Molecular Typing and Identification of Antimicrobial Resistance Markers in *Neisseria gonorrhoeae* positive Clinical Specimens Collected in a Remote Northern Region of Canada

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BACKGROUND

Reported rates of gonorrhea have increased dramatically in Canada,¹ with reports suggesting a high burden in remote northern regions; antimicrobial resistance (AMR) to currently recommended antimicrobials have been reported and the primary diagnostic used is nucleic acid amplification testing (NAAT). Molecular assays were used to provide enhanced molecular typing and predicted molecular AMR data on NAAT specimens from a remote northern region in Canada.

METHOD

Between 2015 and 2017, we evaluated 1,220 (2015, n=335; 2016, n=444; 2017, n=441) *Neisseria gonorrhoeae* positive NAATS (Aptima urine and urogenital swabs) submitted by Northwest Territories for *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST).² Of these, 401 were further analyzed for resistance mutations to cephalosporins (CEPH),³ ciprofloxacin (CIP)⁴ and azithromycin (AZI) ⁵ by single nucleotide polymorphism (SNP) real-time PCR assays (Table 1).

Table 1. Gene Targets and Associated Mutations

Antimicrobial	Gene Target	SNP Detected
-	<i>porA</i>	Positive control for <i>N. gonorrhoeae</i>
CEPH	<i>ponA</i>	L421P
	<i>mtrR</i>	A deletion in promoter
	<i>penA</i>	Mosaic <i>penA</i>
	<i>porB</i>	Wild type G120, A121
CIP	<i>gyrA</i>	Wild type S91, D95
	<i>parC</i>	Wild type D86, S87, S88
AZI	23S	Wild type, 2059, 2611

CEPH: Decreased Susceptibility (DS) = 4/4 SNPs (MIC ≥ 0.125 mg/L); Intermediate (I) = 3/4 SNPs (MIC = 0.032-0.063 mg/L); Susceptible (S) = 0 - 2 SNPs (MIC ≤ 0.016 mg/L)
CIP: Resistant (R) ≥ 1 SNP (MIC ≥ 1 mg/L); S = All wild type (WT) (MIC ≤ 0.5 mg/L)
AZI: R ≥ 1 SNP (MIC ≥ 1 mg/L); S = All WT (MIC ≤0.5 mg/L)

RESULTS

- Sequence types (ST) were obtained for over **90%** (1,127/1,220) of the specimens tested (Table 2).
- Out of a total of 53 STs identified from 2015-2017, **ST4637 (44.3%, 499/1,127)** and **ST10451 (20.6%, 233/1,127)** the most prevalent (Fig. 2).
- 70% of the 401 specimens analyzed for resistance-associated mutations yielded results to predict susceptibility to cephalosporins, ciprofloxacin and azithromycin (Table 3)
- Specimens identified as **ST4637** were predicted to be **susceptible** to cephalosporins, ciprofloxacin and azithromycin based on SNP Assay (Fig. 3).
- Specimens identified as **ST10451** were predicted to have **decreased susceptibility to cephalosporins** (87.3%, 62/71) and **resistance to ciprofloxacin** (100%, 79/79)(Fig. 3).

CONCLUSIONS

- The majority of the *N. gonorrhoeae* strains circulating in this region belong to two sequence types; **ST4637 and ST10451**.
- ST10451** emerged in 2016 and was the most prevalent sequence type in 2017; it is associated with **predicted cephalosporin decreased susceptibility and ciprofloxacin resistance**.
- The enhanced molecular typing and predicted molecular antimicrobial resistance surveillance data will allow public health authorities to better understand the transmission of gonococcal infections and guide public health decision-making in the prevention, treatment and control of gonorrhea in remote regions where cultures are not available.

REFERENCES

1. Public Health Agency of Canada. 2017. Notifiable Diseases On-Line. <http://diseases.canada.ca/notifiable/charts?c=y1> Accessed: 2017/11/14
2. Martin IMC, Ison CA, Aanensen DM, Fenton KA and Spratt BG. 2004. J Infect Dis. 189:1497-1505.
3. Peterson SW, Martin I, Demczuk W, Bharat A, et al. 2015. J Clin Microbiol.53:2042-2048.
4. Peterson SW, Martin I, Demczuk W, Bharat A, et al.. 2015. J Clin Microbiol. 53:3606-3608.
5. Trembizki E, Buckley C, Donovan B, et al. J Antimicrob Chemother. 2015;70:3244-3249.
6. Saitou N. and Nei M. 1987. Mol Biol Evol. 4:406-425.
7. Nei M. and Kumar S. 2000. Mol Evol Phylo. Oxford University Press, New York.
8. Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. 2013. Mol Biol Evol .30: 2725-2729.

Table 2. Northwest Territories' NAAT Samples Tested

Year	Samples Received	Cases	Cases successfully typed by NG-MAST	Number of cases with SNP AMR predictions		
				CEPH	CIP	AZI
2015	346	335	303	59	46	53
2016	454	444	403	138	144	147
2017	446	441	421	110	105	107
TOTAL	1,246	1,220	1,127	307	295	307

Figure 2. Distribution of NG-MAST of NAAT Samples

Table 3. SNP Assay Results

Number of Samples	SNP Assay Predicted Susceptibility			NG-MAST Sequence Type
	CEPH	CIP	AZI	
173	S	S	S	ST4637 (n=38), ST14863 (n=16), ST25 (n=15), ST13808 (n=16) ^a
12	S	R	S	ST15602 (n=5), ST10451 (n=4) ^a
22	I	R	S	ST10451 (n=5), ST9184 (n=6), ST15647 (n=5) ^a
72	DS	R	S	ST10451 (n=61), ST14277 (n=8) ^a

^aplus other STs

Figure 3. Genetic Relationship^{6,7,8} between NG-MASTs of NAATs (N=1,086)^a

^aonly most prevalent STs represented in figure; remaining 40 isolates are distributed among 35 STs with 1 or 2 isolates in each