Assessment of Gentamicin and Ertapenem Susceptibilities of Canadian *Neisseria gonorrhoeae* isolates

P. SAWATZKY[1], I. MARTIN[1], A. YUEN[1], V. ALLEN[2], L. HOANG[3], B. LEFEBVRE[4], M. LOVGREN[5], P. VAN CAESEELE[6], G. HORSMAN[7], S. RATNAM[8], R. GARCEAU[9], T. WONG[10], C. ARCHIBALD[10] , M. R. MULVEY[1]

[1]National Microbiology Laboratory (NML), Public Health Agency of Canada (PHAC), [2]Public Health Ontario Laboratories, Toronto, Ontario, [3]British Columbia Centres for Disease Control Public Health Microbiology & Reference Laboratory, Vancouver, British Columbia, [4]Laboratoire de santé publique du Québec, Ste-Anne-de-Bellevue, Québec, [5]Provincial Laboratory for Public Health, Edmonton, Alberta, [6] Cadham Provincial Laboratory, Winnipeg, Manitoba, [7]Saskatchewan Disease Control Laboratory, Regina, Saskatchewan, [8]Newfoundland & Labrador Public Health Laboratory, St. John's, Newfoundland, [9]Hôpital Dr G.L. Dumont, Moncton, New Brunswick, [10]Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada, Ottawa, Ontario, Canada

ABSTRACT

Background: The emergence of isolates with decreased susceptibilities to the cephalosporins and reports of treatment failures in Canada and around the world has made the concept of untreatable gonorrhea infections a future possibility. Alternative therapies such as gentamicin and ertapenem need to be evaluated for future therapeutic use.
Methods: *Neisseria gonorrhoeae* were collected by Canadian provincial public health laboratories in 2012 and submitted to the National Microbiology Laboratory for testing. *Neisseria gonorrhoeae* multi-antigen sequence types (NG-MAST or STs) and minimum inhibitory concentrations (MICs) were determined using the Etest for gentamicin (n=334) and ertapenem (n=378). Five reference cultures were also tested and their results were compared to established MICs. Currently there are no ertapenem or gentamicin interpretation criteria for *N. gonorrhoeae*.
Results: The MICs of gentamicin ranged from 1 mg/L to 6 mg/L with a modal MIC of 4 mg/L. The MICs of ertapenem ranged from <0.002 mg/L to 0.064 mg/L with a modal MIC of 0.008 mg/L. Isolates with decreased susceptibilities to ceftriaxone and cefixime had a modal MIC for ertapenem of 0.047 mg/L. The gentamicin modal MIC for these isolates remained the same. There were 139 different STs identified among the 378 isolates tested. ST-1407 was found to have the highest prevalence [10.1% (n=39)] with ST-3158, ST-3307, ST-4709 and ST-7986 following at 5.3% (n=18) each. The modal MICs for the ST-1407 isolates were 0.032 mg/L for ertapenem and 3 mg/L for gentamicin.
Conclusions: Modal MICs to gentamicin and ertapenem in a collection of diverse Canadian *N. gonorrhoeae* isolates are similar to that reported in other countries. Gentamicin is already used for gonorrhea treatment in other countries and may be a future option for treatment in combination with azithromycin in Canada. Ertapenem MICs remained low but are slightly elevated in the isolates with decreased susceptibilities to ceftriaxone and cefixime.

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-have-sex-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 *tbp*B (encoding the β 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).

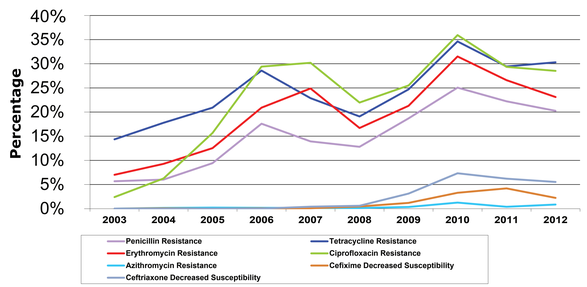
In this study we look for alternative therapies for the treatment of gonorrhea by determining susceptibilities of a diverse group of Canadian *N. gonorrhoeae* isolates to ertapenem and gentamicin.

METHOD

Neisseria gonorrhoeae were collected by Canadian provincial public health laboratories in 2012 and submitted to the National Microbiology Laboratory for testing. Isolates that had previously been tested for minimum inhibitory concentrations (MICs) for penicillin, tetracycline, spectinomycin, erythromycin ceftriaxone, ciprofloxacin, cefixime and azithromycin by agar dilution (Martin et al, 2011; CLSI, 2013) were tested using the Etest (Biomerieux) for gentamicin MICs (n=334) and ertapenem MICs (n=378). Five reference cultures were also tested and their results were compared to established MICs. Currently there are no ertapenem or gentamicin interpretation criteria for *N. gonorrhoeae*. MICs of other antibiotics were 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.

Neisseria gonorrhoeae multi-antigen sequence types (NG-MAST or STs) were determined as previously described (Martin et al, 2004).

Figure 1. Trends of Antimicrobial Susceptibilities of *N. 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

Figure 2. Ertapenem Etest MICs of *N. gonorrhoeae* isolates

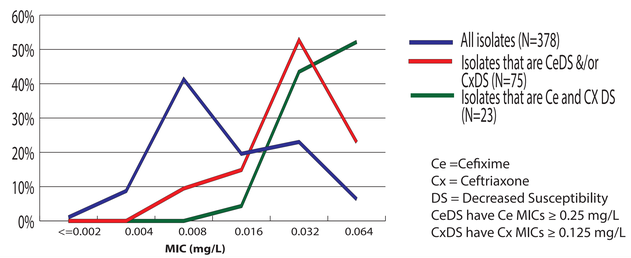


Figure 3. Gentamicin Etest MICs of *N. gonorrhoeae* isolates, N=334

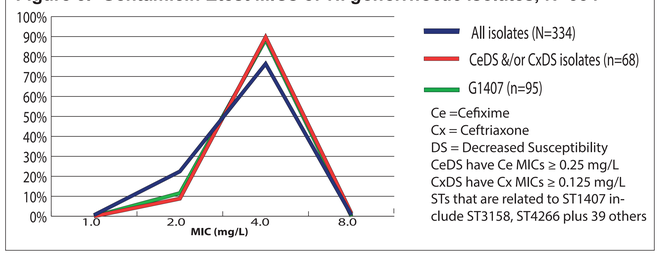


Figure 4. Ertapenem Etest MICs of *N. gonorrhoeae* isolates of ST1407 and related STs (n=103) as compared to all isolates tested (N=378)

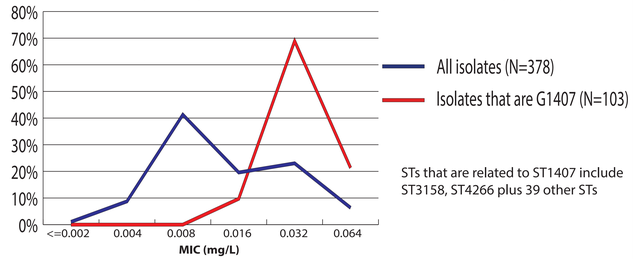
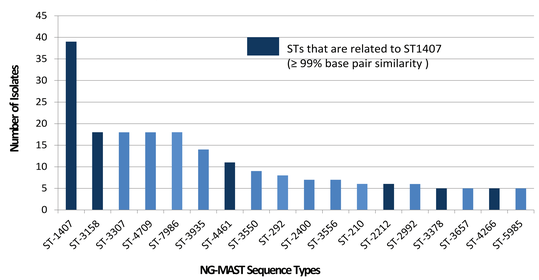
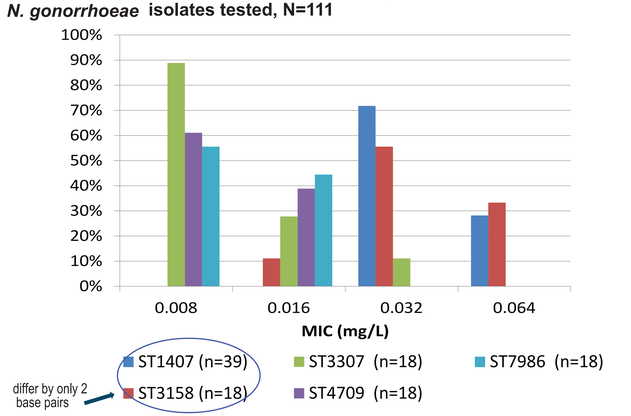


Figure 5. Prevalent *N. gonorrhoeae* NG-MAST Sequence Types within isolates tested, N=205*



*This graph represents 205 isolates of the 378 isolates with STs and Etest MICs for ertapenem and gentamicin. The remaining 173 isolates are dispersed among 121 sequence types (STs) containing 1 to 4 isolates each.

Figure 6. Ertapenem MICs within the 5 most prevalent STs of *N. gonorrhoeae* isolates tested, N=111



RESULTS

- The ertapenem modal MIC for the 376 *N. gonorrhoeae* isolates tested was 0.008 mg/L
- The ertapenem modal MIC for the isolates that had decreased susceptibility (DS) to either ceftriaxone (Cx), cefixime (Ce) or both (n=75) was 0.032 mg/L
- The ertapenem modal MIC for the isolates that had DS to both Cx and CE (n=23) was 0.064 mg/L
- The gentamicin modal MIC for the 334 *N. gonorrhoeae* isolates tested was 4 mg/L
- The gentamicin modal MIC was 4 mg/L for al isolates, including those characterized as CeDS or CxDS.
- Of the 378 isolates tested, 10.1% (39) were ST1407; ST3158, ST3307, ST4709 and ST7986 followed at 5.3% (18) each.
- There were a total of 103 isolates that were closely related molecularly (within 7 base pairs) to ST1407.
- ST1407 is Canada's most common ST and is the internationally identified clone that has been described as a superbug with high-level resistance to both cefixime and ceftriaxone and related isolates (n=103) was 0.032 mg/L (the same as for CxDS &/or CeDS isolates)
- The ertapenem modal MIC for the ST1407 and related isolates (n=103) was 0.032 mg/L (the same as for CxDS &/or CeDS isolates)

CONCLUSION

- Modal MICs to gentamicin and ertapenem in a collection of diverse Canadian *N. gonorrhoeae* isolates are similar to that reported in other countries.
- Ertapenem MICs remained low but are slightly elevated in the isolates with decreased susceptibilities to ceftriaxone and cefixime as well as isolates that were ST1407 or related (n=103).
- Gentamicin is already used for gonorrhea treatment in other countries and may be a future option for treatment in combination with azithromycin in Canada.

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