

# National Surveillance of Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Annual Summary 2012

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

**Professional Guidelines and Public Health  
Practice Division and  
Surveillance and Epidemiology Division  
Centre for Communicable Diseases and  
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Public Health Agency of Canada**

PROTECTING CANADIANS FROM ILLNESS



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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 2008-2012.
- The Canadian reported rate of gonorrhea is on the rise and has more than doubled from 14.9 per 100,000 in 1997 to 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 2008 to 0.063 mg/L in 2012. There was also a shift in the modal MICs of cefixime which increased from 0.032 mg/L in 2008 to 0.125 mg/L in 2010 and 2011 but has since reversed back to 0.032 mg/L in 2012.
- In 2012, 2.2% of isolates (68/3,036) were identified as having decreased susceptibility to cefixime and 5.5% (168/3,036) were identified as having decreased susceptibility to ceftriaxone according to WHO definition (decreased susceptibility MICs  $\geq 0.25$  mg/L for cefixime and  $\geq 0.125$  mg/L for ceftriaxone).
- In response to the increasing MICs and reports of cefixime treatment failures, the Canadian STI Guidelines have been evolving; combination gonorrhea therapy with 250 mg ceftriaxone intramuscularly and azithromycin 1 g orally is recommended as a first-line regimen, with the above combination being the only first-line regimen in the men-who-have-sex-with men population and in pharyngeal infections. Additional information on the treatment of gonococcal infection is available at <http://www.phac-aspc.gc.ca/std-mts/sti-its/alert/2011/alert-gono-eng.php>
- The percentage of azithromycin resistant (MIC  $\geq 2$  mg/L) *N. gonorrhoeae* isolates increased from 0.12% (5/3,907) in 2008 to 0.86% (26/3,036) in 2012. Between 2009 and 2012, five isolates with high level azithromycin resistance (MIC  $\geq 256$  mg/L) were 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. The percentage of ciprofloxacin resistant *N. gonorrhoeae* increased from 1.3% (59/4,458) in 2000 to a high of 35.96% (1,068/2,970) in 2010 decreasing to 28.52% (866/3,036) in 2012.
- 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 study for *N. gonorrhoeae* is being proposed in Canada to collect integrated practice, epidemiological and laboratory information. The objectives of the 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 and to inform Canadian STI management guidelines.



## INTRODUCTION

*Neisseria gonorrhoeae* is the causative agent of gonorrhoea and the second most commonly reported bacterial sexually transmitted infection in Canada, with 11,399 cases reported in 2011 (12). 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 (12). Globally, gonorrhea is a public health threat with 106 million cases estimated each year (20). Recently the World Health Organization released a global action plan to control the spread and impact of antimicrobial resistance in *N. gonorrhoeae* (20) and the CDC reported drug resistant *N. gonorrhoeae* at an urgent hazard level, requiring serious public health attention (4). 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 (2,14). The emergence of isolates with decreased susceptibilities to the cephalosporins (8,9,11,15,19) and reports of treatment failures in Canada (1) and around the world has made the concept of untreatable gonorrhea infections a future possibility. Azithromycin resistant isolates have also been identified. Recently the emergence of high-level azithromycin resistant ( $\geq 256$  mg/L) *N. gonorrhoeae* has been reported internationally (5) and isolates with this high level azithromycin resistance have now been identified in Canada. A further challenge to the laboratories monitoring antimicrobial susceptibilities of gonorrhea is that the number of cultures available 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 National Microbiology Laboratory (NML) has been monitoring the antimicrobial susceptibilities of *N. gonorrhoeae* since 1985; these results inform the gonococcal infection treatment recommendations in the Canadian Guidelines on Sexually Transmitted Infections

## METHODS

In 2012, provincial public health laboratories submitted a total of 1,031 viable *N. gonorrhoeae* isolates 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 the NML (Tables 4 & 5).

*N. gonorrhoeae* isolates are submitted to the 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 is 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 semi-annually. Minimum inhibitory concentration, or 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 outlined in Table 2. Resistance characterization definitions are provided in Table 3. Isolates were also characterized by plasmid profiles analysis, production of  $\beta$ -lactamase and the presence of *tetM* determinant.

*N. gonorrhoeae* isolates were also analyzed by molecular genotyping using the *N. gonorrhoeae* multi-antigen sequence type (NG-MAST) method (10) 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 by Province, 2008-2012**

Province	2008	2009	2010	2011	2012	Total
British Columbia	104	183	256	176	92	811
Alberta	55	91	164	156	94	562
Saskatchewan	1	30	11	35	57	134
Manitoba	3	3	11	12	8	37
Ontario	539	383	383	428	403	2,136
Québec	230	216	335	282	390	1,456
New Brunswick	5	4	9	6	3	27
Nova Scotia	0	2	69	77	0	148
Newfoundland	10	2	7	0	0	19
Prince Edward Island	0	0	0	0	1	1
<b>Total isolates received at NML</b>	<b>947</b>	<b>914</b>	<b>1,245</b>	<b>1,172</b>	<b>1,048</b>	<b>5,331</b>
<b>Total viable isolates available for testing</b>	<b>947</b>	<b>913</b>	<b>1,233</b>	<b>1,158</b>	<b>1,031</b>	<b>5,282</b>
<b>Total isolates resistant to at least one antibiotic</b>	<b>929</b>	<b>873</b>	<b>1,137</b>	<b>1,075</b>	<b>987</b>	<b>5,001</b>
<b>Total number of isolates tested in each province<sup>a</sup></b>	<b>3,907</b>	<b>3,106</b>	<b>2,970</b>	<b>3,360</b>	<b>3,036</b>	<b>16,379</b>
<b>Percentage of isolates resistant to at least one antibiotic</b>	<b>23.78%</b>	<b>28.11%</b>	<b>38.28%</b>	<b>31.99%</b>	<b>32.51%<sup>b</sup></b>	<b>30.53%<sup>b</sup></b>
<b>Percentage of total cases tested</b>	<b>30.71%</b>	<b>27.79%</b>	<b>26.06%</b>	<b>29.49%</b>	<b>27.6%<sup>b</sup></b>	<b>28.39%<sup>b</sup></b>
<b>Total cases reported in Canada</b>	<b>12,723</b>	<b>11,178</b>	<b>11,397</b>	<b>11,394</b>	<b>11,000<sup>b</sup></b>	<b>57,692<sup>b</sup></b>

<sup>a</sup>Total number of isolates tested by the provincial laboratories is used as the denominator in all % resistance calculations.

<sup>b</sup>Number of cases reported in 2012 and percentage of total cases tested is estimated.



**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 (6) except for erythromycin (7) and azithromycin (3,13) and ceftriaxone and cefixime (20).

Antibiotic	Recommended Testing Concentration Ranges (mg/L)	MIC Interpretive Standard (mg/L) <sup>a</sup>				Sources of Antibiotics
		S	DS	I	R	
Penicillin	0.032 – 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	0.001 – 2.0		≥ 0.125			Sigma #C 5793
Cefixime	0.002 – 2.0		≥ 0.25			Wyeth - Ayerst
Azithromycin	0.016 – 32.0	≤ 1.0			≥ 2.0	Pfizer

<sup>a</sup>S= Susceptible, I=Intermediate, R= Resistant, DS= Decreased susceptibility

**Table 3. *Neisseria gonorrhoeae* Antimicrobial Resistance Characterization Definitions**

Characterization	Description	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

In 2012, a total of 3,036 *N. gonorrhoeae* isolates were cultured in public health laboratories across Canada; 1,031 of these were submitted to the NML for antimicrobial susceptibility testing. A total of 987 were found to be resistant to at least one antibiotic tested; this translates to 32.5% of all *N. gonorrhoeae* cases diagnosed by culture as carrying antimicrobial resistance (987/3,036).

Limited demographic data were available for the isolates submitted for testing. Gender and age data available for 97.48% (1,005/1,031) of isolates tested at the NML (Table 4). Of these, 78.91% (793/1,005) were males ranging from 1 week to 79 years of age. A total of 21.10% (212/1,005) of isolates were from females aging 2 months to 66 years.

Anatomic source data were available for 51.79% (534/1,031) of the isolates sent to the NML (Table 5). Of these, 54.87% (293/534) were urethral, 19.66% (105/534) were rectal, 9.18% (49/534) were from the throat, 10.11% (54/534) were cervical, 5.43% (26/534) were vaginal and 1.31% (7/534) were from other sources.

**Table 4. Demographic Data for the *Neisseria gonorrhoeae* isolates tested at the NML, 2012 (N=1,031)**

Age	Male	Female	Not Specified	Totals
Under 15	1	9	0	10
15 - 20	77	60	1	138
21 - 25	200	46	2	248
26 - 35	256	51	8	315
36 - 45	128	27	3	158
46 - 55	98	111	4	113
56 - 65	24	7	0	31
65 +	9	1	0	10
Not Specified	3	0	5	8
<b>Total</b>	<b>796</b>	<b>212</b>	<b>23</b>	<b>1,031</b>

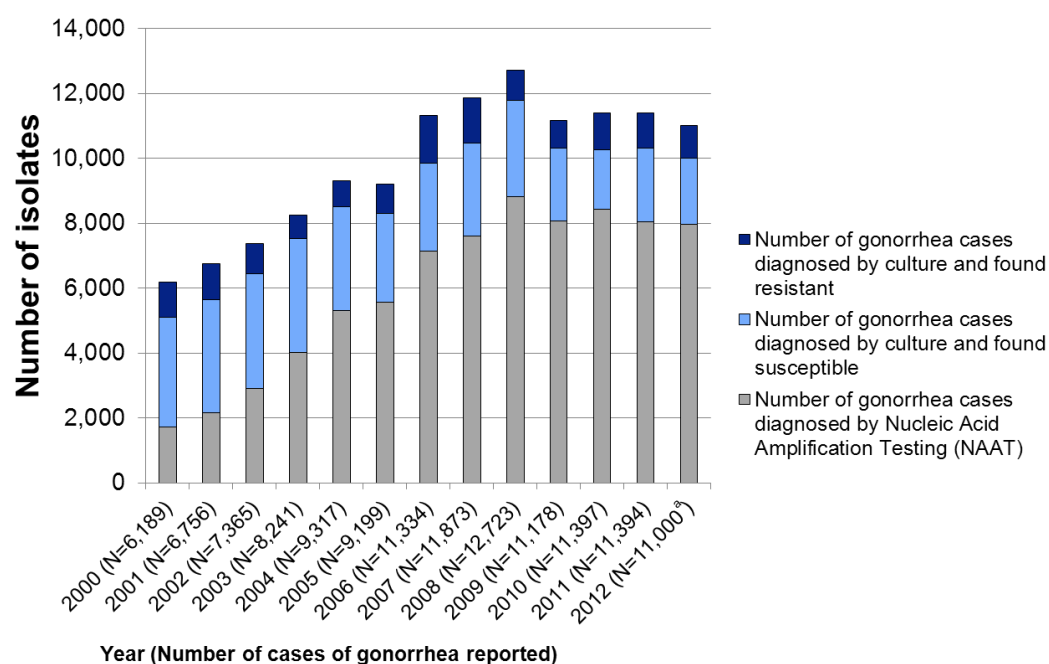
**Table 5. Anatomic Isolation Sites for the *Neisseria gonorrhoeae* isolates tested at the NML, 2012 (N=1,031)**

Isolation Site	Male	Female	Not Specified	Totals
Penis/ Urethra	293	n/a	0	293
Rectum	88	3	14	105
Throat	38	8	3	49
Cervix	n/a	54	0	54
Vagina	n/a	26	0	26
Other	3	4	0	7
Not Specified	374	117	6	497
<b>Total</b>	<b>796</b>	<b>212</b>	<b>23</b>	<b>1,031</b>

n/a=not applicable

Of the 5,282 viable isolates tested at the NML between 2008 and 2012, 94.68% (5,001) were found to be resistant to at least one of the following antibiotics: penicillin, tetracycline, ciprofloxacin, azithromycin, and erythromycin. A total of 5.32% (281/5,282) were found to be susceptible to all of these antibiotics. In 2012, 32.51% (987/3,036) 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 Appendix B.

**Figure 1. *Neisseria gonorrhoeae* Isolates in Canada, 2000 to 2012**



<sup>a</sup>number of gonorrhea cases reported in 2012 is estimated.

## TRENDS IN ANTIMICROBIAL SENSITIVITIES

In 2012, 15.28% (464/3,036) of isolates were classified as Chromosomal Mediated Resistant *Neisseria gonorrhoeae* (CMRNG), while the plasmid-mediated resistant strains occurred at lower rates. Penicillinase Producing *Neisseria gonorrhoeae* (PPNG) accounted for 0.59% (18/3,036) and Tetracycline Resistant *Neisseria gonorrhoeae* (TRNG) for 3.39% (103/3,036) of isolates (Figure 2).

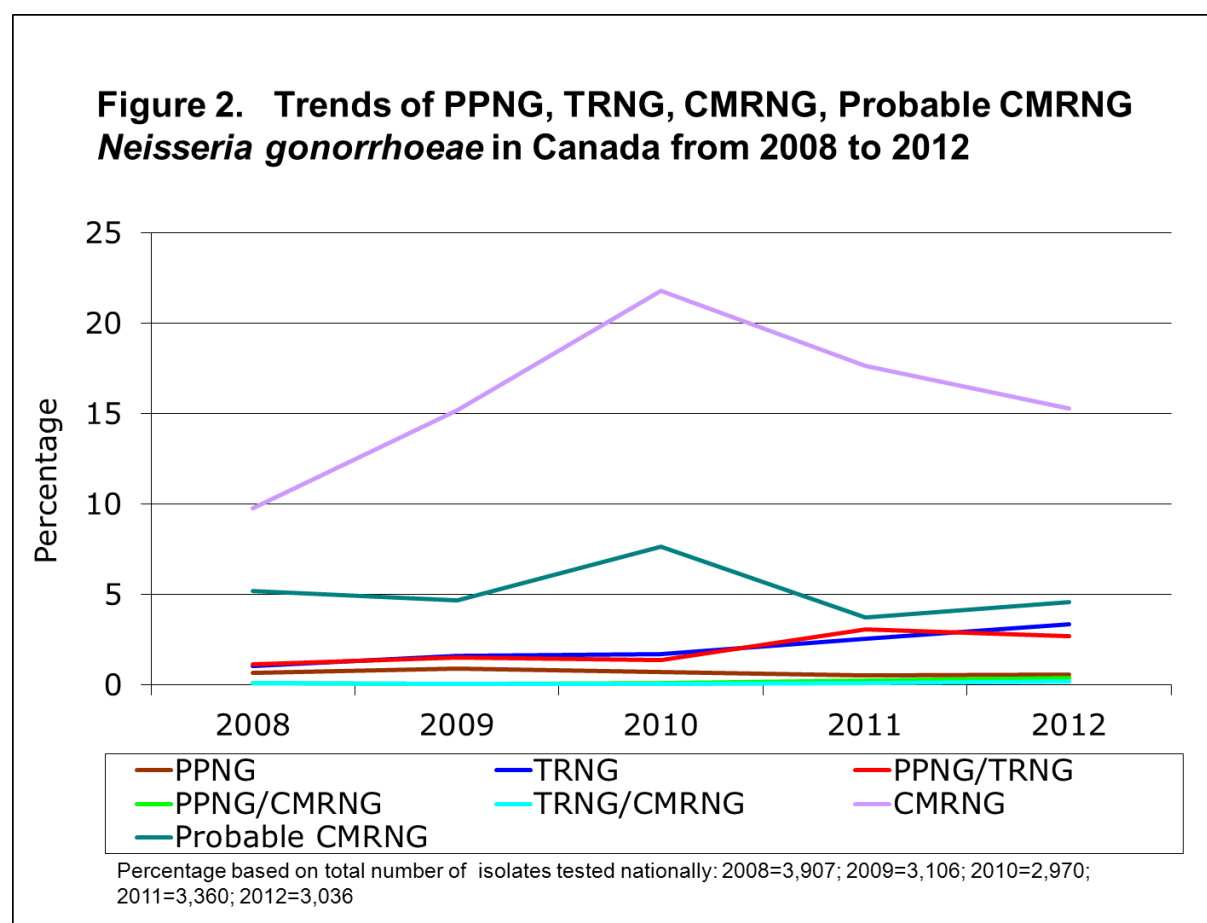
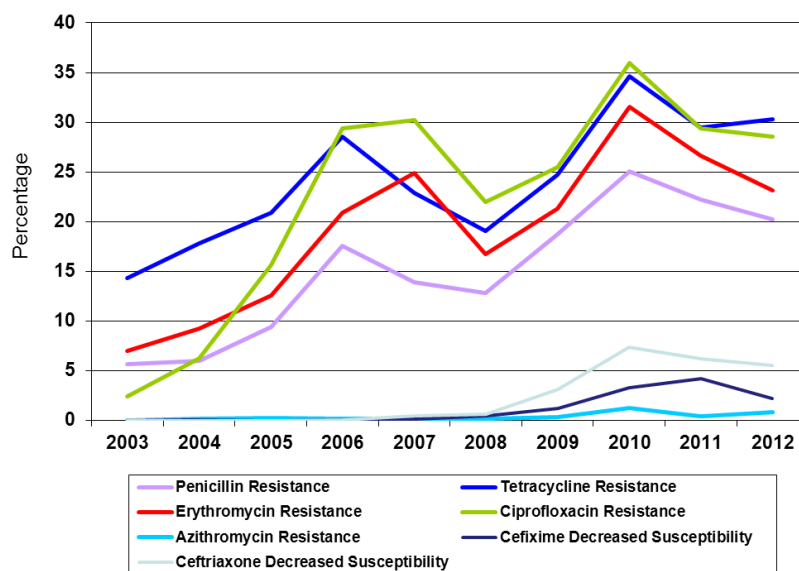


Figure 3 shows the trends of antimicrobial susceptibilities of *N. gonorrhoeae* tested in Canada from 2003 to 2012. In 2003, only 7.01% (297/4,235) of Canadian isolates were found to be erythromycin resistant. This percentage increased to 31.52%, (936/2,970) by 2010 but then decreased to 23.12% (702/3,036) by 2012. Strains with higher MICs to erythromycin also have higher MICs to azithromycin. The percentage of azithromycin resistant (MIC  $\geq 2$  mg/L) *N. gonorrhoeae* isolates increased from 0.12% (5/3,907) in 2008 to 0.86% (26/3,036) in 2012. Between 2009 and 2012, five isolates with high level azithromycin resistance (MIC  $\geq 256$  mg/L) were identified in Canada.

Penicillin and ciprofloxacin resistance followed a similar trend to erythromycin. Penicillin resistance increased from 5.67% (240/4,235) in 2003 to 25.05% (744/2,970) in 2010 and then decreased to 20.26% (615/3,036) in 2012. The ciprofloxacin resistance rate was at 2.41% (102/4,235) in 2003, increased to 35.93% (1,067/2,970) in 2010 and decreased to 28.52% (866/3,036) in 2012. Tetracycline resistance increased from 14.36% (608/4,235) in 2003 to 34.61% (1,028/2,970) in 2010. It decreased to 29.38% (987/3,360) in 2011 but then increased slightly to 30.3% (920/3,036) in 2012. Of the 5,282 viable isolates tested at NML between 2008 and 2012, none were identified with resistance to spectinomycin.

**Figure 3. Trends of Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Tested in Canada, 2003-2012**



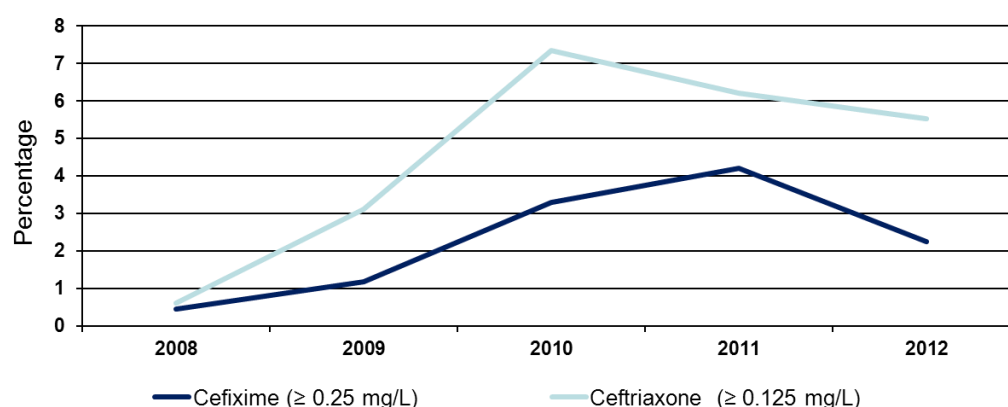
Percentage based on total number of isolates tested nationally: 2003=4,235; 2004=4,018; 2005=3,619; 2006=4,201; 2007=4,275; 2008=3,907; 2009=3,106; 2010=2,970; 2011=3,360; 2012=3,036

## THIRD GENERATION CEPHALOSPORINS

In 2012, according to WHO (20) definitions (MICs  $\geq 0.25$  mg/L for cefixime and  $\geq 0.125$  mg/L for ceftriaxone), 2.24% of isolates (68/3,036) were identified as having decreased susceptibility to cefixime and 5.53% (168/3,036) were identified as having decreased susceptibility to ceftriaxone. These rates are higher than they were in 2008 [0.46% (18/3,907) and 0.61% (24/3,907), respectively] but have decreased slightly since 2011 [4.17% (140/3,360) and 6.19% (208/3,360), respectively] (Figure 4).

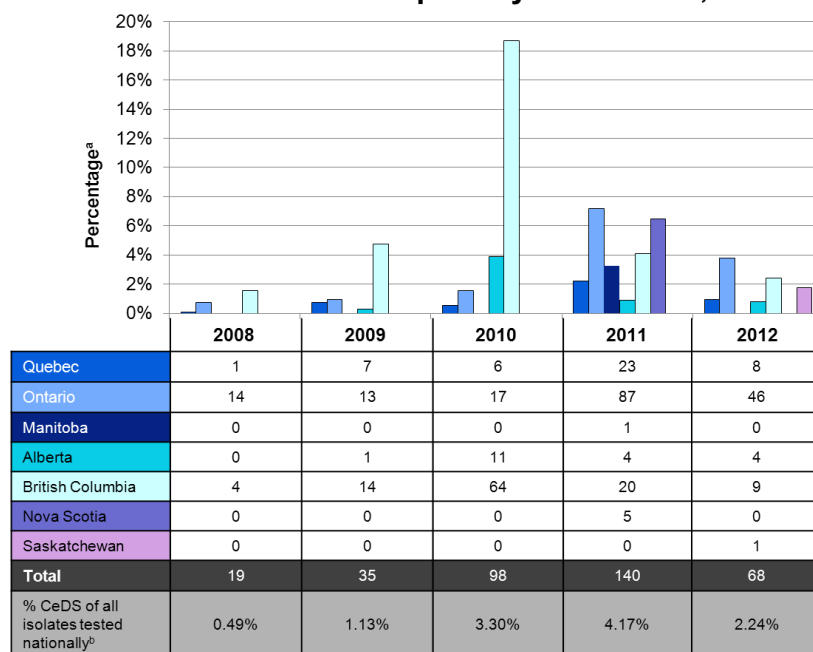
The geographical distribution of *N. gonorrhoeae* isolates with decreased susceptibility to cefixime and ceftriaxone within Canada are represented in Figures 5 and 6.

**Figure 4. *Neisseria gonorrhoeae* Isolates Received by the NML between 2008 and 2012 with Decreased Susceptibility to Cefixime and Ceftriaxone**



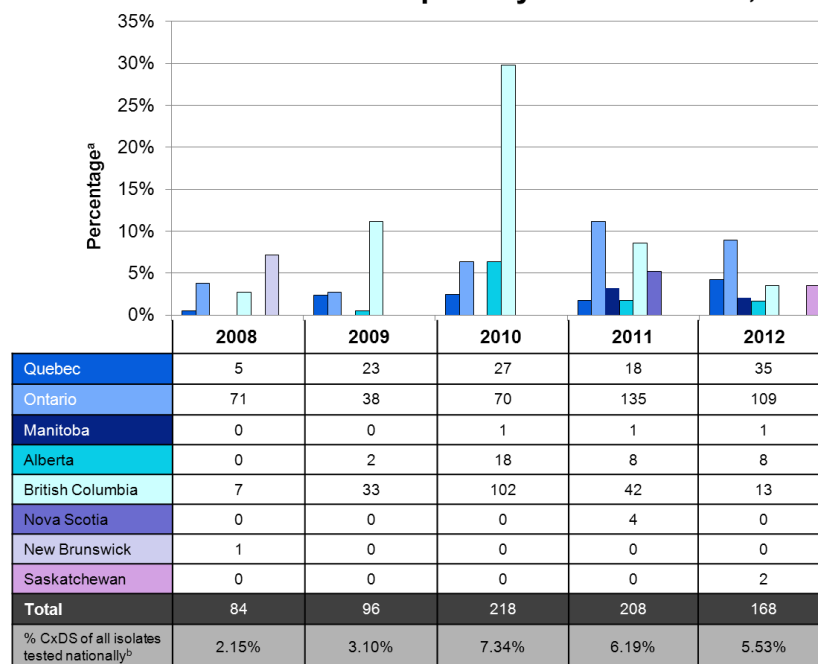
Percentage based on total number of isolates tested nationally: 2008=3,907; 2009=3,106; 2010=2,970; 2011=3,360; 2012=3,036



**Figure 5. Geographical Distribution of *Neisseria gonorrhoeae* Isolates with Decreased Susceptibility to Cefixime, 2008 to 2012**

<sup>a</sup>Denominators used to determine percentages are the number of cultures tested in each province listed in Appendix A.

<sup>b</sup>Percentage based on total number of isolates tested nationally: 2008=3,907; 2009=3,106; 2010=2,970; 2011=3,360; 2012=3,036

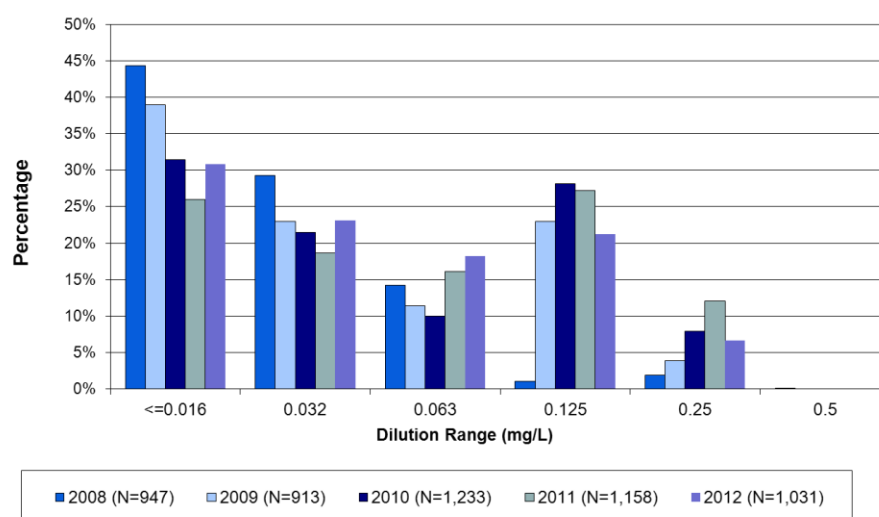
**Figure 6. Geographical Distribution of *Neisseria gonorrhoeae* Isolates with Decreased Susceptibility to Ceftriaxone, 2008 to 2012**

<sup>a</sup>Denominators used to determine percentages are the number of cultures tested in each province listed in Appendix A.

<sup>b</sup>Percentage based on total number of isolates tested nationally: 2008=3,907; 2009=3,106; 2010=2,970; 2011=3,360; 2012=3,036

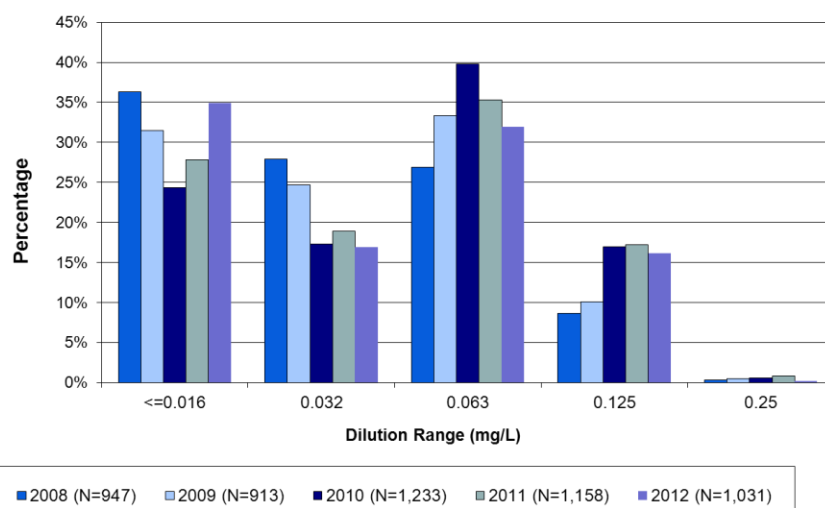
In 2008 the modal MIC for cefixime was 0.032 mg/L increasing to 0.125 mg/L in 2010 and 2011. In 2012 it reversed back to 0.032 mg/L. Ceftriaxone had a modal MIC of 0.032 mg/L in 2008, which increased to 0.063 mg/L in 2009 and has remained at 0.063 mg/L thru 2012 (Figures 7, 8 & 9).

**Figure 7. Trends of Cefixime Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by the NML from 2008 to 2012**

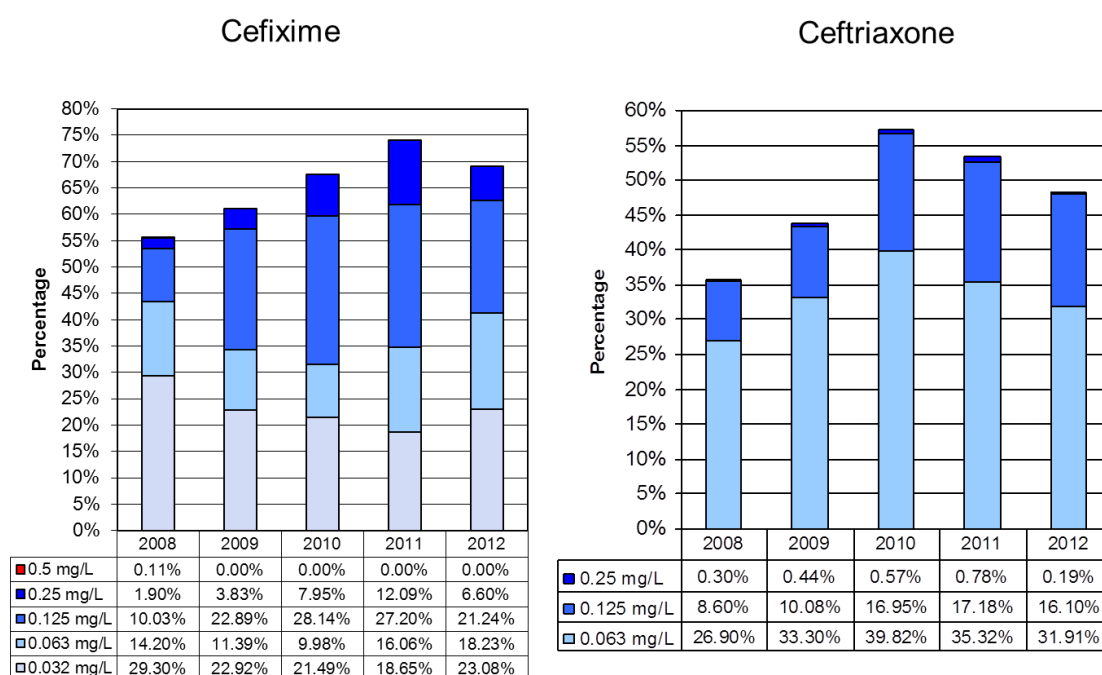


Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N).

**Figure 8. Trends of Ceftriaxone Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by the NML from 2008 to 2012**



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

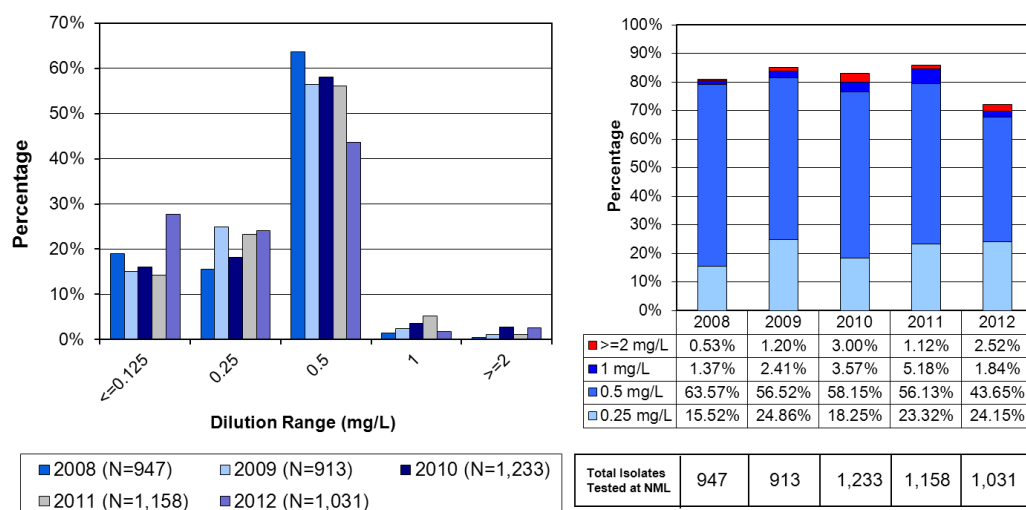
**Figure 9. Cefixime and Ceftriaxone Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by the NML from 2008 to 2012**

Percentages were calculated using the total number of viable isolates (both resistant and susceptible isolates) tested by NML as the denominator (N): 2008=947; 2009=913; 2010=1,233; 2011=1,158; 2012=1,031

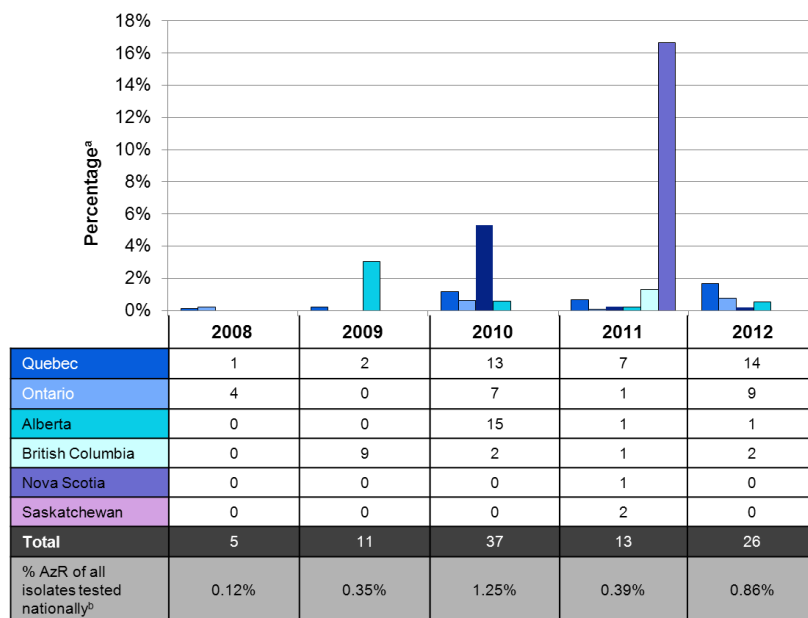
## AZITHROMYCIN

The percentage of all isolates tested nationally that were azithromycin resistant *N. gonorrhoeae* increased from 0.12% (5/3,907) in 2008 to 0.86% (26/3,036) in 2012. Overall, azithromycin resistant *N. gonorrhoeae* accounted for 0.56% (92/16,379) of all strains isolated between 2008 and 2012 (Figure 10). The modal MIC for azithromycin has remained at 0.5 mg/L each year since 2008. Each of the 92 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 11.

**Figure 10. Trends of Azithromycin Susceptibilities of *Neisseria gonorrhoeae* Isolates Received by the NML from 2008 to 2012**



**Figure 11. Geographical Distribution of Azithromycin Resistant *Neisseria gonorrhoeae* Isolates, 2008 to 2012**



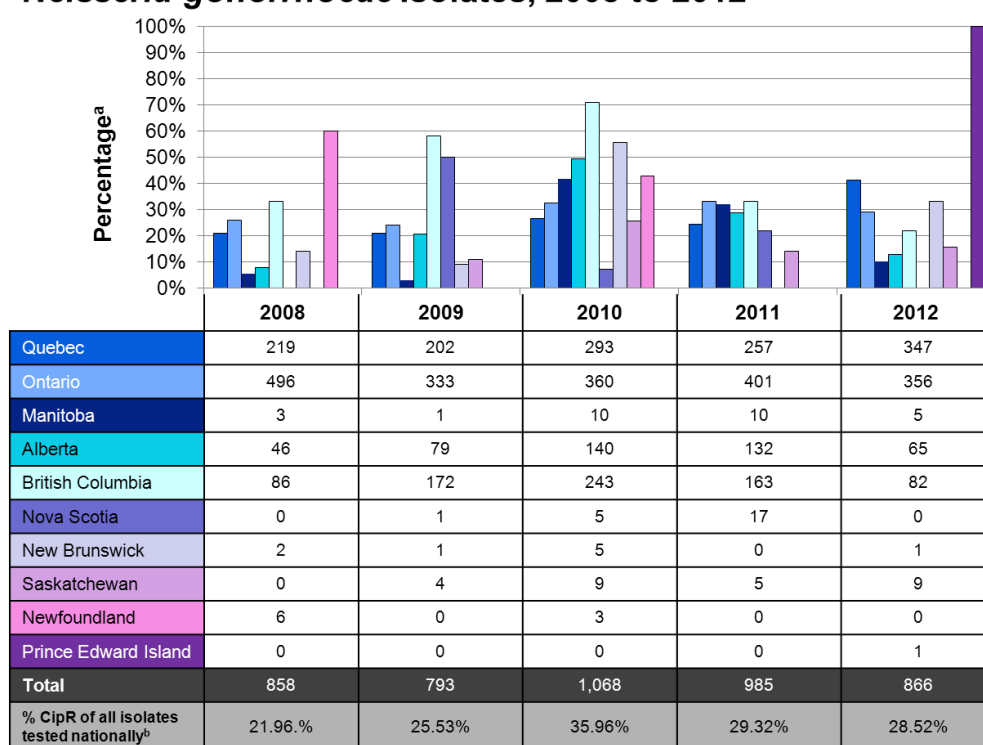
<sup>a</sup>Denominators used to determine percentages are the number of cultures tested in each province listed in Appendix A.

<sup>b</sup>Percentage based on total number of isolates tested nationally: 2008=3,907; 2009=3,106; 2010=2,970; 2011=3,360; 2012=3,036

## CIPROFLOXACIN

Ciprofloxacin resistant *N. gonorrhoeae* accounted for 27.9% (4,570/16,379) of all strains isolated nationally between 2008 and 2012. The percentage of isolates increased from 1.32% (59/4,458) in 2000 to 28.52% (866/3,036) in 2012. Percentage rates for each province are represented in Figure 12. The modal MIC of ciprofloxacin has shifted dramatically from 0.008 mg/L in 2004 to 4 mg/L in 2008 and now to 16.0 mg/L in 2012 (Table 13). Of the 866 ciprofloxacin resistant isolates identified in 2012, 94.92% (n=822) were also resistant to at least one other antibiotic; 54.85% (475/866) were characterized as CMRNG.

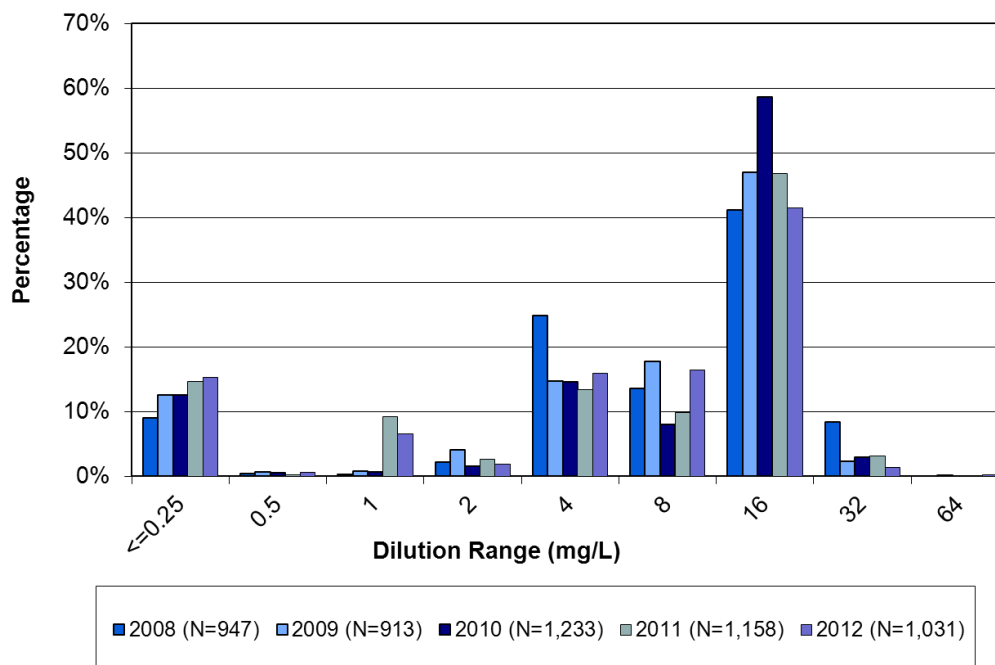
**Figure 12. Geographical Distribution of Ciprofloxacin Resistant *Neisseria gonorrhoeae* Isolates, 2008 to 2012**



<sup>a</sup>Denominators used to determine percentages are the number of cultures tested in each province listed in Appendix A.

<sup>b</sup>Percentage based on total number of isolates tested nationally: 2008=3,907; 2009=3,106; 2010=2,970; 2011=3,360; 2012=3,036

**Figure 13. Trends in Ciprofloxacin MICs of *Neisseria gonorrhoeae* Isolates, 2008 to 2012**

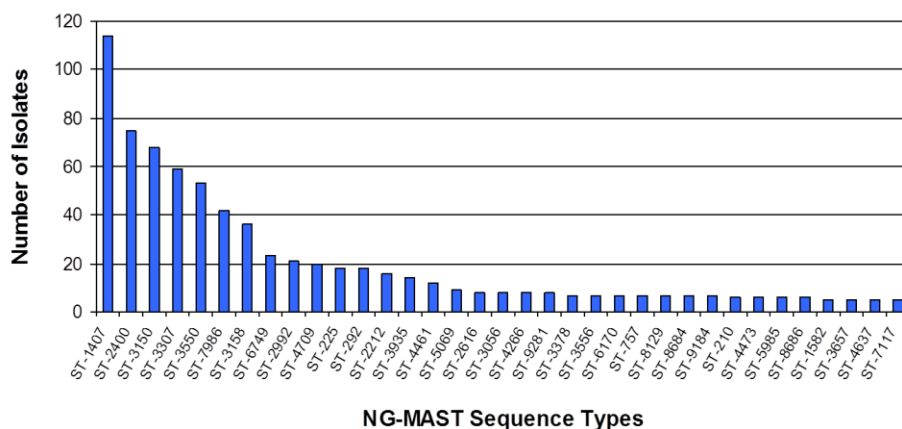


## NG-MAST

NG-MAST molecular-based sequence typing provides a substantial level of discrimination between isolates. In 2012, the most common sequence types (STs) identified by the NML were ST-1407, ST-2400 and ST-3150 at 11.06% (114/1,031), 7.27% (75/1,031) and 6.6% (68/1,031), respectively (Figure 14). These STs were also seen in previous years in Canada. ST-1407 is of particular interest since it is an internationally identified clone that has been described as a superbug, harboring high-level resistance to cephalosporins and is threatening the last recommended first-line therapy options for gonorrhea treatment (1,16,17,18). Isolates that are highly related to ST-1407 include ST-3158 (n=36), ST-2212 (n=16), ST-4461 (n=12), ST-8911 (n=11) plus 23 other STs with 1 to 8 isolates each. These highly related isolates are all characterized with the *tbpB*110 allele and have *por* alleles that differ by up to 5 nucleotide base pairs. A total of 23.57% of isolates (243/1,031) were either ST-1407 or highly related sub-types of ST-1407.



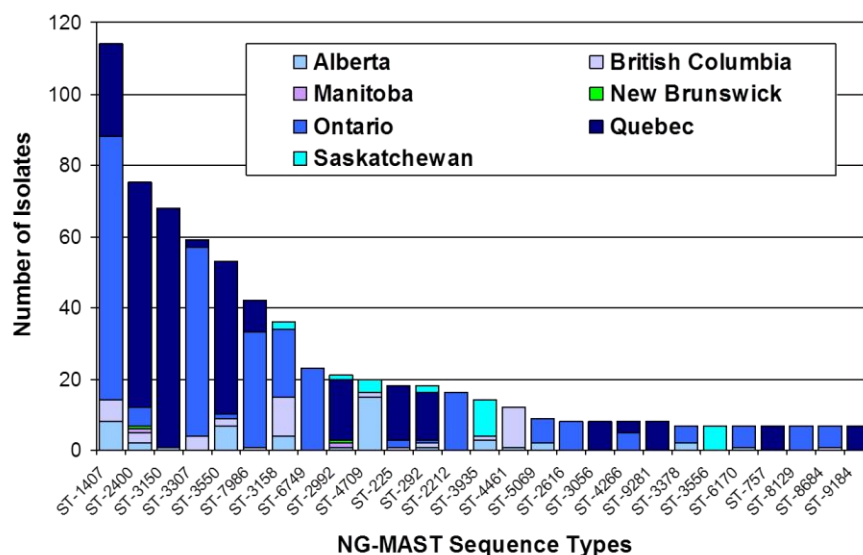
**Figure 14. Prevalent NG-MAST Sequence Type Distribution of *Neisseria gonorrhoeae* Isolates Received by the NML, 2012; N=1,031<sup>a</sup>**



<sup>a</sup>This graph represents 723 isolates. The remaining 308 isolates are dispersed among 223 sequence types (STs) containing 1 to 4 isolates each.

Distribution of STs within provinces is represented in Figures 15 and 16. ST-1407 was identified in four provinces including ON [64.91% (74/114)], QC [22.81% (26/114)], AB [7.02% (8/114)] and BC [5.26% (6/114)]. ST-2400, the next most prevalent ST type, was identified primarily in QC [84.0% (63/75)] followed by ON [6.67% (5/75)], BC [4.0% (3/75)], AB [2.67% (2/75)], MB and NB [1.33% each (1/75)]. All but one of the ST-3150 isolates were identified in QC [98.53% (67/68)] with the one being identified in AB [1.47% (1/68)].

**Figure 15. Provincial Distribution within *Neisseria gonorrhoeae* NG-MAST Sequence Types, 2012; N=1,031<sup>a</sup>**

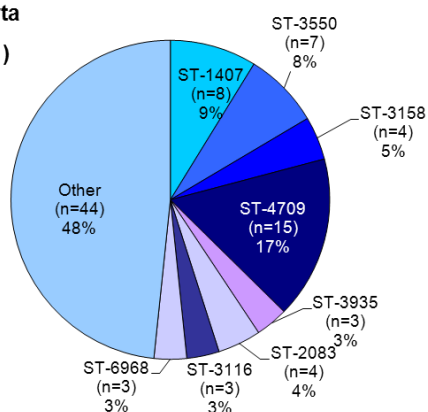


<sup>a</sup>This graph represents 679 isolates. The remaining 352 isolates are dispersed among 231 sequence types (STs) containing 1 to 6 isolates each.

**Figure 16. Distribution of *Neisseria gonorrhoeae* NG-MAST Sequence Types within Provinces, 2012; N=1,031<sup>a</sup>**

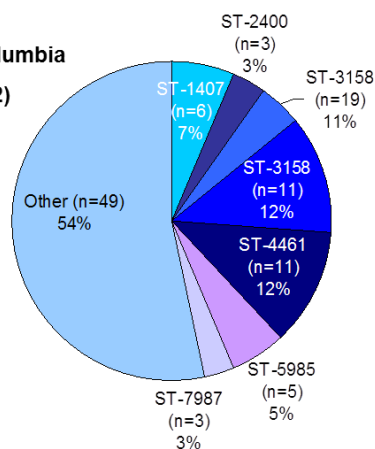
**Alberta**

(n=91)



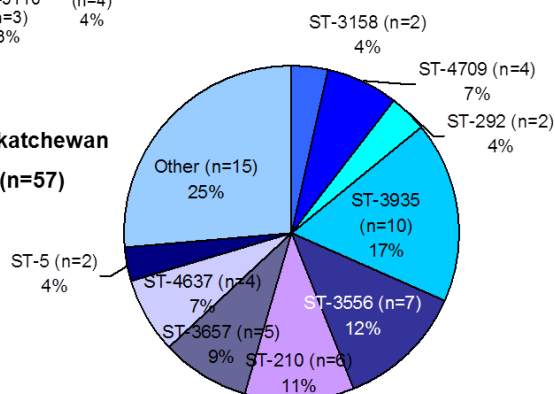
**British Columbia**

(n=92)



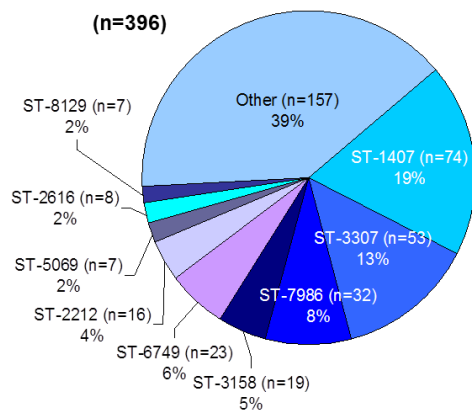
**Saskatchewan**

(n=57)



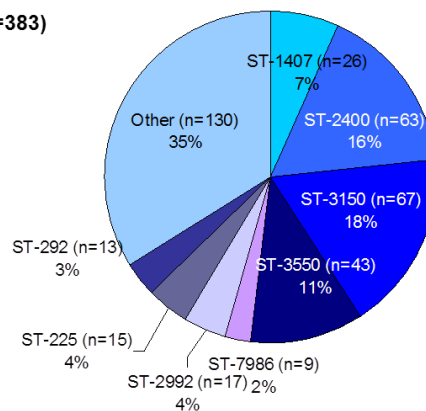
**Ontario**

(n=396)



**Quebec**

(n=383)



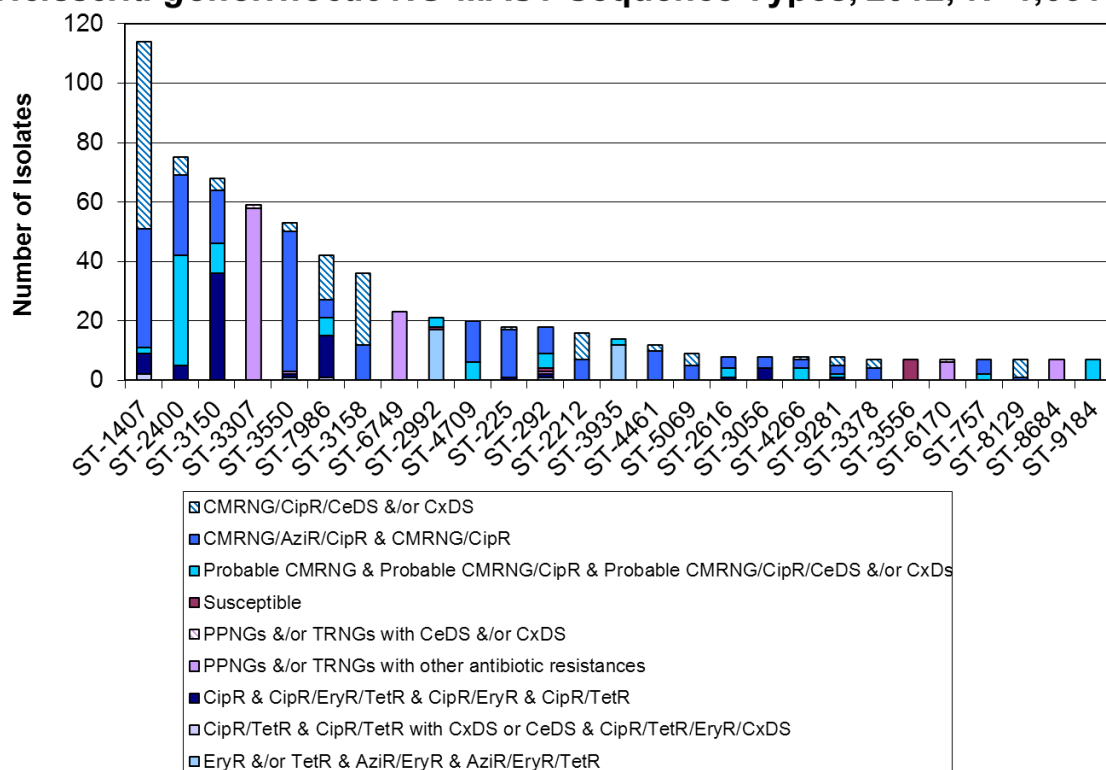
<sup>a</sup> **Manitoba (N=8):** ST-6154 (n=2), ST-2400 (n=1), ST-2992 (n=1), ST-340 (n=1), ST-5082 (n=1), ST-6633 (n=1), ST-7856 (n=1)

**New Brunswick (N=3):** ST-2400 (n=1), ST-2992 (n=1), ST-2 (n=1)

**Prince Edward Island (N=1):** ST-5005 (n=1)

Figure 17 provides a distribution of resistance characterizations among specific ST types. ST-1407 and ST-2400 are predominantly comprised of multi-drug resistant profiles including CMRNG/Probable CMRNG along with CipR and/or CeDS and/or CxDS.

**Figure 17. Distribution of Resistance Characterizations within *Neisseria gonorrhoeae* NG-MAST Sequence Types, 2012; N=1,031<sup>a</sup>**



The most prevalent STs of isolates with decreased susceptibility to cefixime and ceftriaxone were ST-1407 [48.53% (33/68) and 36.31% (61/168), respectively] followed by ST-3158 [16.18% (11/68) and 13.69% (23/168), respectively] and ST-2212 [7.35% (5/68)] for cefixime and ST-7986 [10.12% (17/168)] for ceftriaxone (Figure 18a & b). The ST types identified among the AziR isolates are displayed in Figure 18c. ST-2992 [30.76% (8/26)], ST-3158 [23.08% (6/26)] and ST-1407 [7.69% (2/26)] are the most prevalent. Of all the susceptible isolates available for testing, ST-3556 [18.18% (8/44)] was the most prevalent followed by ST-210 (13.64%, 6/44) and then ST-4637 [9.09% 4/44], (Figure 18d).

## Figure 18. NG-MAST Sequence Types of 2012 *Neisseria gonorrhoeae* Isolates

Figure 18a. Decreased Susceptibility to Cefixime (MIC  $\geq 0.25$  mg/L), n=68

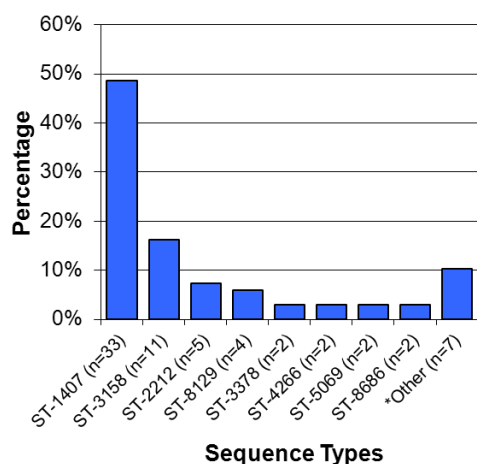


Figure 18b. Decreased Susceptibility to Ceftriaxone (MIC  $\geq 0.125$  mg/L), n=168

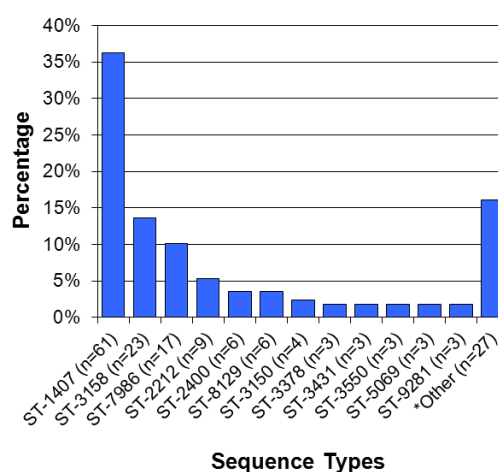


Figure 18c. Azithromycin Resistant (MIC  $\geq 2$  mg/L), n=26

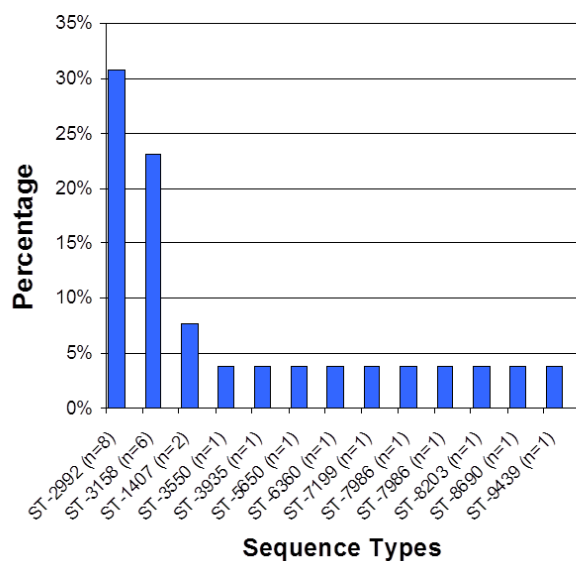
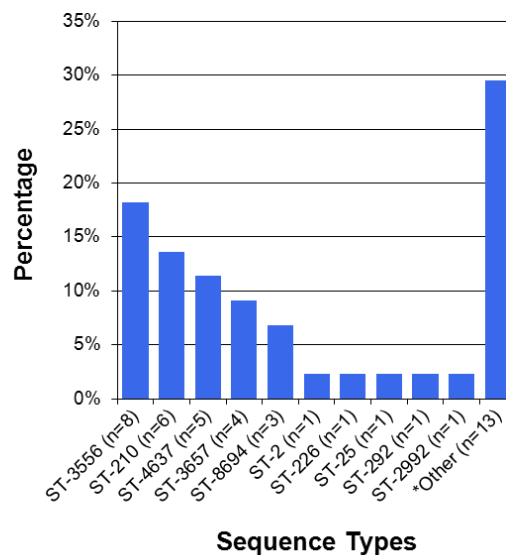


Figure 18d. Susceptible Isolates, n=44

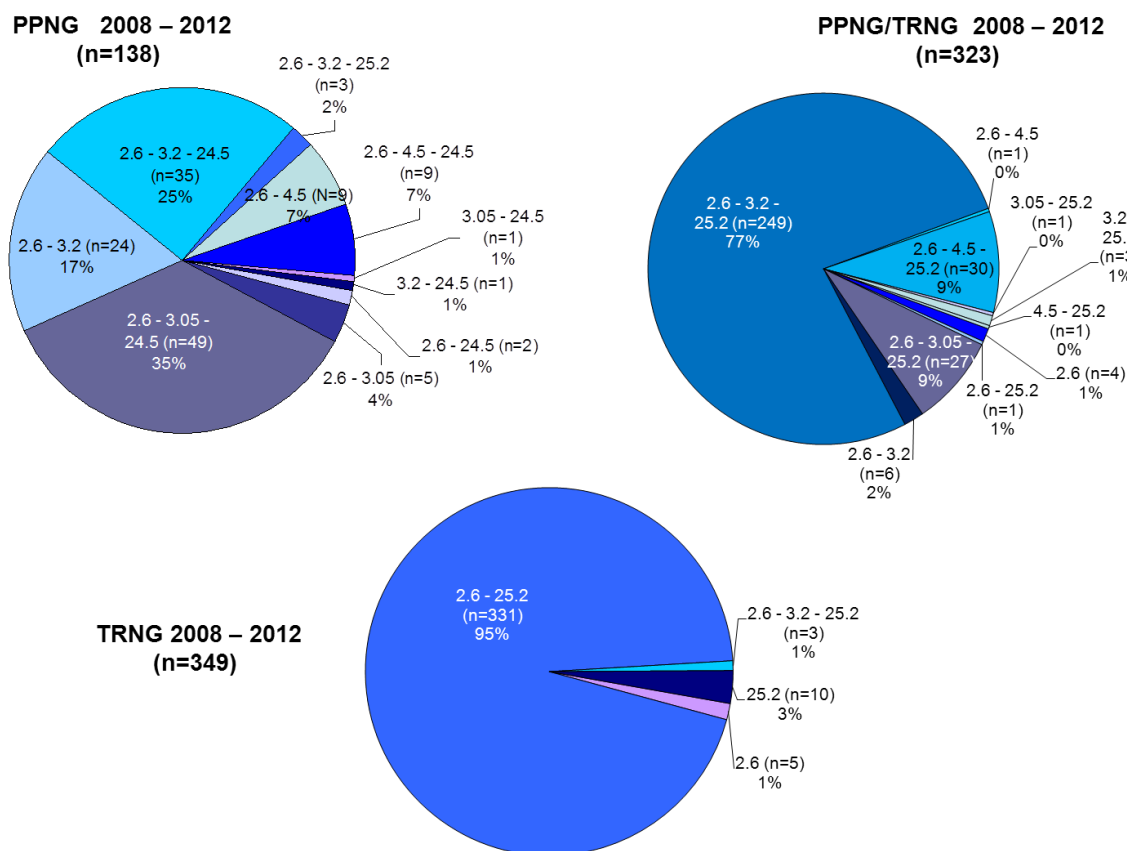


\*Other sequence types contain 1 isolate each.

## PLASMIDS

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. Between 2008 and 2012, the 3.2 Mda plasmid was the most common type amongst the 138 PPNG strains isolated at 45.65% (63/138), followed by the 3.05 Mda plasmid at 39.85% (55/138) and then the 4.5 Mda plasmid at 13.04% (18/138). 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 79.88% (258/323). 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 2008 and 2012, 97.13% (339/349) had the 2.6 and 25.2 Mda plasmids. TRNG isolates accounted for 82.96% of all the plasmid mediated resistance in *N. gonorrhoeae* between 2008 and 2012 (672/810 PPNG, PPNG/TRNG and TRNG strains).

**Figure 19. Plasmid Distribution within Antimicrobial Classifications of *Neisseria gonorrhoeae* Isolats Received by the NML, 2008 – 2012**



Plasmid sizes displayed in megadaltons (Mda).

## CONCLUSION

Continued surveillance and monitoring of the antimicrobial susceptibilities and sequence types of *N. gonorrhoeae* is necessary to inform and subsequently mitigate the impact of antimicrobial resistance in gonorrhea. It 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. Surveillance is also necessary for early detection of novel and emerging resistances. Enhancing surveillance to include linked epidemiological and laboratory data will assist with the limitations in the current passive surveillance system regarding data representativeness and interpretation. 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 A

*Neisseria gonorrhoeae* culture isolates in Canada, 2008 – 2012<sup>a</sup>

Province	2008 GC Cultures			2009 GC Cultures			2010 GC Cultures			2011 GC Cultures			2012 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	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	258	104	40.31%	296	183	61.82%	342	256	74.85%	490	176	35.92%	372	92	24.73%
Alberta	583	55	9.4%	383	90	23.50%	283	164	57.95%	457	156	34.14%	497	94	18.91%
Saskatchewan	55	1	1.8%	36	30	83.33%	35	11	31.43%	35	35	100.00%	57	57	100.00%
Manitoba	53	3	5.7%	34	3	8.82%	24	11	45.83%	31	12	38.71%	49	8	16.33%
Ontario	1,900	539	28.4%	1,383	383	27.69%	1,101	383	34.79%	1,212	428	35.31%	1,218	403	33.09%
Québec	1034	230	22.2%	959	216	22.52%	1,100	335	30.45%	1,045	282	26.99%	838	390	46.54%
New Brunswick	14	5	35.7%	11	4	36.36%	9	9	100.00%	12	6	50.00%	3	3	100.00%
Nova Scotia	0	0	n/a	103	2	1.94%	69	69	100.00%	77	77	100.00%	0	0	n/a
Newfoundland	10	10	100.0%	2	2	100.00%	7	7	100.00%	1	0	0.00%	1	0	0.00%
Prince Edward Island	0	0	n/a	0	0	n/a	0	0	n/a	0	0	n/a	1	1	100.00%
<b>Totals</b>	<b>3,907</b>	<b>947</b>	<b>24.2%</b>	<b>3,207</b>	<b>913</b>	<b>28.5%</b>	<b>2,970</b>	<b>1,245</b>	<b>41.9%</b>	<b>3,360</b>	<b>1,172</b>	<b>34.9%</b>	<b>3,036</b>	<b>1,048</b>	<b>34.5%</b>

<sup>a</sup>no *Neisseria gonorrhoeae* cultures were reported to the NML or received from the Northwest Territories, Nunavut or the Yukon in 2008 to 2012.

n/a-not applicable

## APPENDIX B

Characterization of all *Neisseria gonorrhoeae* Isolates Submitted to the NML, 2008-2012

Characterization		2008	2009	2010	2011	2012	Totals
Plasmid Mediated Resistances	PPNG	10	8	7	2	3	30
	PPNG/CipR	13	11	10	9	6	49
	PPNG/EryR	3	2	1	6	1	13
	PPNG/EryR/CipR	0	0	1	0	2	3
	PPNG/TetR	1	3	1	0	2	7
	PPNG/TetR/CipR	0	2	1	1	4	8
	PPNG/CMRNG	0	0	0	2	2	4
	PPNG/CMRNG/CipR	5	3	3	6	10	27
	PPNG/CMRNG/CxDS/CipR	0	0	0	1	0	1
	PPNG/TRNG	10	11	0	2	11	34
	PPNG/TRNG/CeDS/CipR	0	0	0	2	0	2
	PPNG/TRNG/CeDS/CipR/EryR	0	0	0	6	0	6
	PPNG/TRNG/CeDS/CxDS/CipR/EryR	0	0	0	3	0	3
	PPNG/TRNG/CipR	31	33	32	43	49	188
	PPNG/TRNG/EryR	0	1	0	0	0	1
	PPNG/TRNG/CipR/EryR	4	4	7	34	22	71
	PPNG/TRNG/CxDS/CipR/EryR	0	0	2	14	0	16
	PPNG/TRNG/AziR/CipR/EryR	0	1	0	0	0	1
	PPNG/TRNG/AziR/CipR/CxDS/EryR	0	1	1	0	0	2
	TRNG	29	28	12	21	36	126
	TRNG/CipR	8	12	22	15	16	73
	TRNG/CipR/EryR	2	4	11	36	47	100
	TRNG/CipR/EryR/CeDS	0	0	0	1	0	1
	TRNG/CipR/EryR/CeDS/CxDS	0	0	0	1	0	1
	TRNG/CipR/EryR/CxDS	1	0	0	8	0	9
	TRNG/CxDS	0	0	0	0	1	1
	TRNG/EryR	0	5	6	1	1	13
	TRNG/CipR/PenR	1	0	0	1	0	2
	TRNG/CxDS/CipR	0	0	0	1	1	2
	TRNG/CMRNG	0	0	0	0	1	1
	TRNG/CMRNG/CipR	5	2	1	2	2	12
	TRNG/CMRNG/CxDS/CipR	0	0	1	2	3	6
	TRNG/PenR	0	0	0	1	1	2
Total		123	131	119	221	221	815

Characterization of all *Neisseria gonorrhoeae* isolates submitted to the NML, 2008-2012 (continued)

Characterization		2008	2009	2010	2011	2012	Totals
Chromosomal Mediated Resistances	AziR/EryR	0	0	21	5	0	26
	AziR/EryR/TetR	0	1	2	2	14	19
	AziR/CipR/EryR	0	0	0	0	0	0
	AziR/EryR/PenR	0	0	0	1	0	1
	CeDS/CipR/EryR	0	0	1	0	0	1
	CeDS/CipR/TetR	0	0	0	0	1	1
	CeDS/CxDS/CipR/EryR	1	0	0	0	0	1
	CeDS/CipR/PenR/TetR	0	0	0	1	0	1
	CxDS/CipR/EryR/TetR	0	0	0	1	1	2
	CipR	84	40	54	34	44	256
	CipR/CeDS	0	0	0	0	0	0
	CipR/CxDS	3	0	1	0	0	4
	CipR/EryR	62	37	10	16	3	128
	CipR/EryR/TetR	8	8	15	9	4	44
	CipR/PenR	2	0	0	0	0	2
	CipR/PenR/TetR	0	0	0	0	2	2
	CipR/TetR	48	26	26	23	56	179
	CipR/TetR/CxDS	0	0	1	0	3	4
	EryR	0	1	0	9	3	13
	EryR/TetR	0	0	3	16	10	29
	PenR	1	1	0	0	0	2
	PenR/TetR	2	0	0	0	0	2
	TetR	9	9	9	17	21	65
	TetR/CxDS	0	0	0	0	0	0
	TetR/CeDS	0	0	0	0	0	0
	CMRNG	3	3	3	2	4	15
	CMRNG/AziR/CipR	4	8	8	5	5	30
	CMRNG/AziR/CeDS/CxDS/CipR	0	0	0	0	1	1
	CMRNG/AziR/CipR/CxDS	0	0	2	0	6	8
	CMRNG/CipR	302	367	443	387	294	1,793
	CMRNG/AziR	0	0	1	0	0	1
	CMRNG/CeDS/CipR	2	3	8	32	7	52
	CMRNG/CeDS/CxDS/CipR	11	32	79	88	55	265
	CMRNG/CxDS/CipR	60	60	104	80	92	396
	Probable CMRNG	5	6	4	3	9	27
	Probable CMRNG/AziR/CipR	1	0	2	0	0	3
	Probable CMRNG/CeDS	0	0	0	0	1	1
	Probable CMRNG/CipR	188	137	194	110	123	752
	Probable CMRNG/CeDS/CipR	2	0	0	5	2	9
	Probable CMRNG/CeDS/CxDS/CipR	3	0	10	1	1	15
	Probable CMRNG/CxDS/CipR	5	3	17	7	4	36
	Susceptible Strain	18	40	96	83	44	281
	Total	824	782	1,114	937	810	4,467
Total	947	913	1,233	1,158	1,031	5,282	

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