# External Quality Assurance and Comparability of Antimicrobial Susceptibility Testing of *Neisseria gonorrhoeae* in Canada

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## ABSTRACT

**Background:** The National Microbiology Laboratory (NML), Public Health Agency of Canada conducts ongoing monitoring of antimicrobial susceptibilities in *Neisseria gonorrhoeae*. To standardize the susceptibility testing data and maintain the comparability of data generated from each province in Canada, the NML offers a proficiency testing program conducted two times a year.

**Method**: Eight provincial public health laboratories from across Canada participated in 10 proficiency panel distributions between 2008 and 2012. Control strains included a combination of 4 of the following isolates: WHO B, WHO C, WHO F, WHO K, ATCC 49226. Each distribution included 5 *N. gonorrhoeae* test isolates. Minimum inhibitory concentrations (MICs) were determined using either the agar dilution testing method or the Etest method. MIC interpretations were based on the criteria of the Clinical Laboratory Standards Institute (CLSI, 2013) for all antibiotics except azithromycin (CDC, 2007), erythromycin (Ehret, 1996) and decreased susceptibility to cephalosporins (WHO, 2012).

**Results:** A total of 90 isolates, including control strains and test isolates were included from 2008 to 2012. The overall agreement of MIC results (MICs±1 $\log_2$ dilutions) between all laboratories when compared to the calculated modal MICs was 95.1% (4,243/4,462). Percentage agreements between agar dilution and Etest MIC results were calculated for all strains tested by all laboratories during this time with the following results: penicillin, 100% (51/51); spectinomycin, 98.4% (61/62); tetracycline, 82.3% (56/68); ceftriaxone, 93.1% (54/58); ciprofloxacin, 96.7% (58/60); cefixime 100% (23/23); and azithromycin 98.5% (67/68); erythromycin, 100% (28/28).

**Conclusions**: Laboratories participating in this proficiency testing program achieved a degree of correlation of greater than 95% for antimicrobial susceptibility testing results. Participating in a proficiency testing program is beneficial to each laboratory as possible discrepancies in results are identified giving each laboratory the opportunity to improve testing protocols. This contributes to improved quality of results and leads to better quality patient care and public health prevention programs.

## INTRODUCTION

*Neisseria gonorrhoeae*, the bacterial agent causing gonorrhea infection, is the second most common bacterial pathogen in Canada with over 12,000 reported cases in 2012 (Public Health Agency of Canada, unpublished data). Worldwide cases are estimated at 106 million (World Health Organization, 2012). Symptoms include pelvic inflammatory disease, infertility and ectopic pregnancy in women and epididymitis in men (Public Health Agency of Canada, 2008). Gonorrhea infection has been associated with an increased risk of HIV infection and transmission. (Lewis DA, 2010).

Over time, gonorrhea has developed resistance to many of the antimicrobials used for treatment. Monitoring the antimicrobial susceptibilities of gonorrhea through surveillance programs is of upmost importance as data will inform treatment guidelines. In 2012, in response to decreasing susceptibilities to the 3rd generation cephalosporins (Martin, 2011) and reports of cefixime treatment failures (Allen, 2013), Canada updated its treatment guideline to combination gonorrhea therapy with 250 mg ceftriaxone intramuscularly and azithromycin 1 g orally as the first-line regimen in the men-who-have-sex-with men population and in pharyngeal infections (Public Health Agency of Canada 2011).

The National Microbiology Laboratory (NML) at the Public Health Agency of Canada has conducted ongoing monitoring of antimicrobial susceptibilities in *N. gonorrhoeae* isolates as part of the National *Neisseria gonorrhoeae* Surveillance Program since 1985. Provincial public health laboratories across Canada also monitor antimicrobial susceptibilities and do so using different methodologies. To standardize the susceptibility testing data and maintain the comparability of data generated from each province, the NML offers a proficiency testing program conducted two times a year.

## METHOD

**Participant Performance:** Eight provincial public health laboratories from across Canada participated in 10 proficiency panel distributions between 2008 and 2012. Five *N. gonorrhoeae* isolates with antimicrobial susceptibility profiles similar to current strains circulating in Canada were chosen from the NML *N. gonorrhoeae* culture collection. The NML determined minimum inhibitory concentrations (MICs) for these isolates using the agar dilution testing method as well as the Etest method for all panels (Biomerieux). The isolates were sent to the participating laboratories to be tested with up to 8 antibiotics along with 4 control isolates using the method and media of their choice following CLSI guidelines (CLSI, 2013). Their results were sent to the NML and Etest MICs were rounded up to the closest MIC values for comparison with two-fold agar dilution results. The 50 test isolates resulted in over 2500 data points which were analyzed to determine modal MICs and interpretations for each isolate and antibiotic. MICs interpreted as recommended by CLSI (2013) except for erythromycin (Ehret, 1996), azithromycin (CDC, 2007) and ceftriaxone and cefixime (WHO, 2012). Each participant's results were compared to the modal MICs.

**Comparison of Etest and Agar Dilution Methods:** Modal MICs for Etest results and agar dilution results were determined separately for each isolate and antibiotic. MICs that had "≤" or "≥" signs were excluded from the calculation. The number of dilution differences between the 2 modes for each isolate and antibiotic were determined (Table 1). The results were grouped by antibiotic to determine percent agreement. The Pearson correlation coefficient (Liu, 2014) and T-Test for 2 dependent means (p<0.05) (SocialScienceStatistics) were calculated. Modal interpretations were also determined and compared. Percent discordance, Very Major Errors (VMaE), Major Errors (MaE), Minor Errors (MiE) and Cohen's kappa values (Rosser, 1999) were studied.

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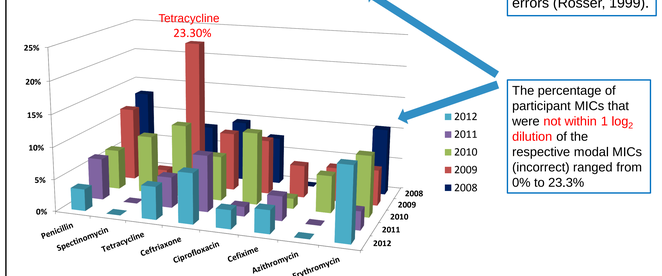
## RESULTS and DISCUSSION

### Figure 1. Combined MIC Determination Performance of Participants of the Canadian National Gonococcal Antimicrobial Susceptibility Comparison

| Antibiotic | MIC s number incorrect*/number tested; percentage incorrect* 2008 | 2009 | 2010 | 2011 | 2012 | Interpretations (2008 to 2012 combined) # discordant/# observations percentage discordant |
|---|---|---|---|---|---|---|
| Penicillin | 8/61 | 9/77 | 5/80 | 5/77 | 2/58 | 36/459 |
| | 13.1% | 11.7% | 6.3% | 6.5% | 3.4% | 7.8% |
| Spectinomycin | 0/55 | 1/49 | 6/66 | 0/60 | 0/49 | 4/338 |
| | 0.0% | 2.0% | 9.1% | 0.0% | 0.0% | 1.2% |
| Tetracycline | 7/84 | 14/60 | 10/88 | 3/63 | 2/39 | 75/404 |
| | 8.3% | 23.3% | 11.4% | 4.8% | 5.1% | 18.6% |
| Ceftriaxone | 8/83 | 7/76 | 7/99 | 7/80 | 7/90 | 27/502 |
| | 9.6% | 9.2% | 7.0% | 8.8% | 7.8% | 5.4% |
| Ciprofloxacin | 6/80 | 7/81 | 8/71 | 1/65 | 2/68 | 30/508 |
| | 7.5% | 8.6% | 11.3% | 1.5% | 2.9% | 5.9% |
| Cefixime | 0/34 | 2/39 | 1/64 | 2/53 | 2/55 | 9/452 |
| | 0.0% | 5.1% | 1.6% | 3.8% | 3.6% | 2.0% |
| Azithromycin | 0/75 | 4/75 | 4/69 | 0/93 | 0/80 | 13/459 |
| | 0.0% | 5.3% | 5.8% | 0.0% | 0.0% | 2.8% |
| Erythromycin | 4/38 | 1/18 | 4/42 | 1/35 | 4/35 | 20/249 |
| | 10.5% | 5.6% | 9.5% | 2.9% | 11.4% | 8.0% |

*Incorrect means not within 1 $\log_2$ dilution of modal MIC

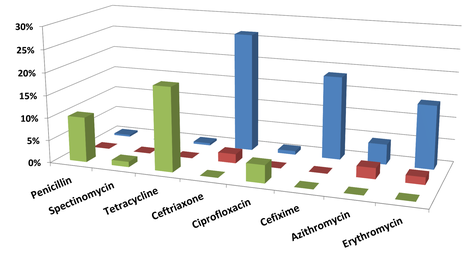
Discordance between participants' interpretations and modal interpretations for the 5 years combined ranged from 1.2% for spectinomycin to18.6% for tetracycline. Tetracycline was the only antibiotic with discordance >10%, the acceptable rate for all interpretation errors (Rosser, 1999).




The percentage of participant MICs that were not within 1 $\log_2$ dilution of the respective modal MICs (incorrect) ranged from 0% to 23.3%

### Figure 2. Percentage of Very Major Errors (VMaE), Major Errors (MaE) and Minor Errors (MiE) between Participants' Interpretations and the Modal Interpretations for Antibiotics Tested, 2008-2012

| Antibiotic | 2008 | 2009 | 2010 | 2011 | 2012 | Type of Error* |
|---|---|---|---|---|---|---|
| Penicillin | - | 2.4% (1/41) | - | - | - | Very Major |
| | 8.2% (7/85) | 11.1% (10/90) | 8.0% (8/100) | 6.3% (6/95) | 5.6% (5/89) | Minor |
| Spectinomycin | - | - | - | - | - | Very Major or Major |
| | - | - | 3.8% (3/80) | - | 1.4% (1/69) | Minor |
| Tetracycline | - | 3.3% (1/30) | - | - | - | Very Major |
| | 16.0% (12/75) | 25.0% (20/80) | 21.1% (19/90) | 15.0% (12/80) | 15.2% (12/79) | Minor |
| Ceftriaxone | 11.1% (1/9) | 7.1% (1/14) | 17.6% (3/17) | 61.5% (8/13) | - | Very Major |
| | 2.4% (2/85) | 8.2% (7/85) | - | - | 5.3% (5/94) | Major |
| Ciprofloxacin | 2.6% (1/38) | - | 2.0% (1/50) | - | - | Very Major |
| | 10.5% (10/95) | 3.0% (3/100) | 9.2% (10/109) | 2.9% (3/105) | 2.0% (2/99) | Minor |
| Cefixime | 27.3% (3/11) | 10.0% (1/10) | - | - | - | Very Major |
| | - | - | 1.0% (1/104) | 4.2% (4/96) | 1.0% (1/98) | Major |
| Azithromycin | 3.4% (2/58) | 4.4% (3/68) | 2.2% (2/92) | - | 2.4% (2/82) | Major |
| | - | - | - | - | 4.0% (4/99) | Minor |
| Erythromycin | 7.7% (2/26) | - | 17.2% (5/29) | 10.3% (3/29) | 29.6% (8/27) | Very Major |
| | - | - | 3.8% (1/26) | - | 4.5% (1/22) | Major |




* **Very Major Interpretation Errors (VMaE)** = false susceptible interpretations with **all resistant interpretations as denominator**
**Major Interpretation Errors (MaE)** = false resistant interpretations with **all susceptible interpretations as denominator**
**Minor Errors (MiE)** = errors involving intermediate interpretations with **all interpretations as denominator**

- The rate of MiE for tetracycline was >10% for all 5 years.
- The rates of VMaE for ceftriaxone (26.4%), cefixime (18.5%), azithromycin (4.49%) and erythromycin (14.6%) were >1.5% which is considered the acceptable rate for VMaE (Rosser, 1999).
- Ceftriaxone, cefixime and erythromycin exhibited a high percentage of VMaE because:
  - they do not have an "Intermediate" classification available to buffer the slight difference between MICs. Many of the DS/R panel isolates' MICs were right at the breakpoint between DS/R and S.
  - Cefixime and ceftriaxone had low numbers of DS isolates (53 and 21, respectively) in the panels. The total number of DS isolates is used as the denominator in calculating the VMaE causing the percentage to be greater.

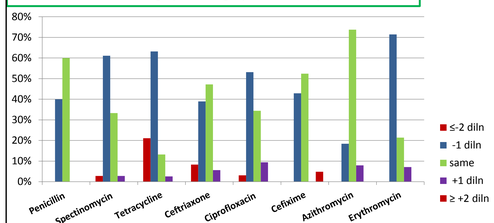
### Figure 3. Percentage of $\log_2$ Differences between the Agar Dilution Modal MICs and the Etest Modal MICs for Proficiency Panel Isolates between 2008 and 2012

| Antibiotic | Percentage of Isolates <= -2 diln | -1 diln | same | +1 diln | >= +2 diln | % Agreement between Agar Diln & Etest* | No. of Modes Compared | Pearson Correlation Coefficient r-value | Correlation | T-Test for 2 Dependent Means, p<0.05 p-value | Significant Difference between Means |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Penicillin | 0.0% | 40.0% | 60.0% | 0.0% | 0.0% | 100% | 30 | 0.83 | Strong positive | 0.1747 | No |
| Spectinomycin | 2.8% | 61.1% | 33.3% | 2.8% | 0.0% | 97.2% | 36 | 0.99 | Strong positive | 0.7158 | No |
| Tetracycline | 21.0% | 63.2% | 13.2% | 2.6% | 0.0% | 79.0% | 38 | 0.93 | Strong positive | 0.0016 | Yes |
| Ceftriaxone | 8.3% | 38.9% | 47.2% | 5.6% | 0.0% | 91.7% | 36 | 0.89 | Strong positive | 0.0046 | Yes |
| Ciprofloxacin | 3.1% | 53.1% | 34.4% | 9.4% | 0.0% | 96.9% | 32 | 0.94 | Strong positive | 0.1710 | No |
| Cefixime | 0.0% | 45.0% | 55.0% | 0.0% | 0.0% | 100% | 20 | 0.90 | Strong positive | 0.0071 | Yes |
| Azithromycin | 0.0% | 18.4% | 73.7% | 7.9% | 0.0% | 100% | 38 | 0.80 | Strong positive | 0.8865 | No |
| Erythromycin | 0.0% | 71.4% | 21.4% | 7.1% | 0.0% | 100% | 14 | 0.94 | Strong positive | 0.5072 | No |

*within 1 $\log_2$ dilution

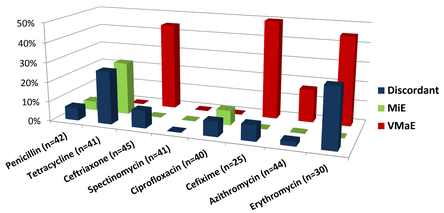
There is a significant difference between the means of the Etest modal MICs and agar dilution modal MICs for tetracycline, ceftriaxone and cefixime with p values <0.05.

The percentage of Etest modal MICs within 1 $\log_2$ dilution of the corresponding agar dilution modal MICs for tetracycline is 79.0%. All other antibiotics have a high percentage (91.7%-100%).




The correlation between agar dilution and Etest modal MICs for all antibiotics tested is high with Pearson correlation coefficients of 0.80 to 0.99.

### Figure 4. Interpretation Errors of Etest compared to Agar Dilution




- Discordance between interpretations by the 2 methods is >10% for tetracycline (26.8%) and erythromycin (30.0%).
- The rates of VMaE for ceftriaxone, cefixime, azithromycin and erythromycin were >10% and tetracycline had 26.8% MiE.
- Cohen's kappa values for the interpretations of each antibiotic was low for tetracycline, erythromycin, ceftriaxone and cefixime (0.493 to 0.667) (Rosser, 1999).
- Tetracycline, ceftriaxone and cefixime's significant difference between MICs contributed to interpretation errors
- There were very few DS/R panel isolates for ceftriaxone (5), cefixime (4) and azithromycin (6) and the MICs of these isolates were right at the breakpoint causing a high percentage of VMaE
- 30% of erythromycin's modal MICs were right at the breakpoint contributing to high percentage of VMaE.

* **Discordant** =interpretations disagree with all interpretations as denominator
**Very Major Interpretation Errors (VMaE)** = false susceptible interpretations with **all resistant interpretations as denominator**
**Minor Errors (MiE)** = errors involving intermediate interpretations with **all interpretations as denominator**

### Table 1. Number of MICs used to Calculate the Modal MICs for Comparison

| Antibiotic | No. Etest MICs | No. Agar Dilution MICs | Isolates with Modal MICs for both methods |
|---|---|---|---|
| Penicillin | 172 (2-7 MICs/isolate) | 78 (2-4 MICs /isolate) | 30 |
| Spectinomycin | 12 (3-4 MICs/isolate) | 112 (2-4 MICs/isolate) | 36 |
| Tetracycline | 197 (5-6 MICs/isolate) | 112 (2-4 MICs/isolate) | 38 |
| Ceftriaxone | 223 (5-7 MICs/isolate) | 114 (2-4 MICs/isolate) | 36 |
| Ciprofloxacin | 189 (2-7 MICs/isolate) | 104 (2-5 MICs/isolate) | 32 |
| Cefixime | 77 (2-7 MICs/isolate) | 46 (2-4 MICs/isolate) | 21 |
| Azithromycin | 230 (5-7 MICs/isolate) | 117 (2-4 MICs/isolate) | 38 |
| Erythromycin | 33 (2-3 MICs/isolate) | 35 (2-3 MICs/isolate) | 14 |

### Table 2. Test Isolates Results Compared to Test Isolate & Controls

| Antibiotic | % Agreement between Etest modal MICs and Agar dilution modal MICs: Test Isolates & Controls | | Test Isolates only | |
|---|---|---|---|---|
| Penicillin | 100.0% | 51/51 | 100.0% | 30/30 |
| Spectinomycin | 98.4% | 61/62 | 97.2% | 35/36 |
| Tetracycline | 82.3% | 56/68 | 79.0% | 30/38 |
| Ceftriaxone | 93.1% | 54/58 | 91.7% | 33/36 |
| Ciprofloxacin | 96.7% | 58/60 | 96.9% | 31/32 |
| Cefixime | 100.0% | 23/23 | 100.0% | 20/20 |
| Azithromycin | 98.5% | 67/68 | 100.0% | 38/38 |
| Erythromycin | 100.0% | 28/28 | 100.0% | 14/14 |

## CONCLUSIONS

- Participating in a proficiency testing program is important in order to identify possible problems with protocols, techniques and/or reagents.
- Laboratories participating in this proficiency testing program achieved 93.7% (2404/2564) agreement when all data from test isolates was combined. When controls were included in totals the percentage agreement went up to 95.1% (4,243/4,462).
- Although there is strong correlation between the Etest and agar dilution MICs for all antibiotics tested, Etest modal MICs differed from agar dilution modal MICs by ≥ 2 $\log_2$ dilutions for cefixime, ceftriaxone and tetracycline in 4.8%, 8.3% and 21.0% of the comparisons, respectively.
- This may have contributed to the higher level of disagreement for these 3 antibiotics in the participant's performance results and interpretation errors.
- Continued surveillance and quality testing programs for antimicrobial resistant *N. gonorrhoeae* lead to better quality patient care and public health prevention programs