# Canada

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### ABSTRACT

Background: Neisseria gonorrhoeae have acquired resistance to many antibiotics and developed decreasing susceptibilities to third generation cephalosporins. Method: NG-MAST sequence types and minimum inhibitory concentrations (MICs) were determined by agar dilution for 3422 N. gonorrhoeae isolates submitted to the National Microbiology Laboratories identify resistance to at least one antibiotic or if the provincial laboratories do not conduct antimicrobial susceptibility testing. MIC interpretations were based on the criteria of the Clinical Laboratory Standards Institute (CLSI) and the World Health Organization (WHO) criteria for decreased susceptibility to cephalosporins. Results: Among all the isolates tested in Canada during 2010-2012, 22.0% (2103/9366) to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefixime (MIC  $\geq$  0.25 mg/L) was identified in 3.3% (98/2970) of isolates in 2010; this increased to 4.2% to erythromycin. Decreased susceptibility to cefix and the erythromycin. Decreas (140/3360) in 2011 and decreased again to 2.2% (68/3036) in 2012. In 2010, 249 sequence types (STs) were identified: the most common STs were ST1407, ST3150 and ST3158 at 13.3%, 11.3% and 9.0% respectively. In 2010, 249 sequence types (STs) were identified: the most common STs were ST1407, ST3150 and ST3158 at 13.3%, 11.3% and 9.0% respectively. In 2010, 249 sequence types (STs) were identified: the most common STs were ST1407, ST3150 and ST3158 at 13.3%, 11.3% and 9.0% respectively. In 2010, 249 sequence types (STs) were identified: the most common STs were ST1407, ST3150 and ST3158 at 13.3%, 11.3% and 9.0% respectively. 2011, 238 STs were identified: the most common STs were ST1407, ST3307 and ST3550 at 15.3%, 9.3% and 5.9% respectively. In 2012, 258 STs were ST1407, ST2400 and ST3150 at 11.1%, 7.3% and 6.6% respectively. Conclusions: Comparing 2010 and 2012 there has been a decline in the proportion of N. gonorrhoeae isolates in Canada is imperative and is associated with modifications of treatment guidelines.

### INTRODUCTION

Neisseria gonorrhoeae, the causative agent of gonorrhea remains a global public health issue and is the second most commonly reported bacterial sexually transmitted infection in Canada with over 12,000 cases reported in 2012 (Public Health Agency of Canada, unpublished data).

Canada conducts surveillance of antimicrobial susceptibilities in N. gonorrhoeae isolates to support development of treatment guidelines. The Canadian STI Guidelines have been evolving; combination gonorrhea therapy with 250 mg ceftriaxone intramuscularly plus azithromycin 1 g orally is recommended as the first-line regimen in the men-who-havesex-with men population and in pharyngeal infections (Public Health Agency of Canada 2011).

*N. gonorrhoeae* has evolved over the years and developed resistance to many of the antibiotics used to treat it including penicillins, tetracyclines, macrolides and quinolones. There are reports of decreased susceptibilities to third generation cephalosporins and treatment failures (Allen et al, 2013; Unemo et al, 2012; Ohnishi et al, 2011). In addition to monitoring the antimicrobial susceptibilities of *N. gonorrhoeae* it is also important to follow the spread of antimicrobial resistant isolates by characterizing each isolate. *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) is a highly discriminatory molecular typing method based on the sequences of the *por* (encoding the gonococcal outer membrane porin) and tbpB (encoding the  $\beta$ subunit of the transferrin-binding protein) genes. NG-MAST can be used to monitor the spread of antimicrobial resistant clones, to determine a re-infection vs treatment failure in a test-of-cure case, and to identify transmission patterns within sexual networks (Unemo, 2011). In this study we present the antimicrobial susceptibilities and sequence type distributions of N. gonorrhoeae isolated in 2010-2012 from across Canada. A baseline of circulating sequence types is established and antimicrobial resistant phenotypes within the most prevalent sequence types are identified

### METHOD

A total of 9366 N. gonorrhoeae isolates were cultured across Canada between 2010 and 2012, and of those 3422 viable isolates were submitted to the National Microbiology Laboratory, Public Health Agency of Canada as part of the National *N. gonorrhoeae* Surveillance Program for antimicrobial susceptibility testing. Strains submitted to the Surveillance Program are almost all resistant to at least one antibiotic tested.

The antibiotic susceptibilities to penicillin, tetracycline, erythromycin, cefixime, ceftriaxone, azithromycin, ciprofloxacin, and spectinomycin were determined using agar dilution as previously described (Martin et al. 2011). Interpretation of the MIC was based on the criteria of the Clinical Laboratory Standards Institute (CLSI, 2013) except for erythromycin (Ehret, 1996) and azithromycin (CDC, 2007). Recent WHO guidelines have provided updated cephalosporin definitions: cefixime decreased susceptibility MIC≥0.25 mg/L and ceftriaxone decreased susceptibility MIC ≥0.125 mg/L (World Health Organization 2012). Plasmid profiles were determined (Promega Wizard Plus, Fisher Scientific, Ottawa, Ontario) and ß-lactamase production was analyzed using nitrocefin (Oxoid Ltd. Ottawa, Ontario).

NG-MAST was done as previously described (Martin et al. 2004). Concatenated sequences of the *por* and *tbp*B alleles were created for the most prevalent STs identified in this study to create a phylogenetic tree.

## Multi-Antigen Sequence Types (NG-MAST) and Antimicrobial Susceptibilities on Neisseria gonorrhoeae isolated in Canada, 2010-2012



2012=1031). The remaining 1751 isolates are dispersed among 599 sequence types (STs) containing 1 to 51 isolates each

Description	Definition
Penicillinase Producing Neisseria gonorrhoeae	Pen MIC $\geq$ 2.0 mg/L, $\beta$ -lactamase positive, $\beta$ -lactamase plasmid (3.05, 3.2 or 4.5 Mdal plasmid)
Tetracycline Resistant Neisseria gonorrhoeae	Tet MIC ≥ 16.0 mg/L, 25.2 Mdal plasmid, TetM PCR positive
Chromosomal Mediated Resistant <i>Neisseria</i> gonorrhoeae	Pen MIC $\ge$ 2.0 mg/L, Tet MIC $\ge$ 2.0 mg/L but $\le$ 8.0 mg/L, and Ery MIC $\ge$ 2.0 mg/L
Probable Chromosomal Mediated Resistant <i>Neisseria gonorrhoeae</i>	One of the MIC values of Pen, Tet, Ery = 1 mg/L, the other two $\ge 2.0$ mg/L
Penicillin Resistant Neisseria gonorrhoeae	Pen MIC $\geq$ 2.0 mg/L, $\beta$ -lactamase negative
Tetracycline Resistant Neisseria gonorrhoeae	Tet MIC $\geq$ 2.0 mg/L but $\leq$ 8.0 mg/L
Erythromycin Resistant Neisseria gonorrhoeae	Ery MIC ≥ 2.0 mg/L
Ciprofloxacin Resistant Neisseria gonorrhoeae	Cip MIC ≥ 1.0 mg/L
Azithromycin Resistant Neisseria gonorrhoeae	Az MIC ≥ 2.0 mg/L
Spectinomycin Resistant <i>Neisseria gonorrhoeae</i>	Spec R ≥ 128 mg/L
<i>Neisseria gonorrhoeae</i> with Decreased Susceptiblity to Ceftriaxone	$Cx MIC \ge 0.125 mg/L$
<i>Neisseria gonorrhoeae</i> with Decreased Susceptiblity to Cefixime	Ce MIC ≥ 0.25 mg/L



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### RESULTS

In 2012, 32.5% (987/3036) of all *N. gonorrhoeae* isolates tested in all jurisdictions across Canada were found to be resistant to at least one antibiotic.

In 2012, the resistance to penicillin was 20.3% (615/3036), tetracycline was 30.3% (920/3036), erythromycin was 23.1% (702/3036), ciprofloxacin was 28.5% (866/3036) and azithromycin was 0.86% (26/3036).

The MICs of the 3rd generation cephalosporins have been increasing over time. There has been a shift in the modal MICs of ceftraxone 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.

The percentage of isolates with decreased susceptibility to cefixime (MIC ≥0.25) mg/L) was 0.46% in 2008. This increased to 4.2% (140/3360) by 2011 and decreased to 2.2% (68/3036) in 2012.

The percentage of isolates with decreased susceptibility to ceftriaxone (MIC ≥ 0.125 mg/L) was 0.61% in 2008. This increased to 7.2% (218/2970) by 2010 and decreased to 6.2% (208/3360) in 2011 and then again to 5.5% (168/3036) in 2012.

The percentage of azithromycin resistant (MIC ≥2 mg/L) *N. gonorrhoeae* isolates increased from 0.12% (5/3907) in 2008 to 0.86% (26/3036) in 2012.

In 2010, ST1407, ST3150 and ST3158 were the most common STs representing 13.3%, 11.3% and 9.0% respectively. In 2011, ST1407, ST3307 and ST3550 were the most common at 15.3%, 9.3% and 5.9% respectively. In 2012, ST1407, ST2400 and ST3150 were the most prevalent at11.1%, 7.3% and 6.6%, respectively. Isolates that are ST1407 or an ST that is closely related to it have resistance to

multiple antibiotics and are more likely to be CeDS and/or CxDS.

### **CONCLUSION**

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 multidrug resistant gonorrhea. Over time, N. gonorrhoeae has acquired resistance to many antibiotics such as penicillin, tetracycline, erythromycin and ciprofloxacin

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 susceptiblity testing) to Nucleic Acid Amplification Test (NAAT) for the diagnosis of gonorrhea.

Strengthening surveillance mechanisms and correlating antibiotic susceptibilities of *N. gonorrhoeae* with treatment failure is important to ensure that treatment guidelines deal with the decreasing susceptibilities to the recommended treatment for gonorrhea infections and that emerging resistances are identified.

Canada's most common ST type, ST1407 is the internationally identified clone that has been described as a superbug, harboring high-level resistance to both cefixime and ceftriaxone and is threatening the last options for first-line treatment of gonorrhea.

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