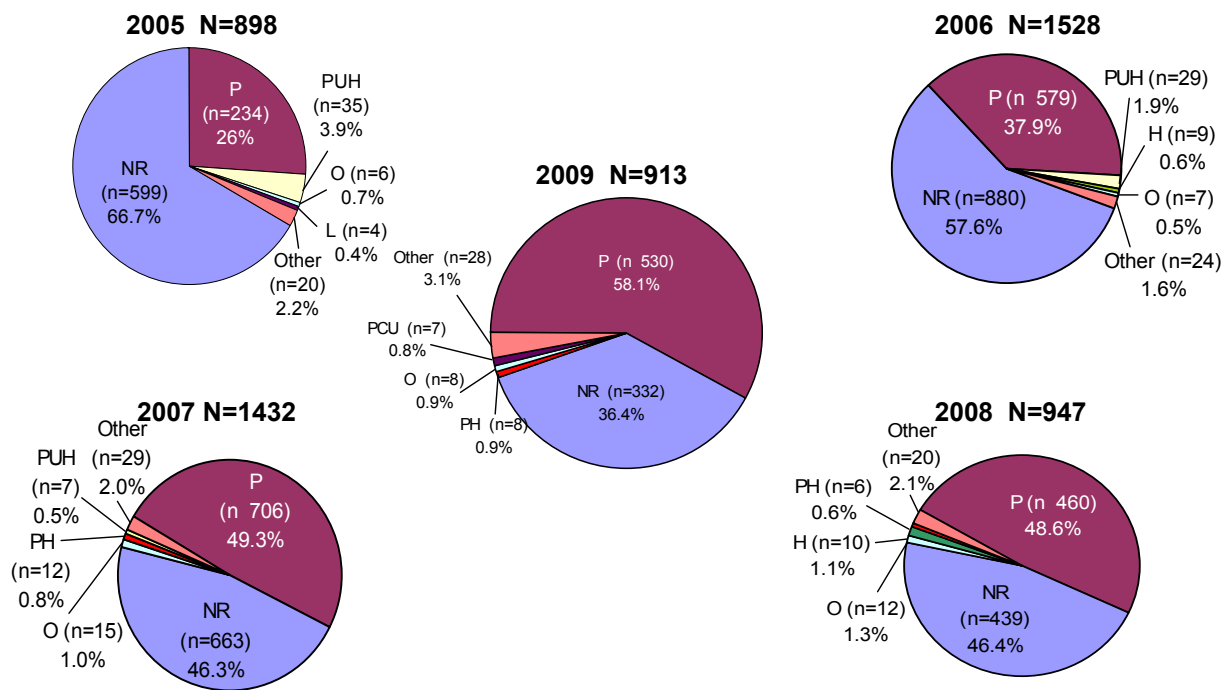


**Figure 10. Trends of Ceftriaxone Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by NML from 2005 to 2009**



Auxotypes for all isolates were also determined. The majority of isolates in 2005 and 2006 were non-requiring (NR) at 66.7% and 57.6%, respectively. The next most common auxotype in 2005 and 2006 was proline-requiring (P) at 26.1% and 37.9%, respectively. By 2007, 2008 and 2009 P-requiring isolates became the most common auxotype at 49.3%, 48.6% and 58.1% respectively followed by NR at 46.3% in 2007, 46.4% in 2008 and 36.4% in 2009. In 2009 a variety of other auxotypes were also identified at a low frequency including ornithine-requiring (O) at 0.9%, proline, hypoxanthine-requiring (PH) at 0.9% and proline, citrulline, uracil-requiring (PCU) at 0.8% (Figure 11).

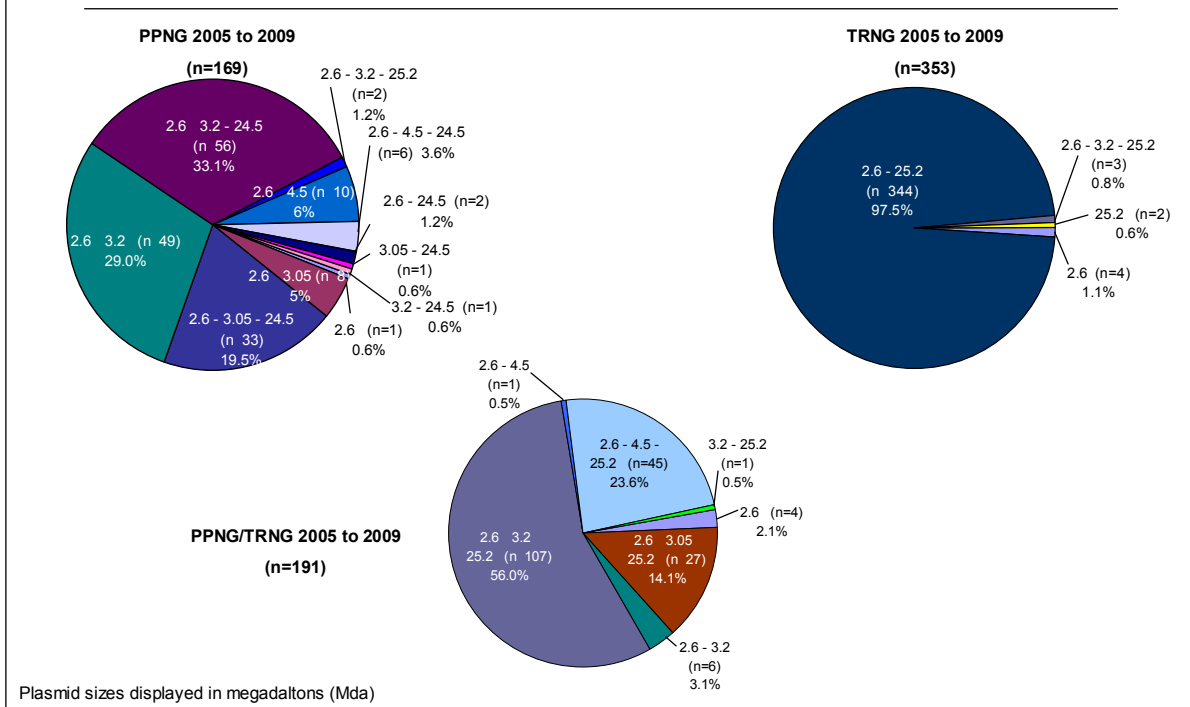
**Figure 11. Auxotype Distribution of *Neisseria gonorrhoeae* Isolates Received by NML, 2005 – 2009**



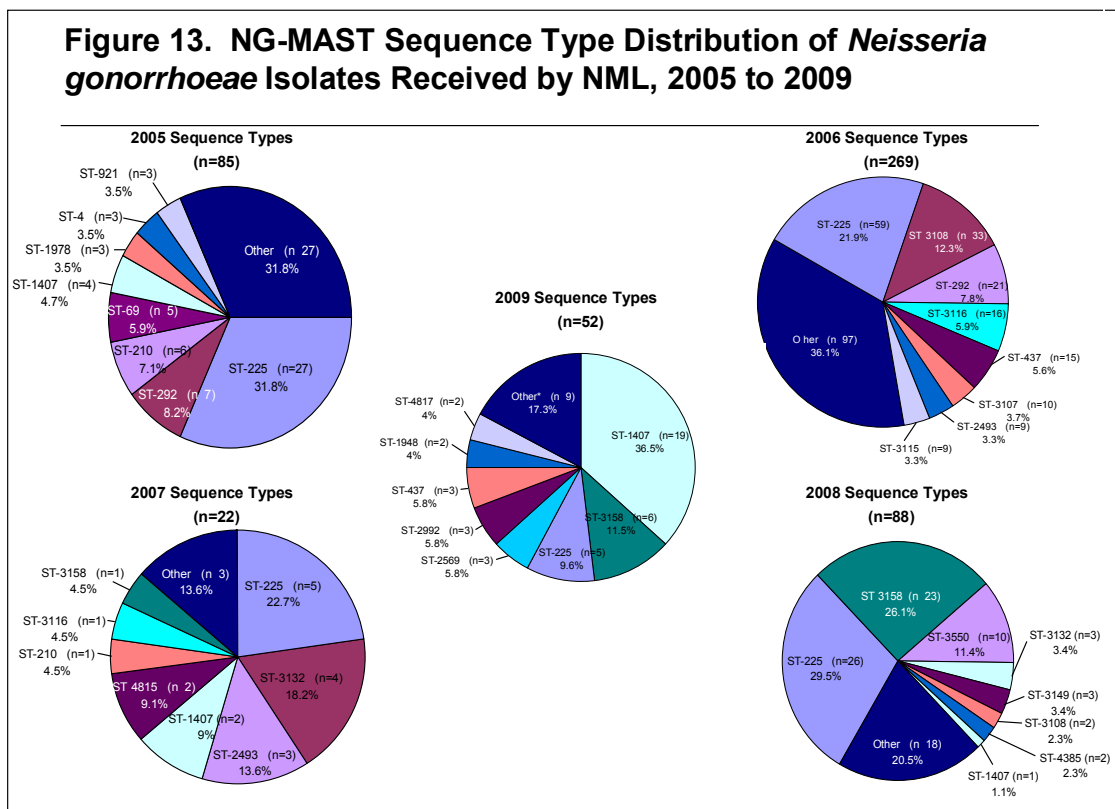
Auxotype Definitions: Non-requiring (NR), leucine (L) - requiring, ornithine (O) - requiring, citrulline (C) - requiring, proline (P) - requiring, arginine (A) - requiring, hypoxanthine (H) - requiring, uracil (U) - requiring and methionine (M) - requiring

Plasmid profiles for PPNG, TRNG and PPNG/TRNG isolates are shown in Figure 12. The  $\beta$ -lactamase gene was encoded in three different types of plasmids of sizes 3.05 Mda, 3.2 Mda and 4.5 Mda. The 3.2 Mda plasmid was the most common type amongst the 169 PPNG strains isolated between 2005 and 2009 at 63.9%, followed by the 3.05 Mda plasmid at 24.9% and then the 4.5 Mda plasmid at 9.5%. 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 59.7%. 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 2005 and 2009, 98.3% had the 2.6 and 25.2 Mda plasmids. TRNG isolates accounted for 76.3% of all the plasmid mediated resistance in *N. gonorrhoeae* between 2005 and 2009 (544 of 713 PPNG, PPNG/TRNG and TRNG strains).

**Figure 12. Plasmid Distribution within Antimicrobial Classifications of *Neisseria gonorrhoeae* Isolates Received by NML, 2005 - 2009**



NG-MAST molecular-based sequence typing provides a substantial level of discrimination between isolates. STs were determined for selected isolates between 2005 and 2009 as shown in Figure 13. In 2010, STs will be determined for all *N. gonorrhoeae* isolates. In 2009, the most common STs were ST-1407 and ST-3158 at 36.5% and 11.5%, respectively. 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 3<sup>rd</sup> generation cephalosporins. ST-1407 has been reported in England (9, 10), the USA (11), Australia (12). ST-1407 is very closely related to ST-3158 and has been reported in Sweden (13).



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## Conclusion

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Although penicillin and tetracycline have not been used in gonorrhoea treatment for many years, the identification of the penicillin resistance phenotypes assist in monitoring the susceptibilities to the 3rd generation cephalosporins since these isolates also exhibit higher MICs of ceftriaxone and cefixime. 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 is not available.

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