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Public Health Agency of Canada
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Canada

National Surveillance of *Neisseria gonorrhoeae* AMR in Canada

Shelley Peterson, Robyn Thorington
Streptococcus and STI Section
Bacterial Pathogens, AMR, and Wastewater Division
Science, Reference, and Surveillance Directorate
National Microbiology Laboratory Branch
Public Health Agency of Canada

Disclosures

- Shelley Peterson: Nothing to declare
- Robyn Thorington: Nothing to declare

Learning Objectives

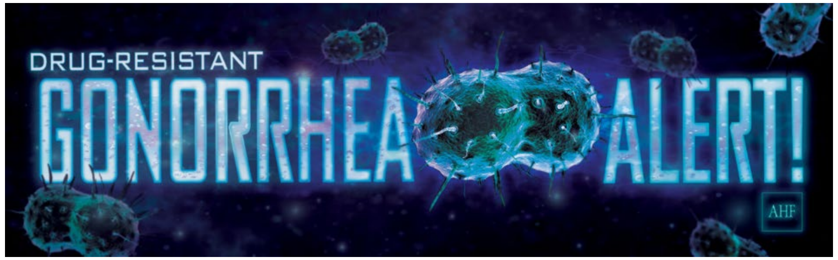
- Summarize recent epidemiological trends in *Neisseria gonorrhoeae* activity in Ontario and the rest of Canada
- Describe the current approach to *Neisseria gonorrhoeae* whole genome sequencing and reporting at the National Microbiology Laboratory
- Recognize the threat of the global spread of XDR *Neisseria gonorrhoeae* strains and the potential impact of increased ceftriaxone resistance on the efficacy of current recommended therapies
- Understand the value of NAAT testing for expanding the breadth of surveillance data where cultures are unavailable, particularly in northern, remote and isolated regions of Canada

We would like to acknowledge that the CSCHAH is located on the traditional Treaty One Territory of the Anishinaabeg, Cree, Oji-Cree, Dakota, and Dene Peoples, and on the homeland of the Métis Nation. We respect the territories and treaties, we acknowledge the harms and mistakes of the past, and wish to move forward in partnership with Indigenous communities in a spirit of reconciliation and collaboration.

Gonococcal Antimicrobial Surveillance Program (GASP-Canada) - Objective

To characterize antimicrobial resistant *Neisseria gonorrhoeae* in order to monitor trends, support outbreak and treatment failure investigations and the development of treatment guidelines.

- *N. gonorrhoeae* is the 2nd most prevalent bacterial STI in Canada
- Canadian gonorrhea isolates have shown increased resistance to frontline therapies in recent years, MDR and XDR isolates, and reported treatment failures to recommended therapies
- **Voluntary, passive surveillance system monitoring antimicrobial susceptibilities of *N. gonorrhoeae* since mid-80's - approx. 3000 isolates submitted annually – database includes 60K isolates**
- **A subset of GASP-Canada isolates are part of the Enhanced Surveillance of Antimicrobial-Resistant Gonorrhea (ESAG) system**

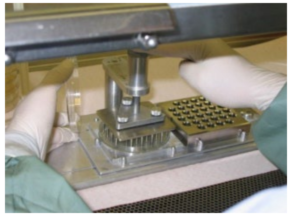


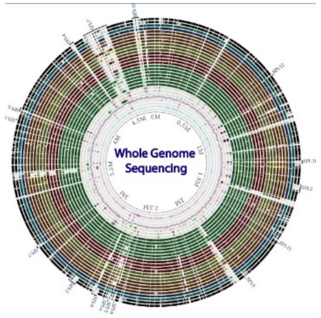
GASP-Canada Program Overview

All Provincial/Territorial Laboratories from across Canada (and NB hospitals)

Resistant or untested cultures and data submitted to NML

National Microbiology Laboratory (NML)





Perform molecular and microbiological characterization of cultures

Reporting

Government of Canada Gouvernement du Canada

Canadian Network for Public Health Intelligence

National Surveillance of Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Annual Summary 2019

Streptococcus and STI Unit
National Microbiology Laboratory
Public Health Agency of Canada

Professional Guidelines and Public Health Practice

UPDATE 2020

Canadian Antimicrobial Resistance Surveillance System Report

07_MS_Gono 2020_AMR_subm Sept06 2022

Antimicrobial susceptibilities of *Neisseria gonorrhoeae* in Canada, 2020

Robyn Thorington[1], Pamela Sawatzky[1], Brigitte Lefebvre[2], Mathew Diggle[3], Linda Hoang[4], Samir Patel[5], Paul Van Caessele[6], Jessica Minion[7], Richard Garceau[8], Myrna Matheson[9], David Haldane[10], Genevieve Gravel[11], Michael R. Mulvey[1], Irene Martin[1*]

Section 5-6: Canadian Guidelines on Sexually Transmitted Infections – Management and treatm
specific infections – Gonococcal Infections

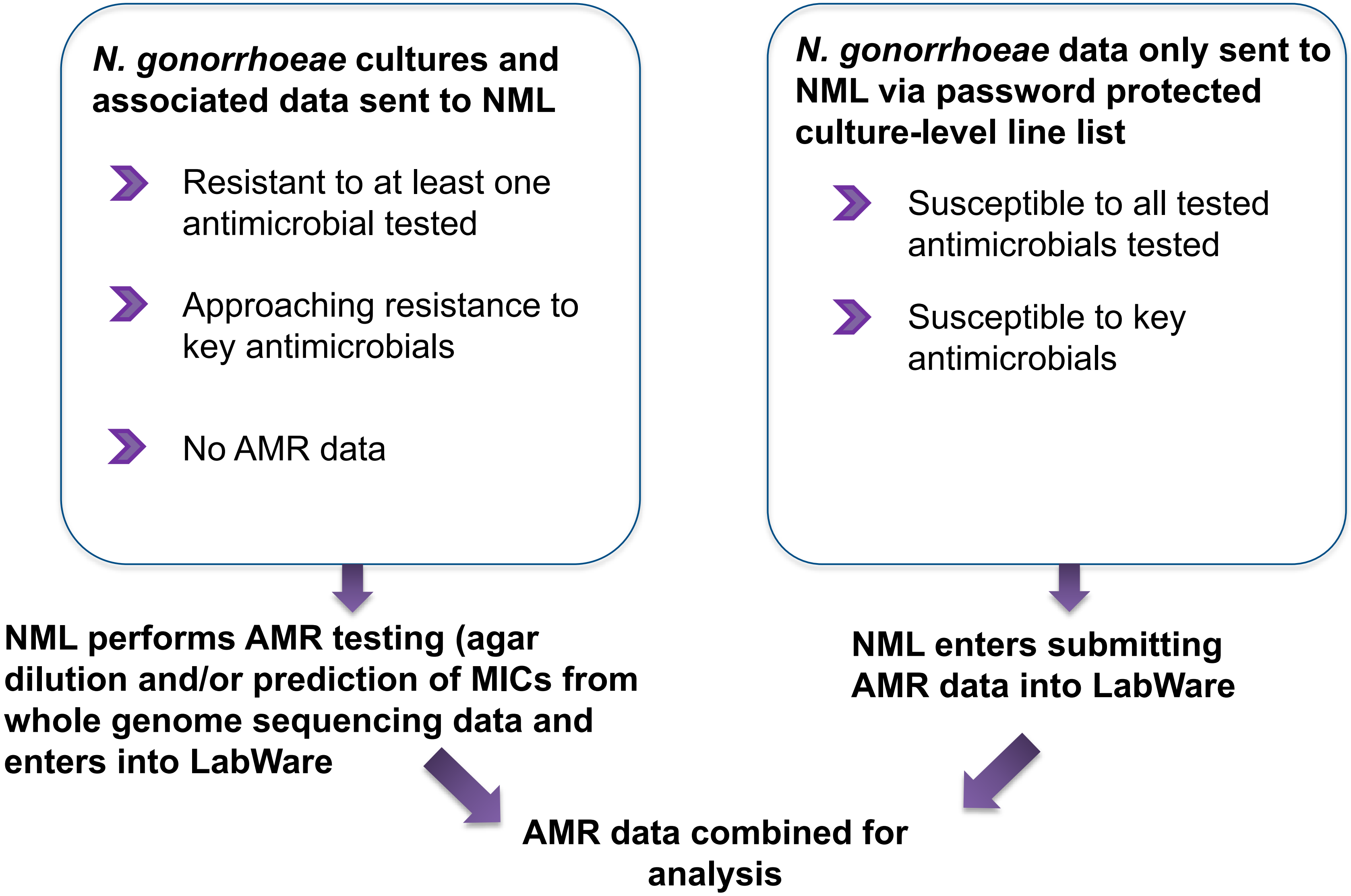
Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report

2021

World Health Organization

Generate, consolidate and report actionable surveillance data/ Provincially/Territorially, Nationally and Internationally

Collaboration between PHAC, provincial/territorial public health laboratories across Canada



***N. gonorrhoeae* cultures and associated data sent to NML**

- Resistant to at least one antimicrobial tested
- Approaching resistance to key antimicrobials
- No AMR data

NML performs AMR testing (agar dilution and/or prediction of MICs from whole genome sequencing data and enters into LabWare

***N. gonorrhoeae* data only sent to NML via password protected culture-level line list**

- Susceptible to all tested antimicrobials tested
- Susceptible to key antimicrobials

NML enters submitting AMR data into LabWare

AMR data combined for analysis

Minimum data submitted to NML along with culture:

- Unique ID
- Submitter
- Province/Territory
- Date of isolation or collection
- Age / Date of birth
- Gender
- Source / Anatomical site of isolation
- MIC results (if available)

Antimicrobial agents are tested based on Clinical and Laboratory Standards Institute (CLSI) guidelines using recommended controls.

NML requests the **total number of cultures** tested in each jurisdiction annually to determine the **denominator** to be used in all AMR trending analysis.

Data Sourcing

- All data presented here was generated by the Streptococcus and STI Section, National Microbiology Lab as part of their work monitoring *N. gonorrhoeae* AMR in Canada. This data is based on cultures and clinical specimens sent to the section from jurisdictions across Canada on a voluntary basis.
- All culture data presented is part of the Gonococcal Antimicrobial Surveillance Programme (GASP-Canada)

Antimicrobials tested

Antimicrobials with MICs from Agar dilution:

- Azithromycin
- Cefixime
- Ciprofloxacin
- Ceftriaxone
- Erythromycin
- Ertapenem (not reported)
- Gentamicin (not reported)
- Penicillin
- Spectinomycin
- Tetracycline

Antimicrobials with MICs predicted from WGS:

- Azithromycin
- Cefixime
- Ciprofloxacin
- Ceftriaxone
- Penicillin
- Spectinomycin
- Tetracycline

NML National Gonococcal Antimicrobial Susceptibility Comparison Program

- NML distributes 10 *N. gonorrhoeae* isolates to 8 participating laboratories annually.
- AST determined by either agar dilution or E-test

- Usually very good agreement between laboratories. The last panel (2023):
  - the overall agreement level between all participating laboratories and the modal MIC of all results submitted was 91.6%
  - The agreement between participating E-Test laboratories and the E-Test mode was 96.7%.
  - The agreement between participating agar dilution laboratories and the agar dilution mode was 98.9%

As per our ISO 17025 requirements, we participate in an annual External Quality Assurance Program administered by the CDC. Previously participated in a program from the UK

ESAG - Enhanced Surveillance of Antimicrobial Resistant Gonorrhea

REPORT ON THE ENHANCED SURVEILLANCE OF ANTIMICROBIAL-RESISTANT GONORRHEA (ESAG)

RESULTS FROM 2015 TO 2017

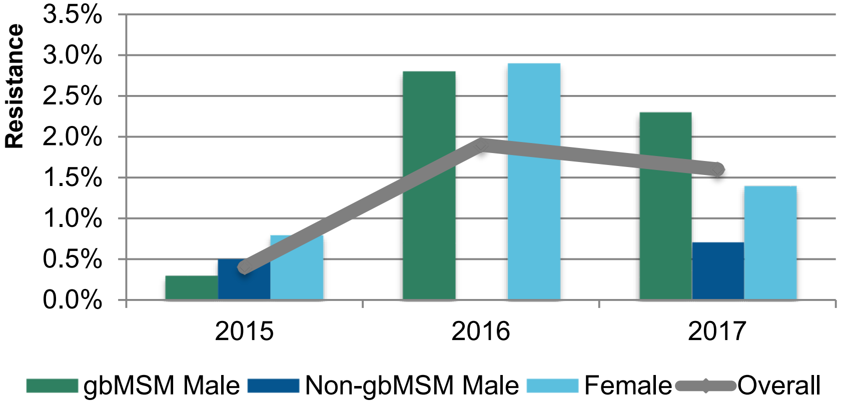
PROTECTING AND EMPOWERING CANADIANS TO IMPROVE THEIR HEALTH

Public Health Agency of Canada
Agence de la santé publique du Canada

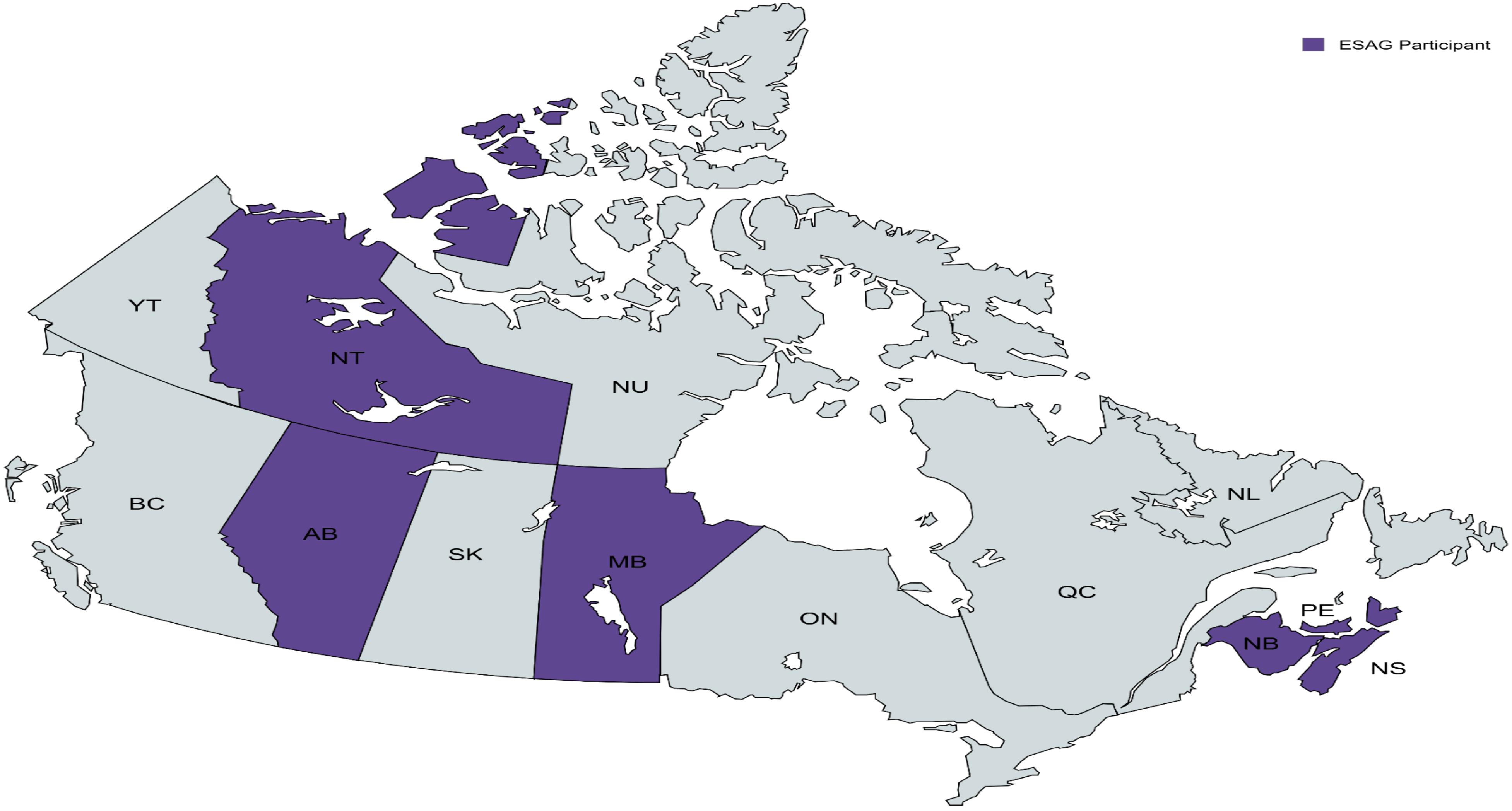
Canada

- Integrates **epidemiology** and **laboratory** surveillance data – hosted on **CNPHI**
- Resistance data will inform development of **Canadian STI Treatment Guidelines**
- Monitors adherence to STI Treatment Guidelines and **antimicrobial use**
- Monitor resistance in **key at-risk populations**
- Identify **treatment failures**
- Identify **outbreak clusters**

Distribution of azithromycin resistance by sex or sexual behaviour, ESAG 2015-2017



Which provinces and territories currently participate in ESAG?



Launched in 2013 with data collection starting in 2014

Created with mapchart.net

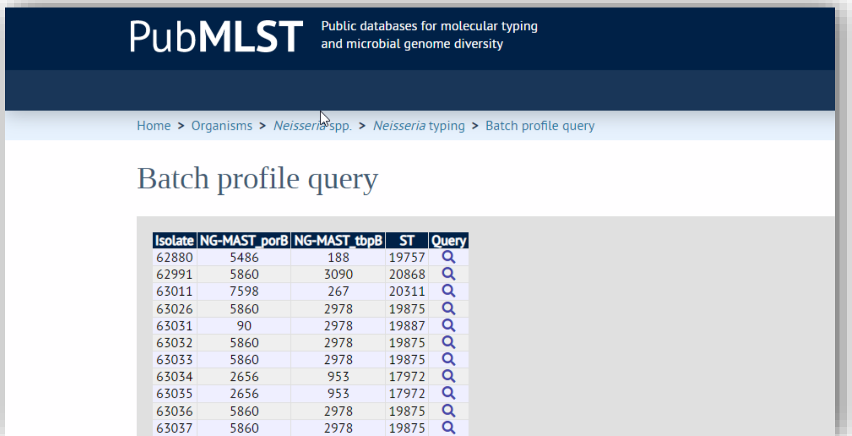
ESAG on Canadian Network for Public Health Intelligence (CNPHI)

Dashboard

- Trends of MDR, XDR, Resistant and Susceptible Neisseria gonorrhoeae
- Neisseria gonorrhoeae Cultures Tested by Year and Province / Territory
- Proportion of Routine vs ESAG Neisseria gonorrhoeae Isolates Submitted to the NML by Year

Neisseria gonorrhoeae Multi-Antigen Sequence Typing (NG-MAST)

- A valuable sequence typing tool that can be used to characterize different strains of GC
- Sequences two highly variable regions of GC/ *porin B (porB)* and *transferrin-binding protein B (tbpB)*
- The identity of the two alleles then make up the sequence type (ST) of the bacterial strain
- Allele IDs and sequence types are assigned on PubMLST (www.pubmlst.org)



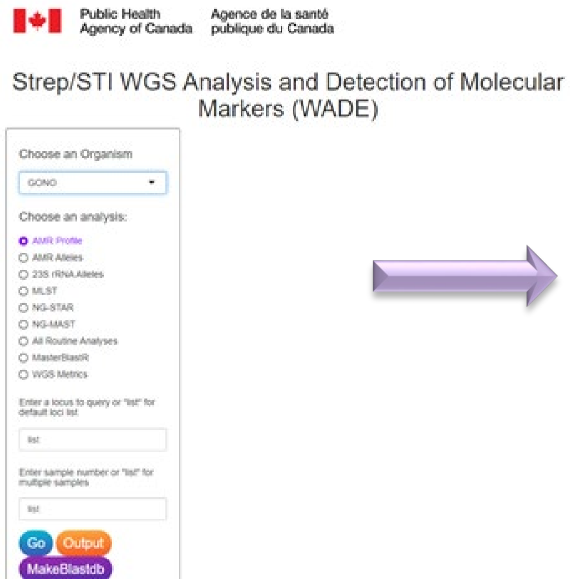
Batch profile query

Isolate	NG-MAST_porB	NG-MAST_tbpB	ST	Query
62880	5486	188	19757	🔍
62991	5860	3090	20868	🔍
63011	7598	267	20311	🔍
63026	5860	2978	19875	🔍
63031	90	2978	19887	🔍
63032	5860	2978	19875	🔍
63033	5860	2978	19875	🔍
63034	2656	953	17972	🔍
63035	2656	953	17972	🔍
63036	5860	2978	19875	🔍
63037	5860	2978	19875	🔍

Routine genomics on all gonorrhea cultures:

- Implemented November 2022
- ISO 17025 April 2023

NexteraXT
NextSeq1000/2000



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Strep/STI WGS Analysis and Detection of Molecular Markers (WADE)

Choose an Organism

GONO

Choose an analysis:

● AMR Profile
○ AMR Alleles
○ 23S rRNA Alleles
○ MLST
○ NG-STAR
○ NG-MAST
○ All Routine Analyses
○ MasterBlastR
○ WGS Metrics

Enter a locus to query or "list" for default loci list

list

Enter sample number or "list" for multiple samples

list

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MECHANISMS OF RESISTANCE

Equations To Predict Antimicrobial MICs in *Neisseria gonorrhoeae* Using Molecular Antimicrobial Resistance Determinants

Walter Demczuk,[a] Irene Martin,[a] Pam Sawatzky,[a] Vanessa Allen,[b] Brigitte Lefebvre,[c] Linda Hoang,[d] Prenilla Naidu,[e]

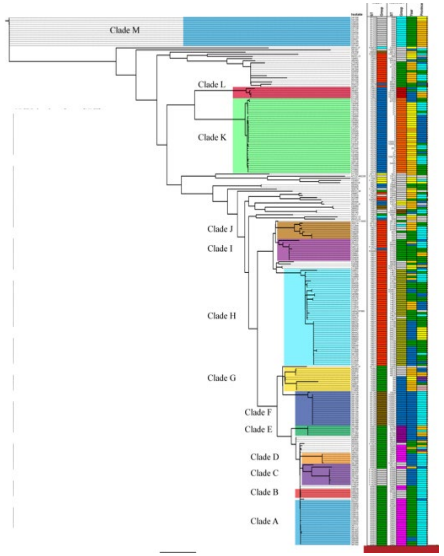
Predict AMR

NGMAST

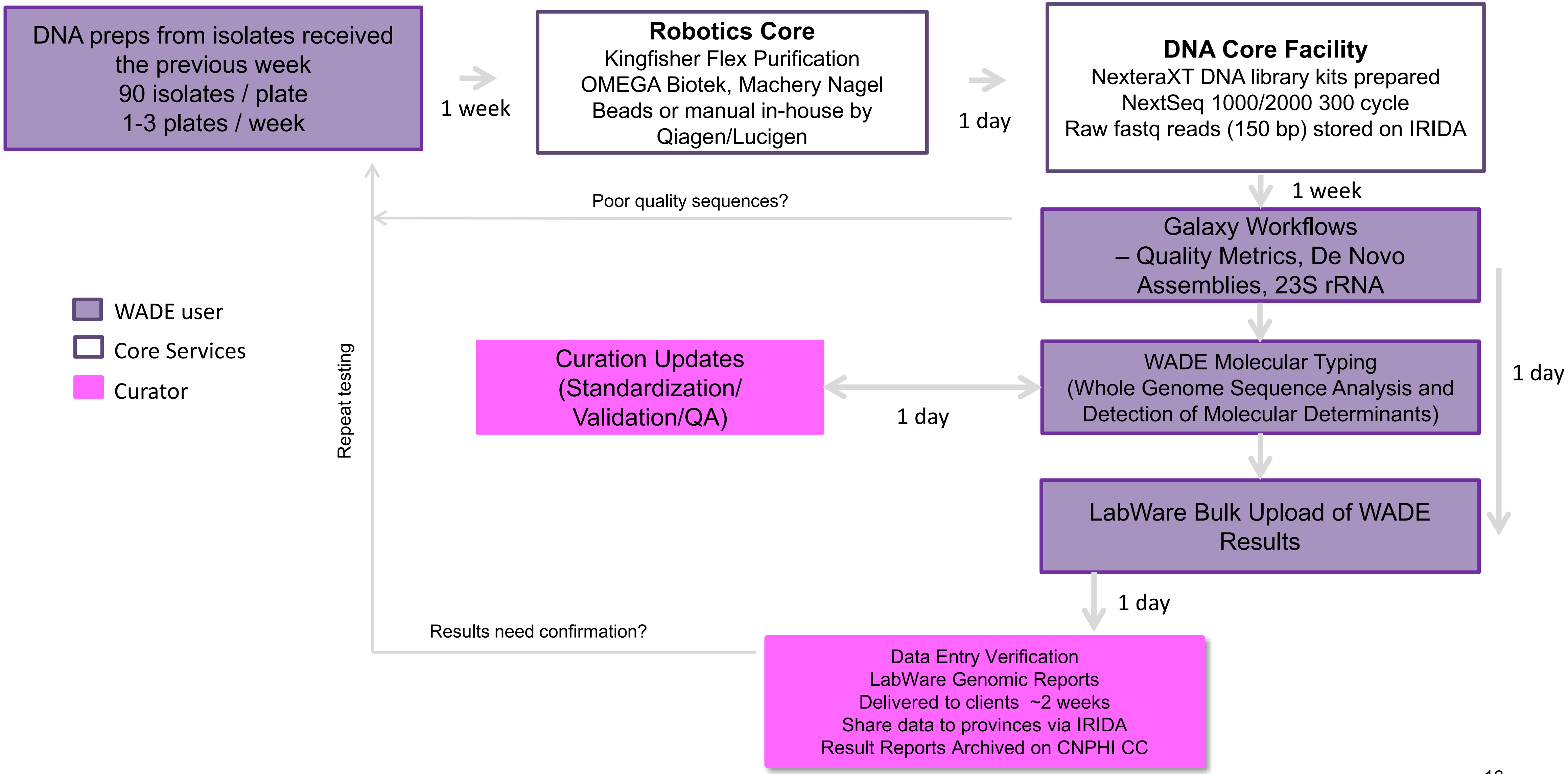
NGSTAR

MLST

Outbreak Support



Estimated batch-based genomic workflow timeline



DNA preps from isolates received the previous week
90 isolates / plate
1-3 plates / week

1 week

Robotics Core
Kingfisher Flex Purification
OMEGA Biotek, Machery Nagel
Beads or manual in-house by Qiagen/Lucigen

1 day

DNA Core Facility
NexteraXT DNA library kits prepared
NextSeq 1000/2000 300 cycle
Raw fastq reads (150 bp) stored on IRIDA

1 week

Galaxy Workflows
– Quality Metrics, De Novo Assemblies, 23S rRNA

Poor quality sequences?

WADE Molecular Typing
(Whole Genome Sequence Analysis and Detection of Molecular Determinants)

1 day

Curation Updates
(Standardization/ Validation/QA)

1 day

LabWare Bulk Upload of WADE Results

1 day

Data Entry Verification
LabWare Genomic Reports
Delivered to clients ~2 weeks
Share data to provinces via IRIDA
Result Reports Archived on CNPHI CC

Results need confirmation?

Repeat testing

🟪 WADE user
☐ Core Services
■ Curator

N. gonorrhoeae Sequence Typing for Antimicrobial Resistance (NG-STAR)

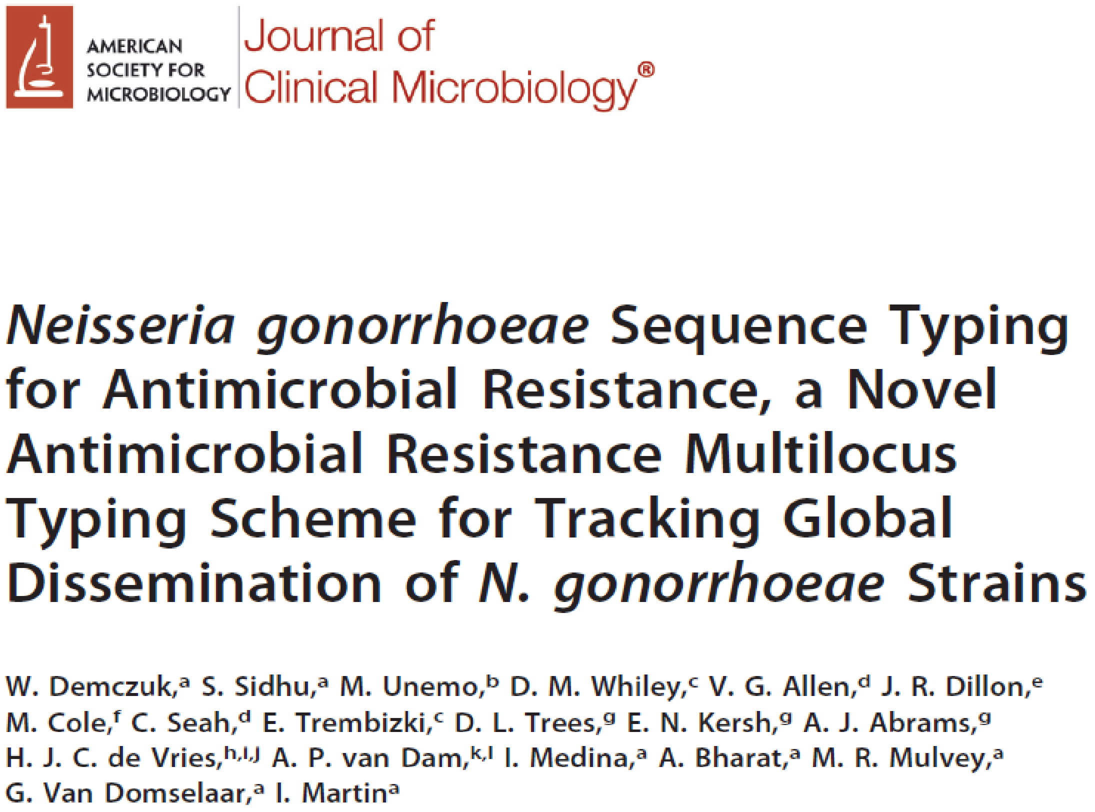
- Tracking the global dissemination of AMR *N. gonorrhoeae* strains using standardized nomenclature

- Seven genes are assigned allele numbers and the combination of alleles produces the NG-STAR type
  - *penA*: cephalosporin decreased susceptibility/ resistance
    - *penA* 60.001 strongly associated with CRO-R
  - *mtrR*: associated with AZI-R
  - *porB*: cephalosporin decreased susceptibility/ resistance
  - *ponA*: associated with elevated cephalosporin MICs
  - *gyrA*: associated with CIP-R
  - *parC*: associated with CIP-R
  - *23S rRNA*: associated with AZI-R

NG-STAR Website

- https://ngstar.canada.ca
- Submissions from 36 countries, across all continents, with most submissions from Canada, Sweden, USA

- Contacts:
  - Shelley Peterson (shelley.peterson@phac-aspc.gc.ca)
  - Nick Nordal-Budinsky (nick.nordal-budinsky@phac-aspc.gc.ca)
  - Robyn Thorington (robyn.thorington@phac-aspc.gc.ca)



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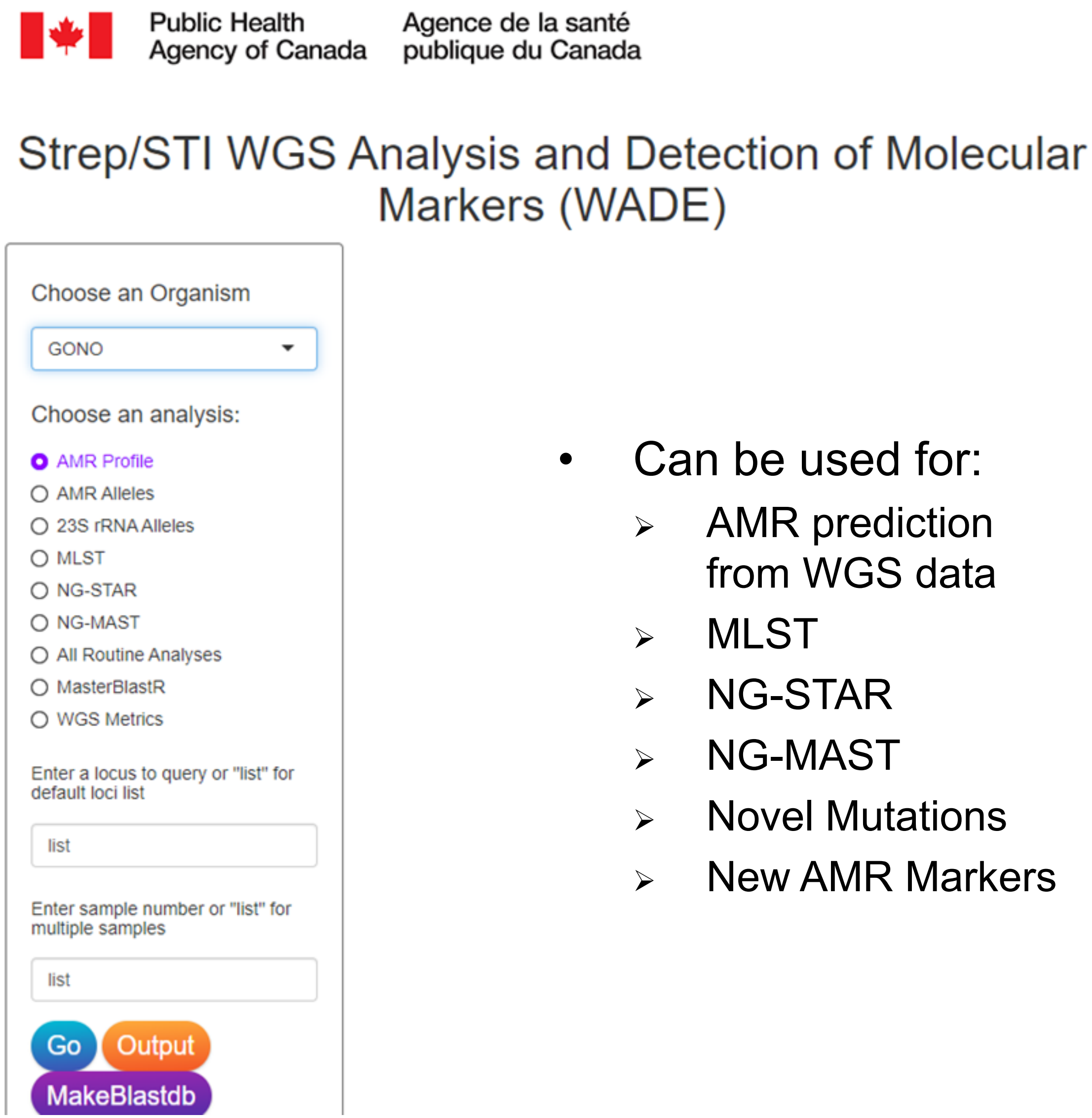
***Neisseria gonorrhoeae* Sequence Typing for Antimicrobial Resistance, a Novel Antimicrobial Resistance Multilocus Typing Scheme for Tracking Global Dissemination of *N. gonorrhoeae* Strains**

W. Demczuk,[a] S. Sidhu,[a] M. Unemo,[b] D. M. Whiley,[c] V. G. Allen,[d] J. R. Dillon,[e] M. Cole,[f] C. Seah,[d] E. Trembizki,[c] D. L. Trees,[g] E. N. Kersh,[g] A. J. Abrams,[g] H. J. C. de Vries,[h,i,j] A. P. van Dam,[k,l] I. Medina,[a] A. Bharat,[a] M. R. Mulvey,[a] G. Van Domselaar,[a] I. Martin[a]

WADE – Whole genome sequence Analysis and DEtection of molecular markers



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Strep/STI WGS Analysis and Detection of Molecular Markers (WADE)

Choose an Organism

GONO

Choose an analysis:

- ● AMR Profile
- ○ AMR Alleles
- ○ 23S rRNA Alleles
- ○ MLST
- ○ NG-STAR
- ○ NG-MAST
- ○ All Routine Analyses
- ○ MasterBlastR
- ○ WGS Metrics

Enter a locus to query or "list" for default loci list

list

Enter sample number or "list" for multiple samples

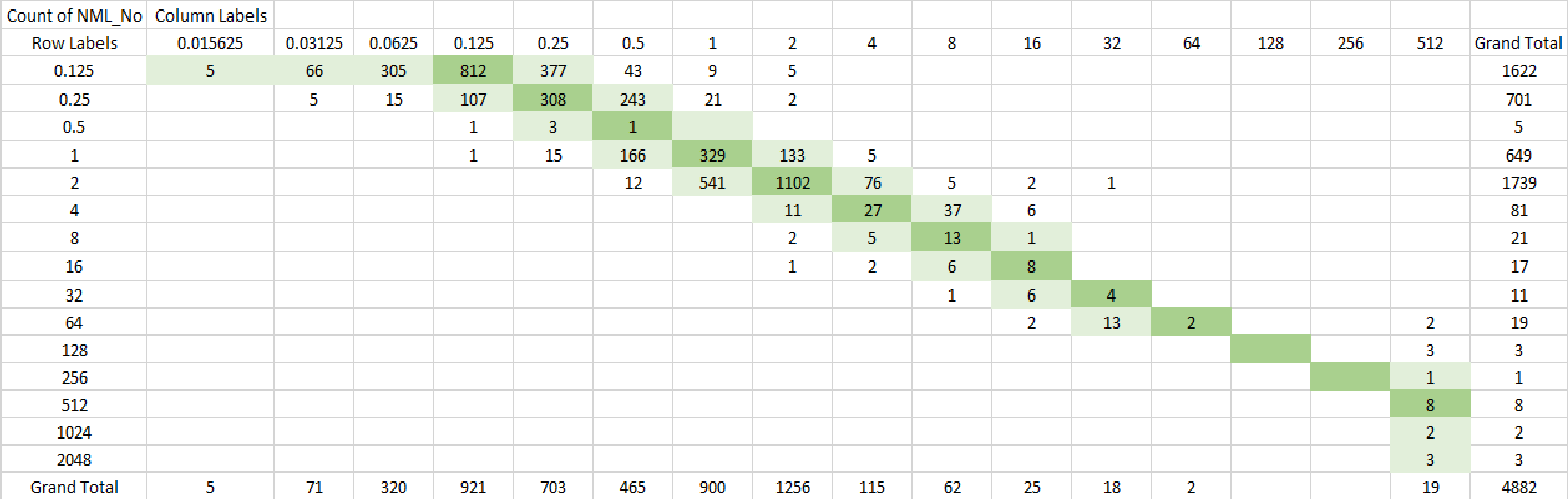
list

- Can be used for:
  - AMR prediction from WGS data
  - MLST
  - NG-STAR
  - NG-MAST
  - Novel Mutations
  - New AMR Markers

- In-house developed R-Shiny App
- Enables analysis by bench-level lab technicians.
  - Uses assembled genomes
    - Assemble once, use many times
  - Can query hundreds of genomes per batch
- Available on GitHub to external collaborators and clients.
  - https://github.com/phac-nml/wade
- Shelley Peterson is the curator of WADE
  - shelley.peterson@phac-aspc.gc.ca

MIC prediction using multivariate logistic regression on Molecular resistance determinants in *N. gonorrhoeae*

- Linear regression analysis to generate predictive equations
- Data sets for testing and training the model are made up of cultures sent to SSTI with both agar dilution and WGS results
- Equations are only as accurate as the data that used to train and test them
- Cultures from across Canada will always be needed to ensure the accuracy of the equations
  - Phenotypic results need to reflect what is currently circulating in Canada



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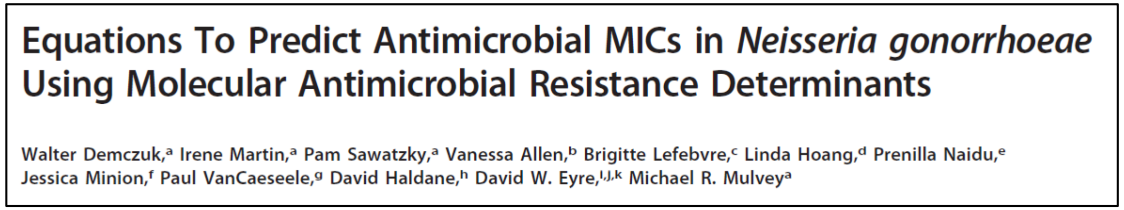
Row Labels	0.015625	0.03125	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	Grand Total
0.125	5	66	305	812	377	43	9	5									1622
0.25		5	15	107	308	243	21	2									701
0.5				1	3	1											5
1				1	15	166	329	133	5								649
2						12	541	1102	76	5	2	1					1739
4								11	27	37	6						81
8								2	5	13	1						21
16								1	2	6	8						17
32										1	6	4					11
64											2	13	2			2	19
128																3	3
256																1	1
512																8	8
1024																2	2
2048																3	3
Grand Total	5	71	320	921	703	465	900	1256	115	62	25	18	2			19	4882

Published MIC prediction algorithms integrated into WADE

- Equations published Feb 2020
- ISO accredited Spring 2023



Equations To Predict Antimicrobial MICs in *Neisseria gonorrhoeae* Using Molecular Antimicrobial Resistance Determinants

Walter Demczuk,[a] Irene Martin,[a] Pam Sawatzky,[a] Vanessa Allen,[b] Brigitte Lefebvre,[c] Linda Hoang,[d] Prenilla Naidu,[e] Jessica Minion,[f] Paul VanCaeseele,[g] David Haldane,[h] David W. Eyre,[i,j,k] Michael R. Mulvey[a]

- Phenotypic testing completed on a sub-set of isolates to support annual validation of AMR prediction pipelines

- Equations are reviewed annually to ensure maintenance of prediction accuracy
  - Incorporate new resistance mutations

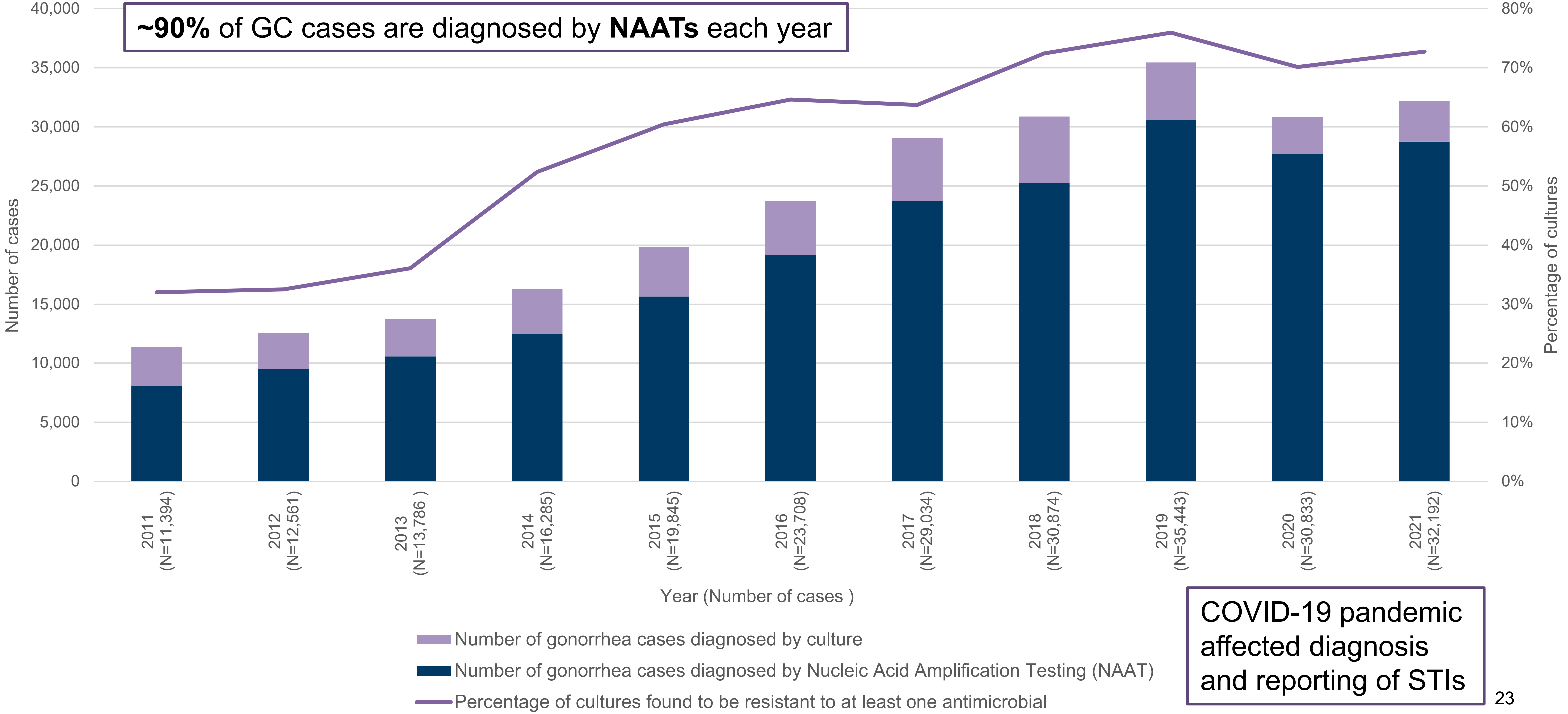
GASP-Canada/ESAG still need cultures

- **Culture submission is required to maintain accuracy of the predicted MICs**
  - *N. gonorrhoeae* is highly competent and acquires new AMR markers easily
  - MIC drift is also closely monitored
  - Cultures are needed from all regions of Canada to ensure accurate prediction of MICs across the country

- **Agar dilution is still used for:**
  - AZI, CRO, CFM approaching and over the resistance breakpoint
  - Confirming results as needed (CRO-R cases)
  - Repeated WGS failures
    - Could indicate a new SNP/allele of interest

Neisseria gonorrhoeae (GC) cases in Canada, 2011-2021

The Canadian reported rate of gonorrhea has more than doubled since 2013 from 40.56 per 100,000 population to 84.21 per 100,000 population in 2021



~90% of GC cases are diagnosed by **NAATs** each year

COVID-19 pandemic affected diagnosis and reporting of STIs

NAAT sample processing and molecular assays

NAAT Specimens

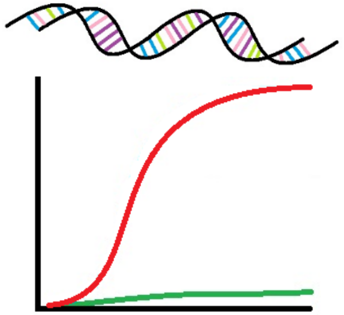
High-throughput Nucleic Acid Extractions

Nested Conventional PCR

NG-MAST
Neisseria gonorrhoeae
Multi Antigen Sequence Typing

PubMLST

Real-Time PCR SNP Detections



These tests are now routine surveillance at NMLB, available on the NML Guide to Services:

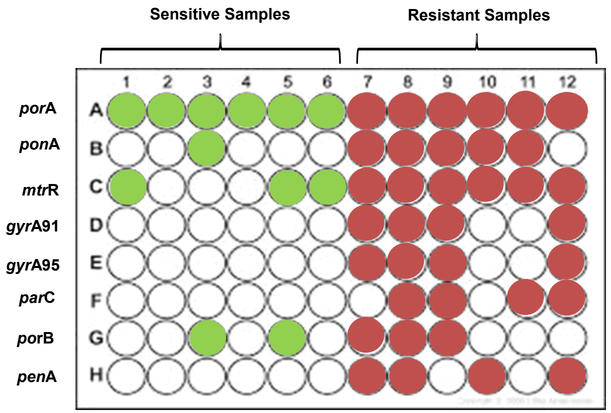
NG-MAST:
https://cnphi.canada.ca/gts/reference-diagnostic-test/15061?alphaReturn=pathogenByLetter&alphaChar=N

AMR Predictions:
https://cnphi.canada.ca/gts/reference-diagnostic-test/15062?alphaReturn=pathogenByLetter&alphaChar=N

Minimum Inhibitory Concentration Ranges

AMR Predictions

Cephalosporins, Ciprofloxacin, and Azithromycin



		Sensitive Samples						Resistant Samples					
		1	2	3	4	5	6	7	8	9	10	11	12
*por*A	A	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥
*pon*A	B	○	○	🟩	○	○	○	🟥	🟥	🟥	🟥	🟥	○
*mtr*R	C	🟩	○	○	○	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥
*gyr*A91	D	○	○	○	○	○	○	🟥	🟥	🟥	○	○	🟥
*gyr*A95	E	○	○	○	○	○	○	🟥	🟥	🟥	○	○	🟥
*par*C	F	○	○	○	○	○	○	○	🟥	🟥	○	🟥	🟥
*por*B	G	○	○	🟩	○	🟩	○	🟥	🟥	🟥	○	○	○
*pen*A	H	○	○	○	○	○	○	🟥	🟥	○	🟥	○	🟥

NAAT AMR Prediction Testing – SNP Assay Targets

- Focuses on antimicrobials that are part of current recommended or alternative treatments
  - Cephalosporins (Ceftriaxone and Cefixime)
  - Azithromycin
  - Ciprofloxacin

- SNPs tested using the assay include:

Cephalosporins

(Ceftriaxone and Cefixime)

- ➢ *ponA* L421P
- ➢ *porB* G120/A121
- ➢ *mtrR* -35delA
- ➢ *penA* A311V
- ➢ *penA* A501
- ➢ *penA* N513Y
- ➢ *penA* G543S

Azithromycin

- ➢ 23S A2059G
- ➢ 23S C2611T
- ➢ *mtrR* promoter variants (meningitidis-like and “disrupted”)

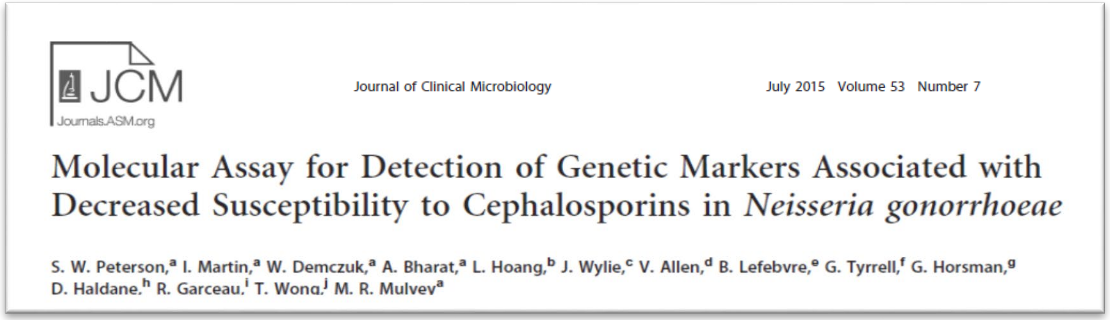
Ciprofloxacin

- ➢ *gyrA* S91
- ➢ *parC* D86/S87/S88

Interpretation of Results from SNP Assays for GC AMR Predictions

Antimicrobial	Interpretative Category	NAATs	Predicted NAAT MICs	
			Ceftriaxone	Cefixime
Cephalosporins	Resistant	penA A311V	≥ 0.25 mg/L	≥ 0.5 mg/L
	Decreased Susceptibility	A501P and 2 SNPs	≥ 0.125 mg/L	≥ 0.25 mg/L
	Intermediate or Decreased Susceptibility	3/7 SNPs (not including penA A311V)	0.032 - ≤ 0.125 mg/L	0.032 - ≤ 0.25 mg/L
		N513Y, L421P, and tbpB-10	N/A	0.032 - ≤ 0.25 mg/L
	Susceptible	0 to 2 SNPs	< 0.032 mg/L	< 0.032 mg/L
Ciprofloxacin	Resistant	≥ 1 SNP	≥ 1 mg/L	
	Susceptible	All WT (wild type)	< 1 mg/L	
Azithromycin	Resistant	≥ 1 SNP	≥ 1 mg/L	
	Susceptible	All WT (wild type)	< 1 mg/L	

SNP Assay Publications

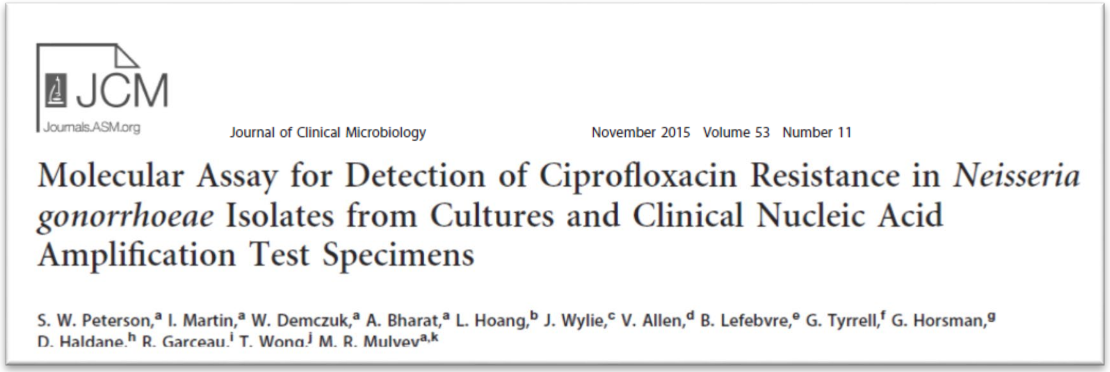


JCM Journals.ASM.org

Journal of Clinical Microbiology July 2015 Volume 53 Number 7

Molecular Assay for Detection of Genetic Markers Associated with Decreased Susceptibility to Cephalosporins in *Neisseria gonorrhoeae*

S. W. Peterson,[a] I. Martin,[a] W. Demczuk,[a] A. Bharat,[a] L. Hoang,[b] J. Wylie,[c] V. Allen,[d] B. Lefebvre,[e] G. Tyrrell,[f] G. Horsman,[g] D. Haldane,[h] R. Garceau,[i] T. Wong,[j] M. R. Mulvey[a]



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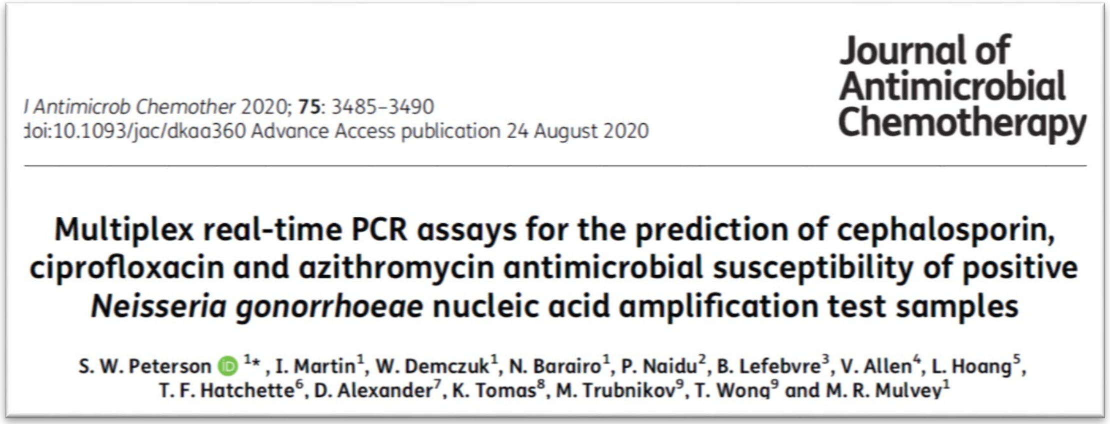
Journal of Clinical Microbiology November 2015 Volume 53 Number 11

Molecular Assay for Detection of Ciprofloxacin Resistance in *Neisseria gonorrhoeae* Isolates from Cultures and Clinical Nucleic Acid Amplification Test Specimens

S. W. Peterson,[a] I. Martin,[a] W. Demczuk,[a] A. Bharat,[a] L. Hoang,[b] J. Wylie,[c] V. Allen,[d] B. Lefebvre,[e] G. Tyrrell,[f] G. Horsman,[g] D. Haldane,[h] R. Garceau,[i] T. Wong,[j] M. R. Mulvey[a,k]



J Antimicrob Chemother 2020; **75**: 3485–3490
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Journal of Antimicrobial Chemotherapy

Multiplex real-time PCR assays for the prediction of cephalosporin, ciprofloxacin and azithromycin antimicrobial susceptibility of positive *Neisseria gonorrhoeae* nucleic acid amplification test samples

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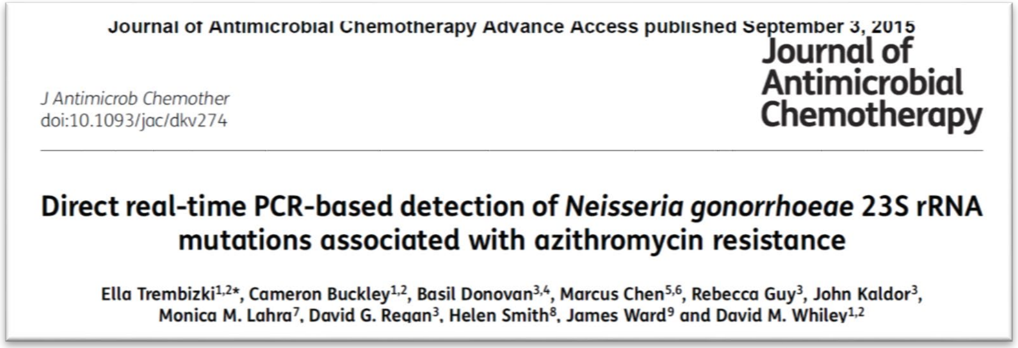


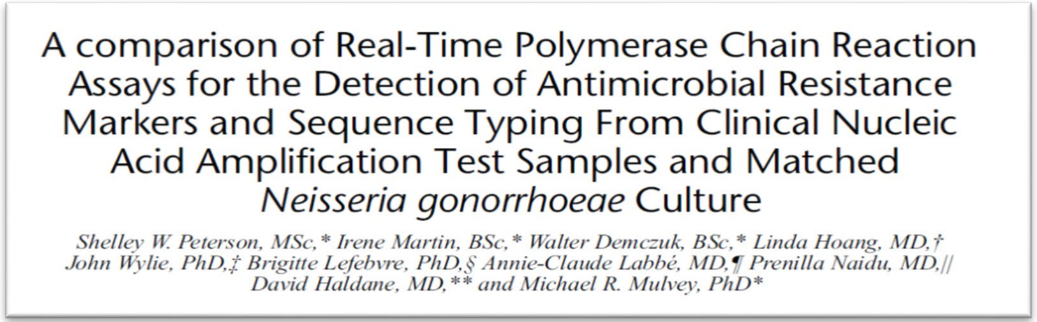
Journal of Antimicrobial Chemotherapy Advance Access published September 3, 2015

J Antimicrob Chemother
doi:10.1093/jac/dkv274

Journal of Antimicrobial Chemotherapy

Direct real-time PCR-based detection of *Neisseria gonorrhoeae* 23S rRNA mutations associated with azithromycin resistance

Ella Trembizki[1,2]*, Cameron Buckley[1,2], Basil Donovan[3,4], Marcus Chen[5,6], Rebecca Guy[3], John Kaldor[3], Monica M. Lahra[7], David G. Regan[3], Helen Smith[8], James Ward[9] and David M. Whiley[1,2]



A comparison of Real-Time Polymerase Chain Reaction Assays for the Detection of Antimicrobial Resistance Markers and Sequence Typing From Clinical Nucleic Acid Amplification Test Samples and Matched *Neisseria gonorrhoeae* Culture

Shelley W. Peterson, MSc, Irene Martin, BSc,* Walter Demczuk, BSc,* Linda Hoang, MD,† John Wylie, PhD,‡ Brigitte Lefebvre, PhD,§ Annie-Claude Labbé, MD,¶ Prenilla Naidu, MD,|| David Haldane, MD,** and Michael R. Mulvey, PhD**

Sexually Transmitted Diseases • Volume 45, Number 2, February 2018

Comparison of GC NAATs vs. cultures for AMR testing

NAATs	Cultures (gold standard)
Collection from GC-positive patients is less invasive and more efficient	Invasive collection method that requires good transport and isolation procedures
Can be used for GC cases from remote regions	Requires resources to collect and transport to maintain viability for testing
Can detect isolates that are not viable, but may not differentiate in a mixed culture	Organisms must be viable for testing. Can separately test organisms in a mixed culture
Molecular tests only detect known targets that predict resistance/susceptibility interpretation to certain antimicrobials, rather than MIC values	Agar dilution or E-test and can test a full range of antimicrobials Whole genome sequencing is only performed on bacterial cultures
Meant for larger scale surveillance; not for individual patient care	Agar dilution for GC cultures can be used for individual patient care

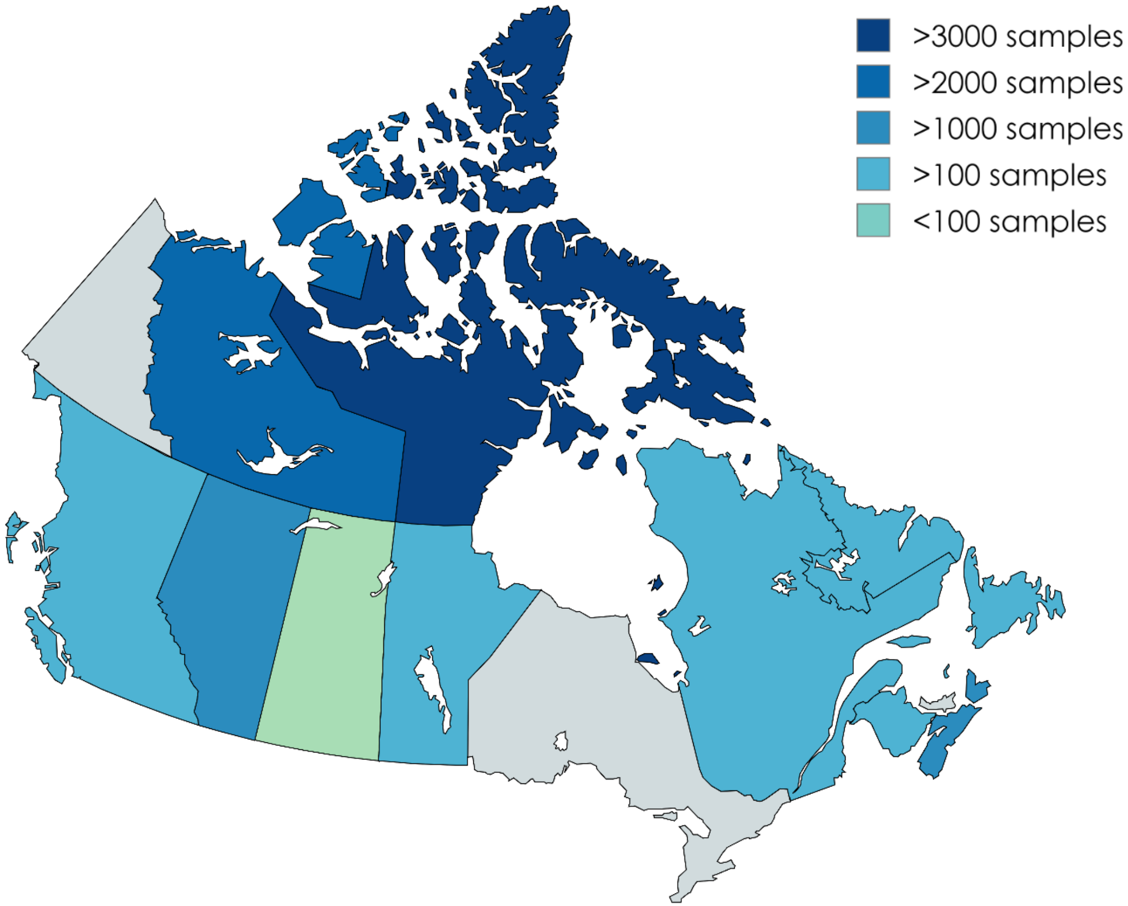
Limitations of GC NAATs for AMR Testing

- Detection of non-GC organisms
  - Not recommended for pharyngeal specimens due to presence of non-GC *Neisseria* species
- Cephalosporin AMR in *N. gonorrhoeae* is determined by combined effects of multiple genes
  - Can lead to incorrect predictions due to organisms with the same SNP profile exhibiting a range of MICs
- Limit of detection
  - Low concentration of GC DNA in NAAT samples can limit SNP detection
- Emergence of new strains
  - Can lead to false negative or false SNP results due to sequence variations in primer or probe regions

SSTI routinely updates these assays to include currently circulating mutations to ensure the assay predictions are as accurate as possible

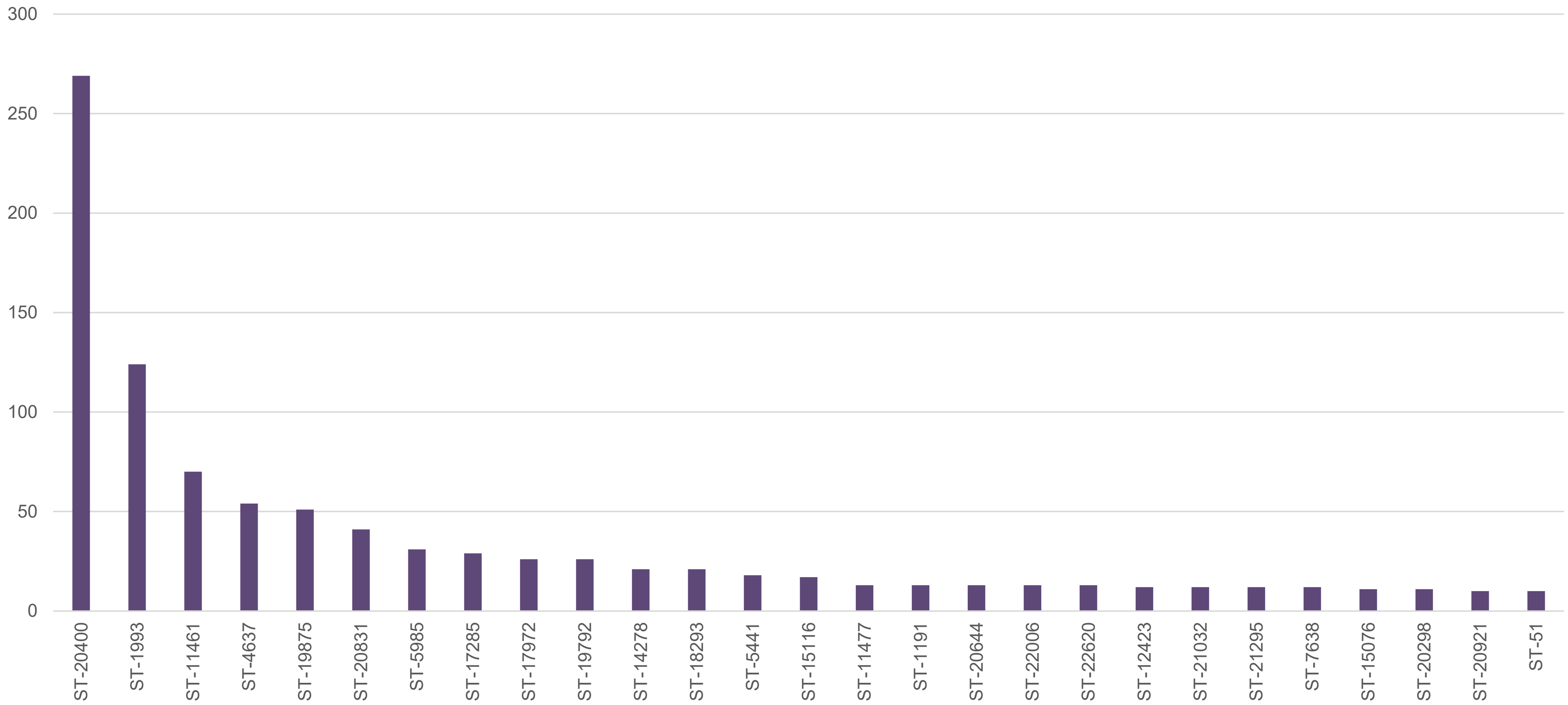
N. gonorrhoeae NAAT specimens

- NMLB has been accepting GC-positive NAAT samples since 2017
- To date we have received 10,636 NAAT specimens from 10 provinces/territories



Province/Territory	# of samples	Proportion
Nunavut	3,463	32.56%
Northwest Territories	2,025	19.04%
Alberta	1,948	18.32%
Nova Scotia	1,552	14.59%
British Columbia	424	3.99%
Manitoba*	363	3.41%
Newfoundland/Labrador*	318	2.99%
New Brunswick*	264	2.48%
Quebec*	206	1.94%
Saskatchewan	73	0.69%
TOTAL	**10,636**	**100%**

GC-NAAT NG-MAST Prevalence, 2023 (N=1,370)



- 230 STs detected in 2023
- Note: This figure only contains STs with ≥10 samples

STs of interest, 2023

ST-20400 and ST-1993

- The most prevalent STs were ST-20400 (19.6%) and ST-1993 (9.1%)
  - To date, these STs have only been detected in the North
  - ST-1993 was first detected in 2017 and is associated with predicted susceptible GC strains
  - ST-20400 was first detected in 2022 is associated with predicted elevated MICs to cephalosporins and ciprofloxacin resistance

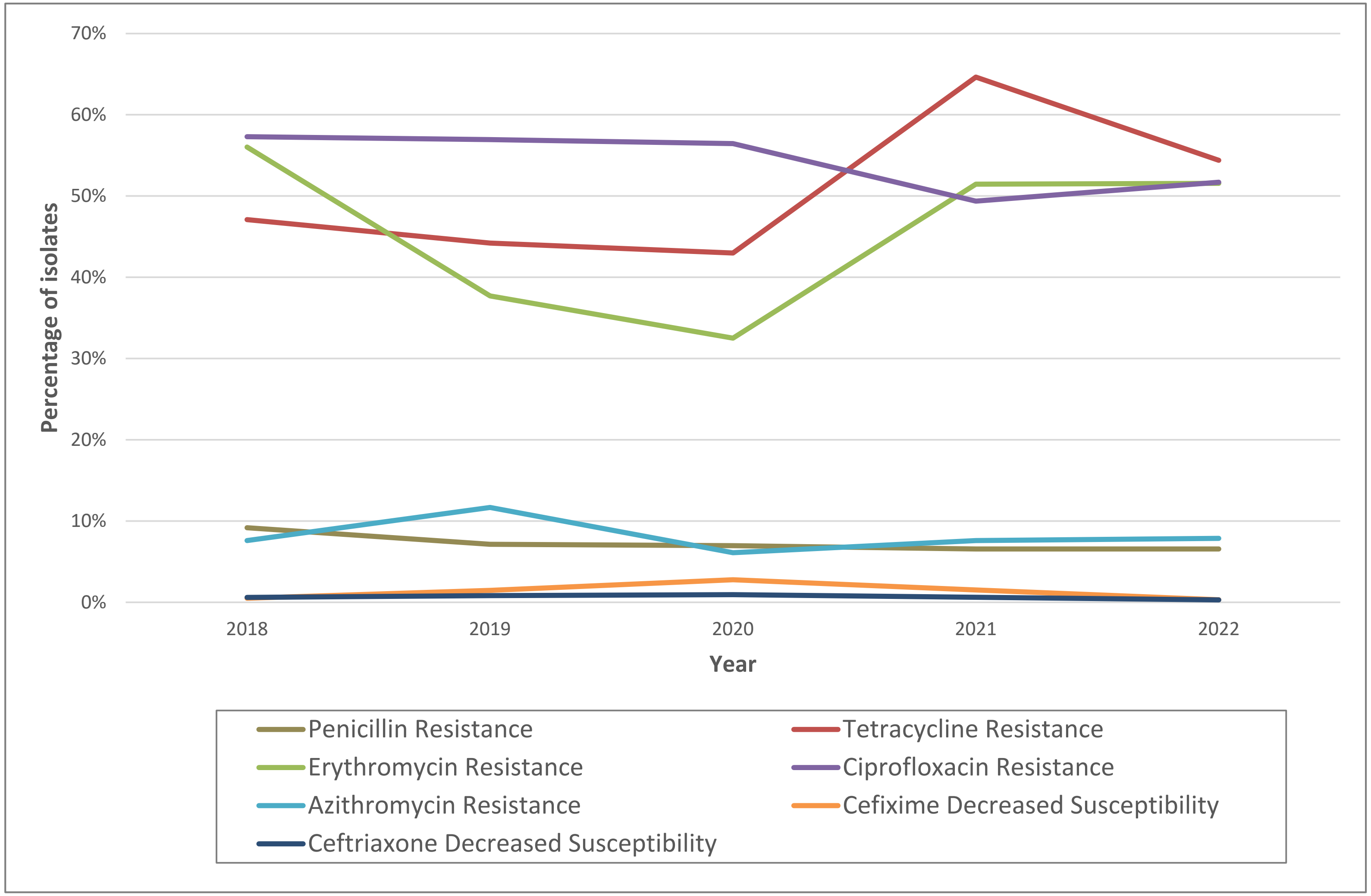
ST-20336

- ST-20336 was detected in the NAATs from P/Ts in the East
  - ST-20336 was first detected in Ontario in 2021 as a GC culture, susceptible to azithromycin
  - In 2023, GC cultures and NAATs in the East with high level azithromycin resistance were detected
    - For GC NAATs, the 23S A2059G SNP was present

N. gonorrhoeae cultures tested in each province, 2018–2022

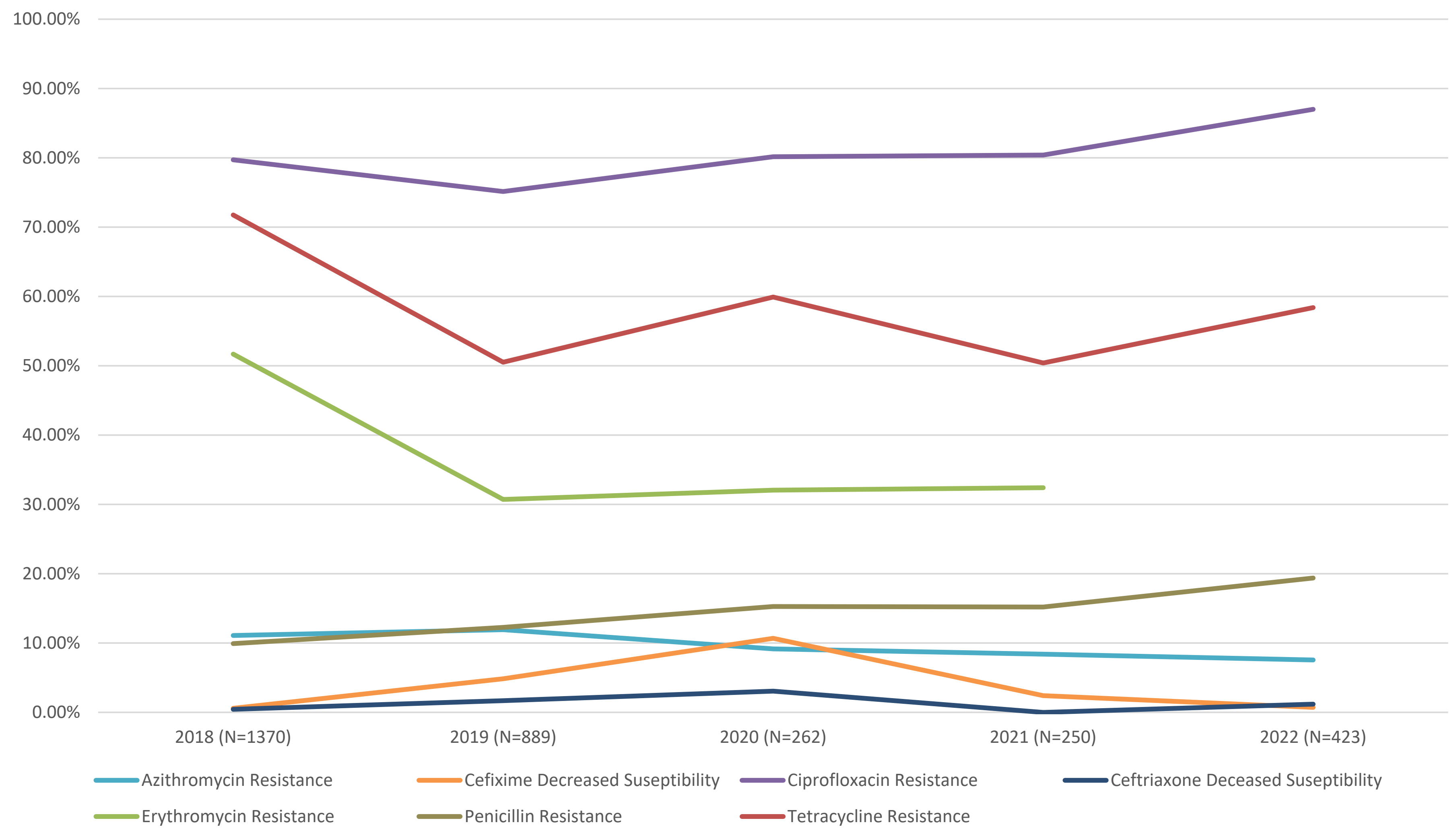
Province/Territory[a]	2018	2019	2020	2021	2022	Total
Alberta	**837**	**926**	**575**	**652**	**651**	**4,252**
-submitted AMR data	**200**	**134**	**102**	**131**	**131**	
British Columbia	**219**	**255**	**129**	**119**	**128**	**2,092**
-submitted AMR data	**296**	**258**	**266**	**210**	**256**	
Manitoba	**195**	**167**	**137**	**44**	**96**	**625**
Nova Scotia	**35**	**30**	**2**	**10**	**10**	**1**
Ontario	**1,370**	**889**	**262**	**250**	**423**	**3,172**
Québec	**805**	**736**	**455**	**985**	**1,271**	**8,174**
-submitted AMR data	**1,060**	**1,051**	**721**	**576**	**524**	
Saskatchewan	**135**	**127**	**51**	**41**	**49**	**400**
Other[b]	**19**	**28**	**17**	**36**	**44**	**140**
Total number of cases diagnosed by culture with either AMR data or cultures submitted to the NML (duplicates, contaminated and no growths excluded)	**4,943**	**4,334**	**2,679**	**2,909**	**3,393**	**18,258**
Total number of cases with cultures tested in each province[d]	5,607	4,859	3,130	3,439	3,855	20,890
Total cultures resistant to at least one antibiotic	4,061	3,690	2,195	2,501	2,910	15,357
Percentage of cultures resistant to at least one antibiotic	72.4%	75.9%	70.1%	72.7%	75.5%	73.3%
Percentage of total reported cases tested	**18.2%**	**13.7%**	**10.2%**	**10.7%**	N/A	N/A
Total cases reported in Canada	30,874	35,443	30,833	32,192	N/A	N/A

Antimicrobial Susceptibilities of *N. gonorrhoeae* in Canada, 2018-2022



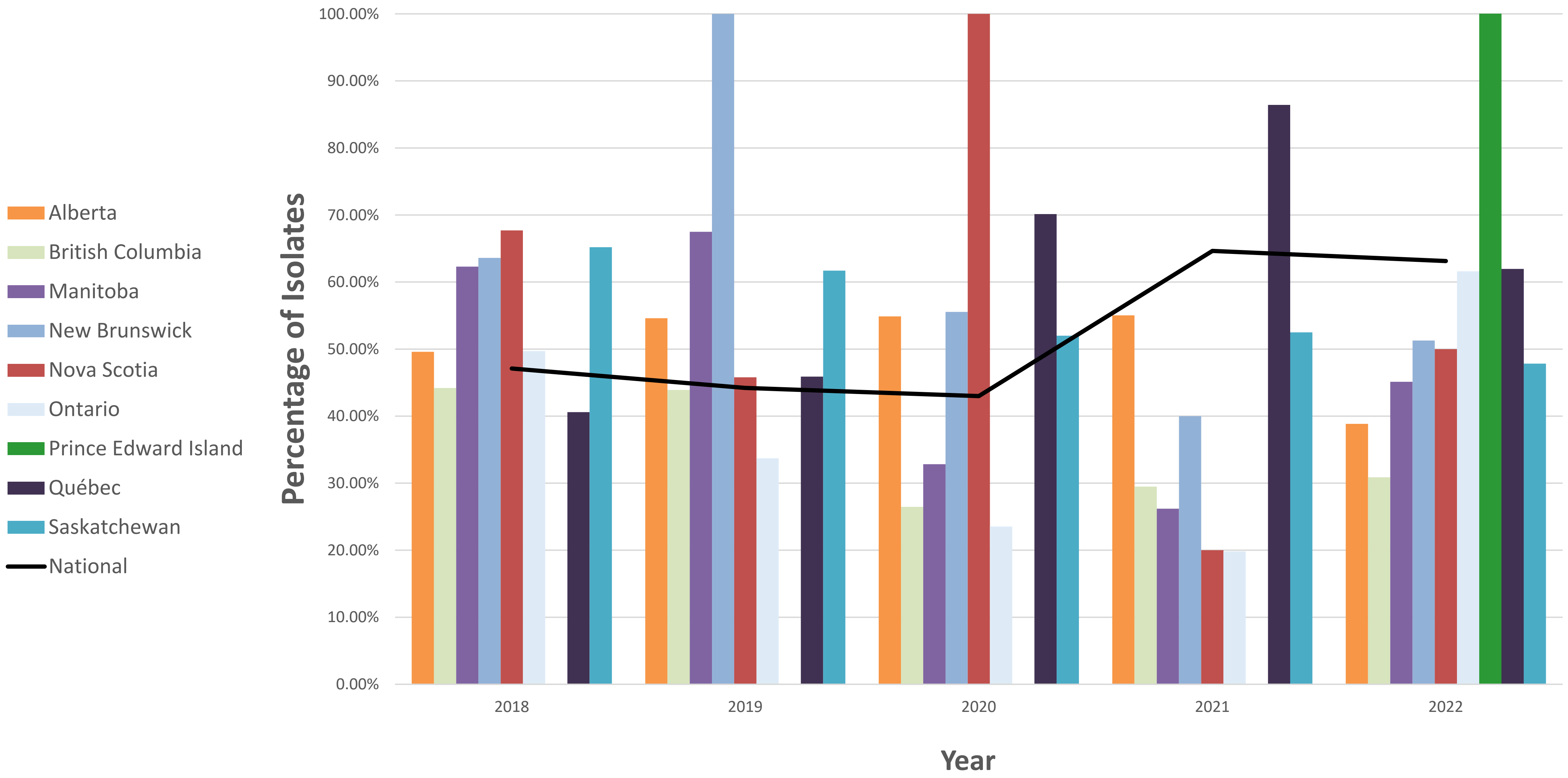
Antimicrobial	2021	2022
Penicillin[b]	6.5%	6.5%
Tetracycline[b]	64.6%	54.4%
Erythromycin[b]	51.5%	51.6%
Ciprofloxacin	49.4%	51.7%
Azithromycin	7.6%	8.1%
Cefixime	1.51%	0.3%
Ceftriaxone	0.61%	0.3%

Antimicrobial Susceptibilities of *N. gonorrhoeae* in Ontario, 2018-2022

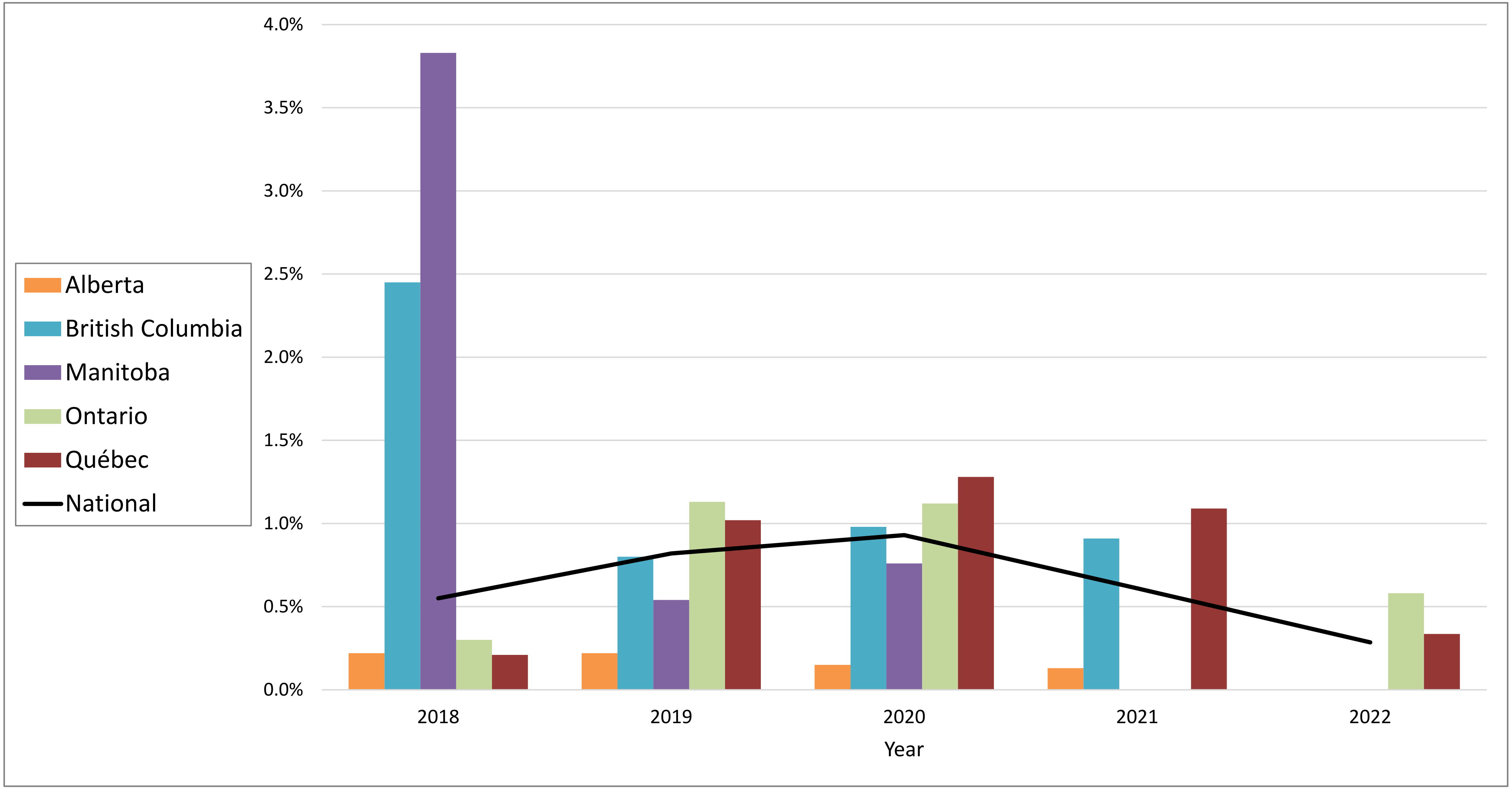


ERY MICs not predicted by WGS. No data from 2022 included here due to the large decrease in denominator size and bias to testing resistant samples

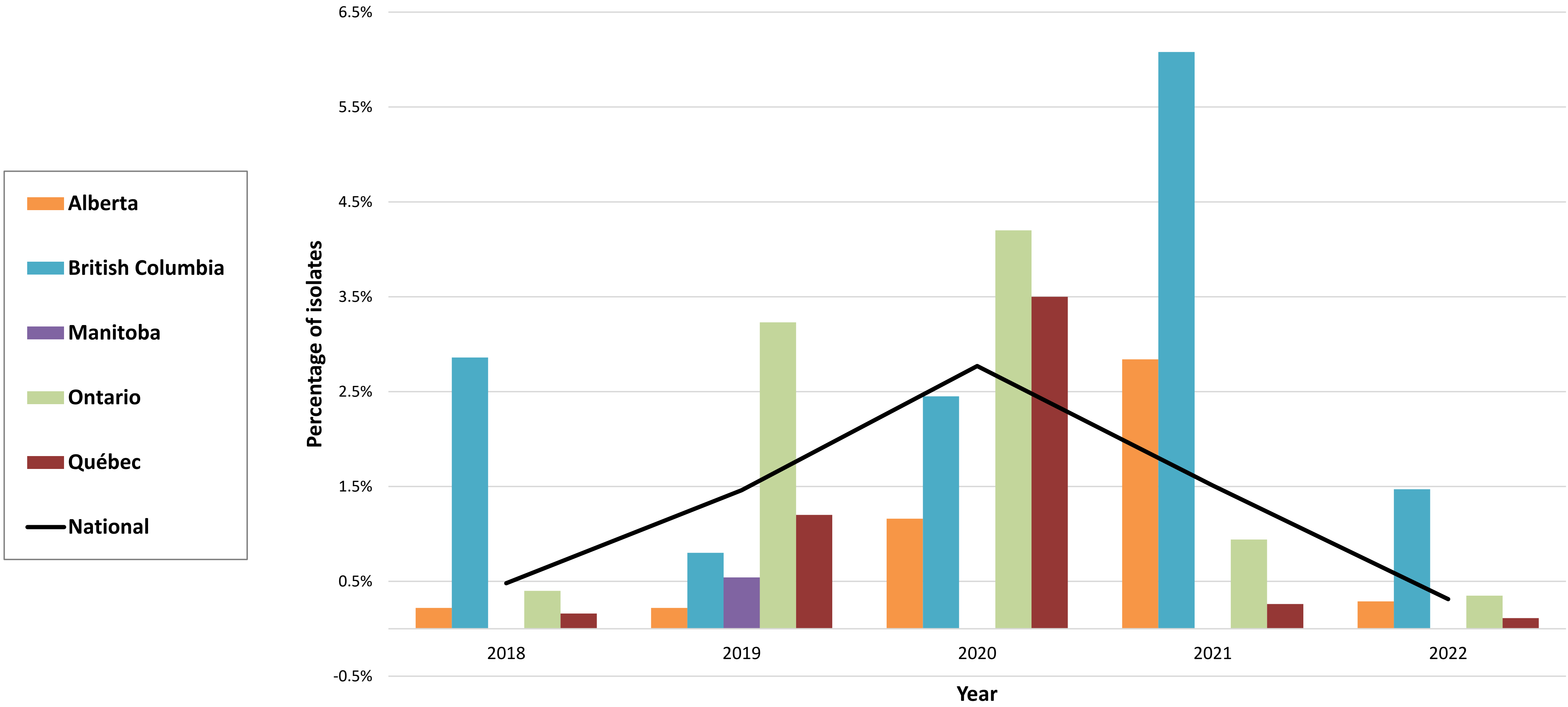
N. gonorrhoeae Isolates with Resistance to Tetracycline, 2018 to 2022



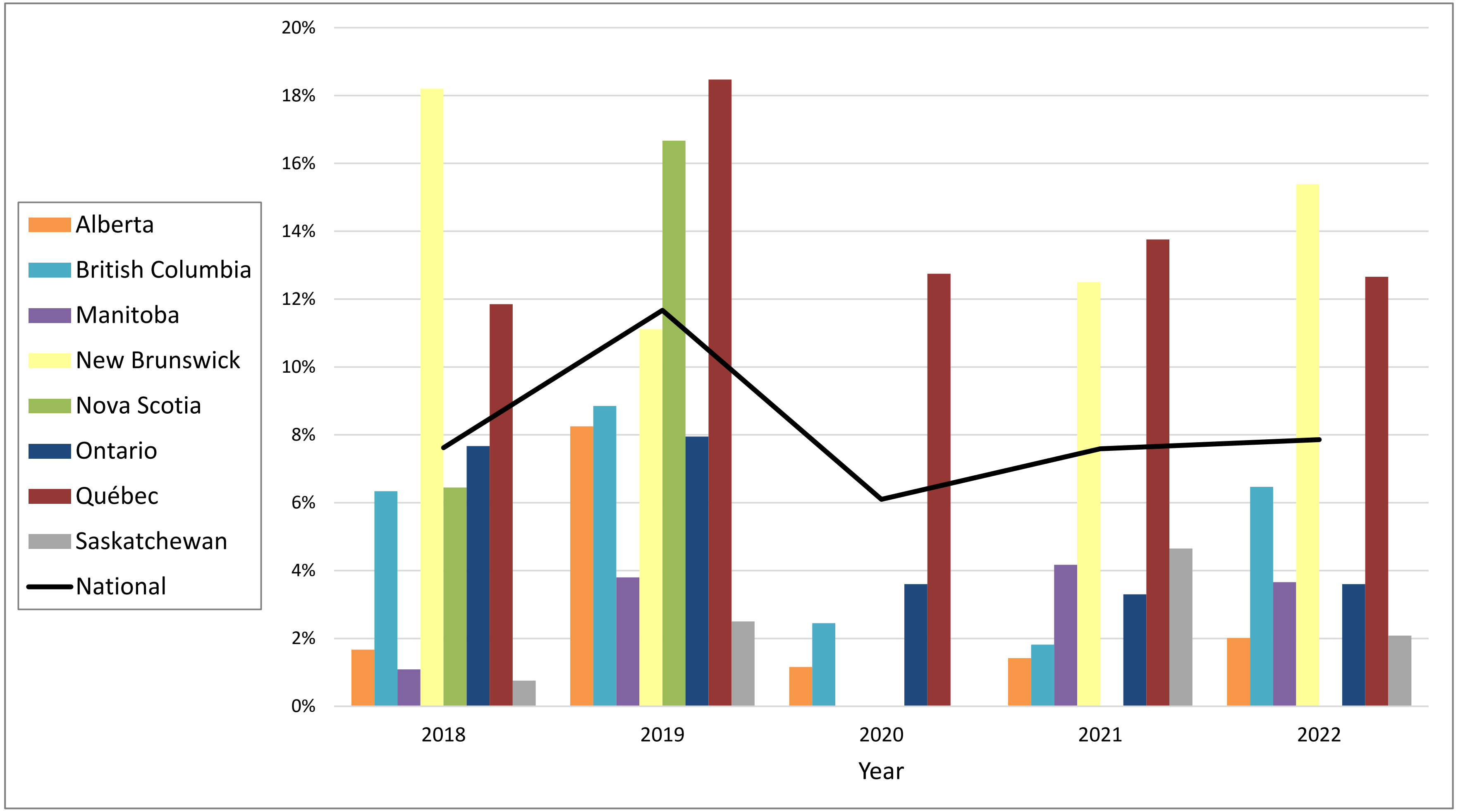
N. gonorrhoeae with Decreased Susceptibility to Ceftriaxone, 2018 to 2022



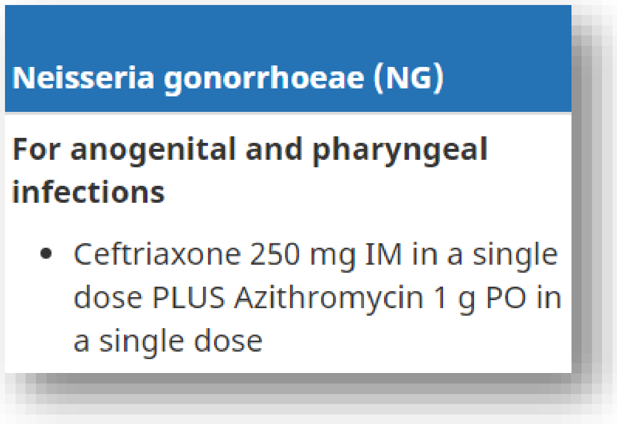
N. gonorrhoeae with Decreased Susceptibility to Cefixime, 2018 to 2022



Azithromycin Resistant *N. gonorrhoeae*, 2018 to 2022



Canadian Guidelines for
Sexually Transmitted Infections



Neisseria gonorrhoeae (NG)

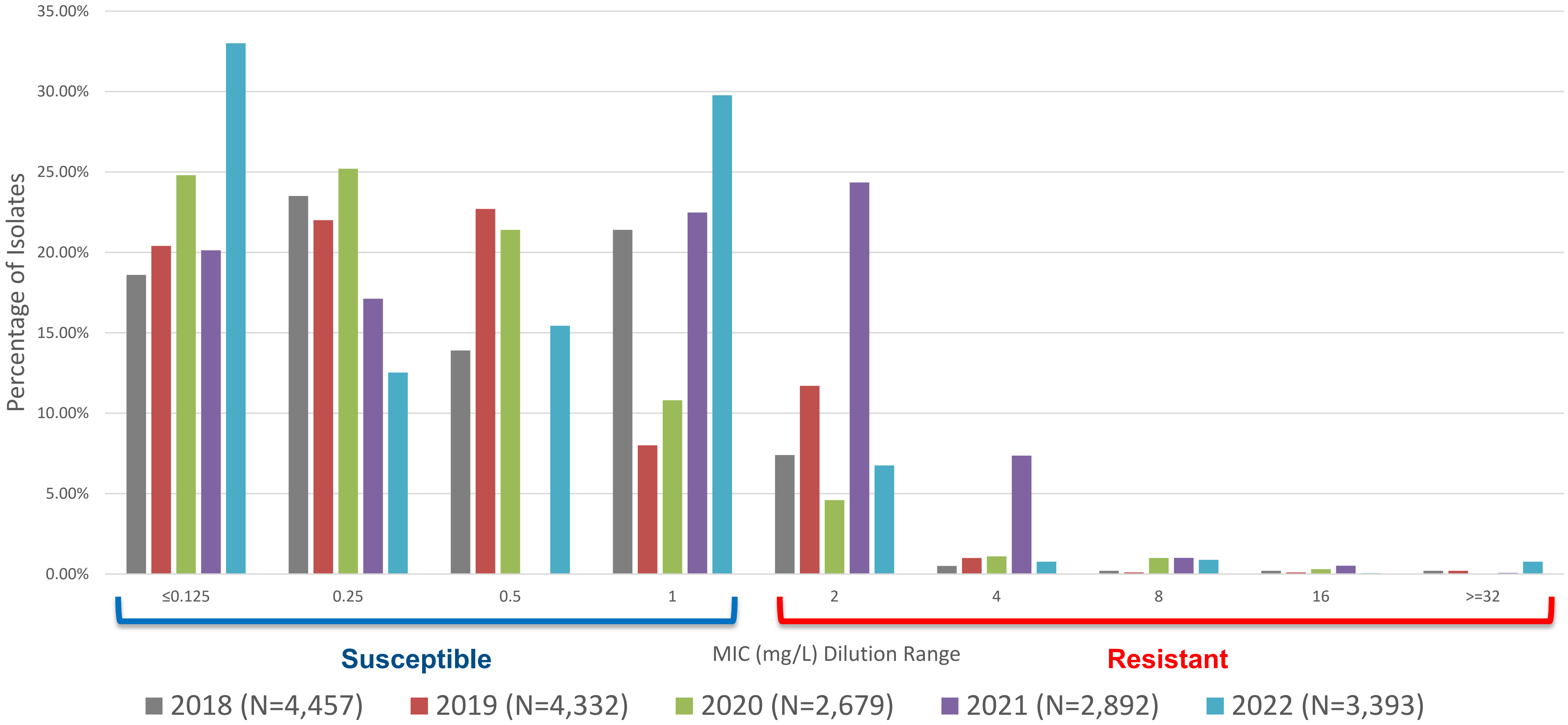
For anogenital and pharyngeal infections

- Ceftriaxone 250 mg IM in a single dose PLUS Azithromycin 1 g PO in a single dose

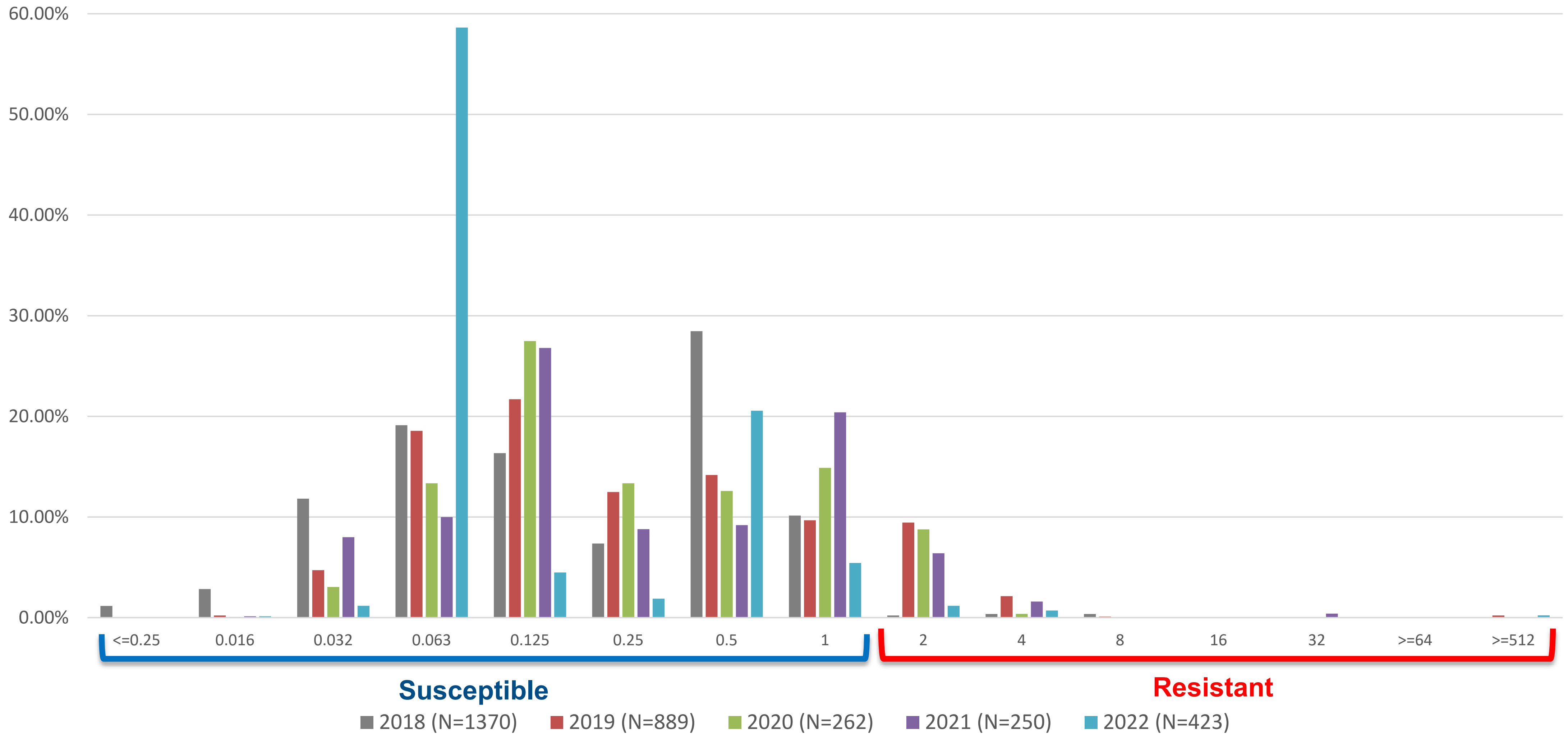
5% WHO

Gonorrhea chapter is currently under review again.

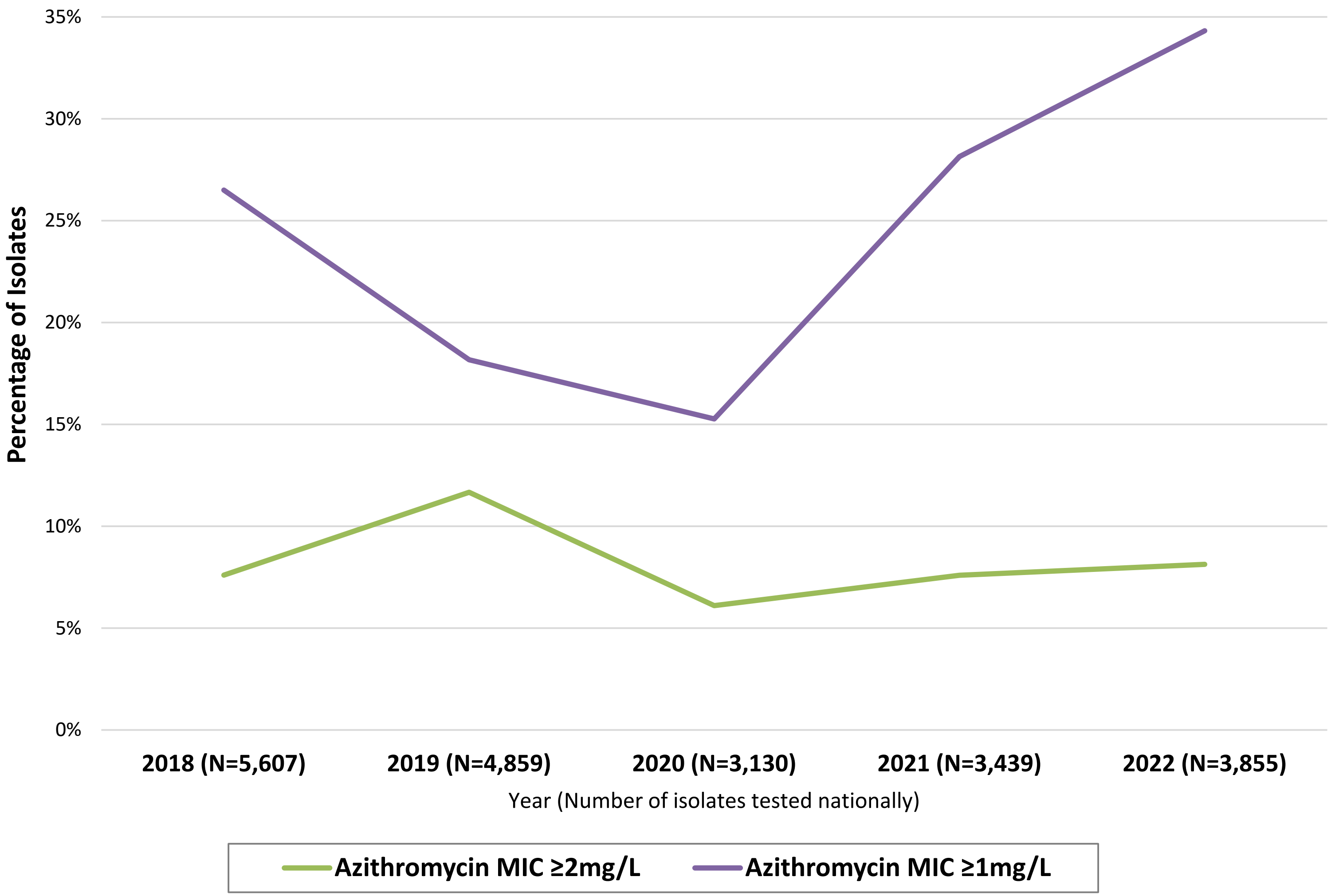
Azithromycin Susceptibilities of *Neisseria gonorrhoeae* from 2018 to 2022



Azithromycin Susceptibilities of *Neisseria gonorrhoeae* in Ontario 2018-2022

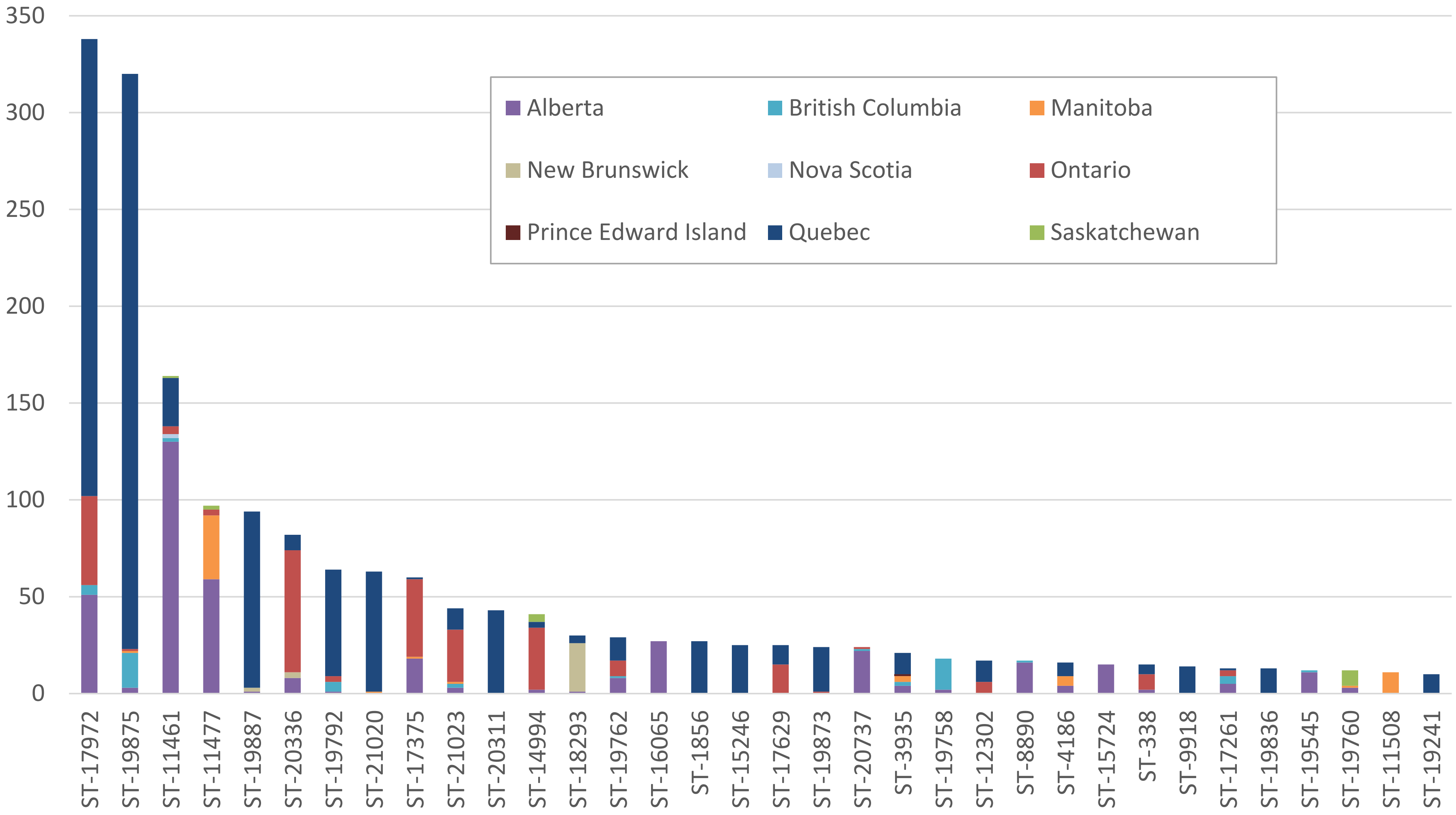


Percentage of azithromycin resistant *N. gonorrhoeae* isolates using susceptibility breakpoints of ≥ 1 mg/L and ≥ 2mg/L

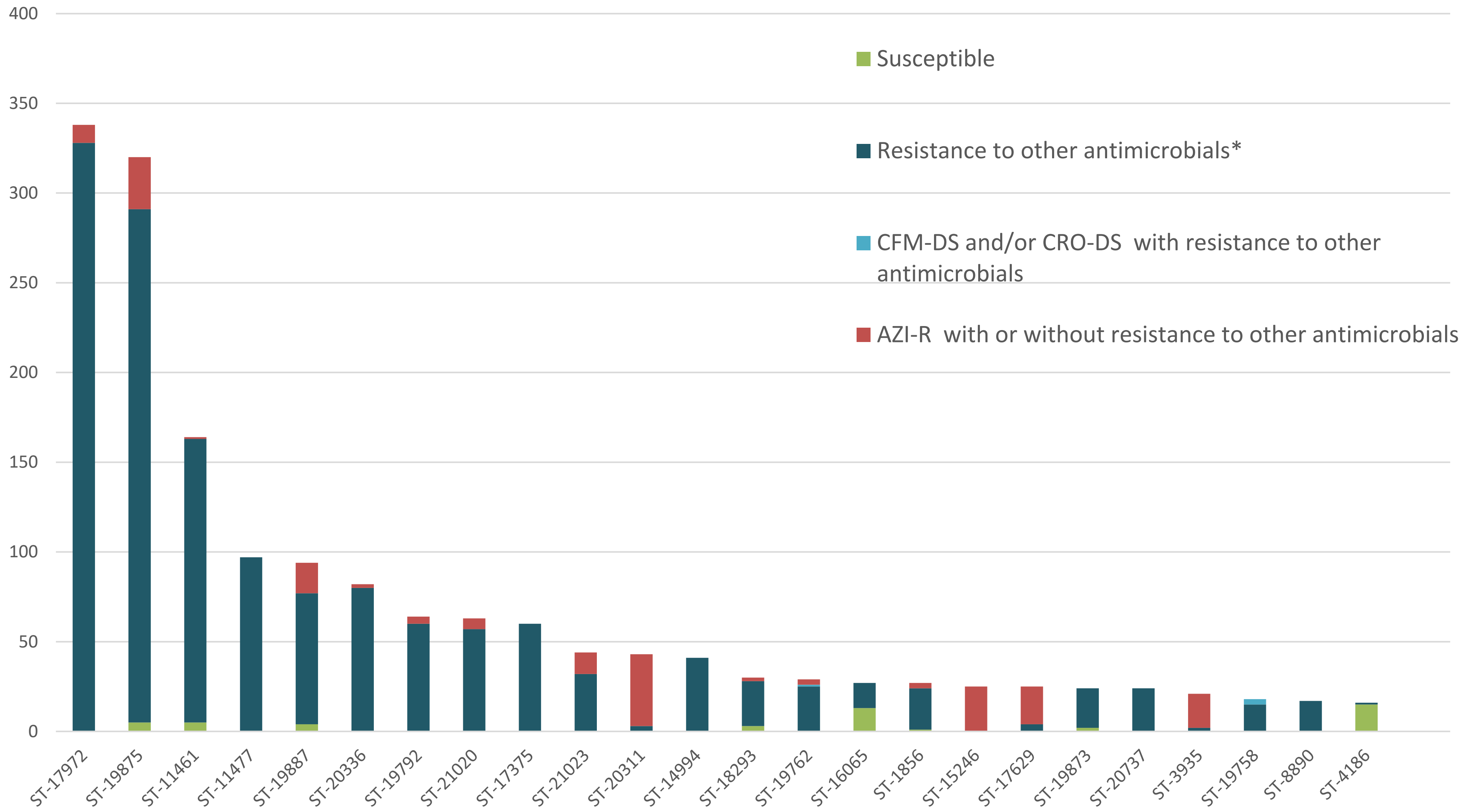


- Other regions, (eg. **Australia, UK**) have set the azithromycin breakpoint at **1 mg/L.**
- This is the epidemiological cut-off value (**ECOFF**) from the European Committee on Antimicrobial Susceptibility Testing (**EUCAST**)

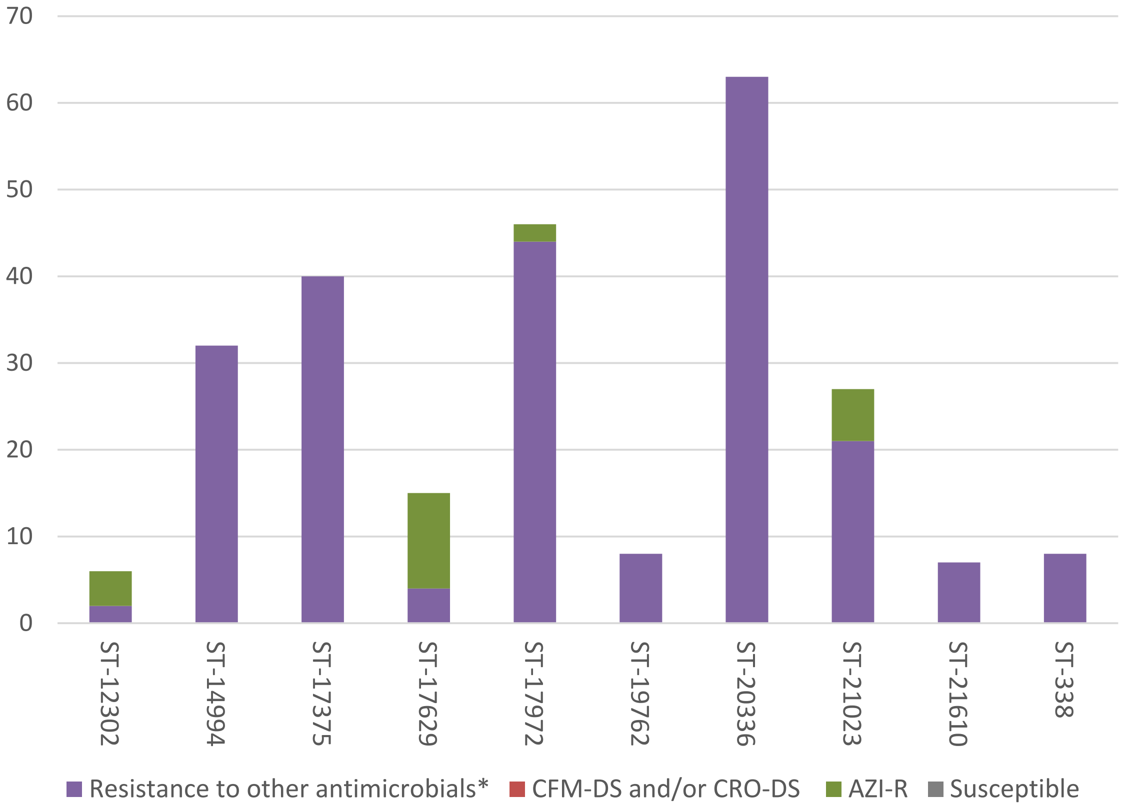
Provincial distribution within *N. gonorrhoeae* multi-antigen sequence types (NG-MAST), 2022 (N=2,544)

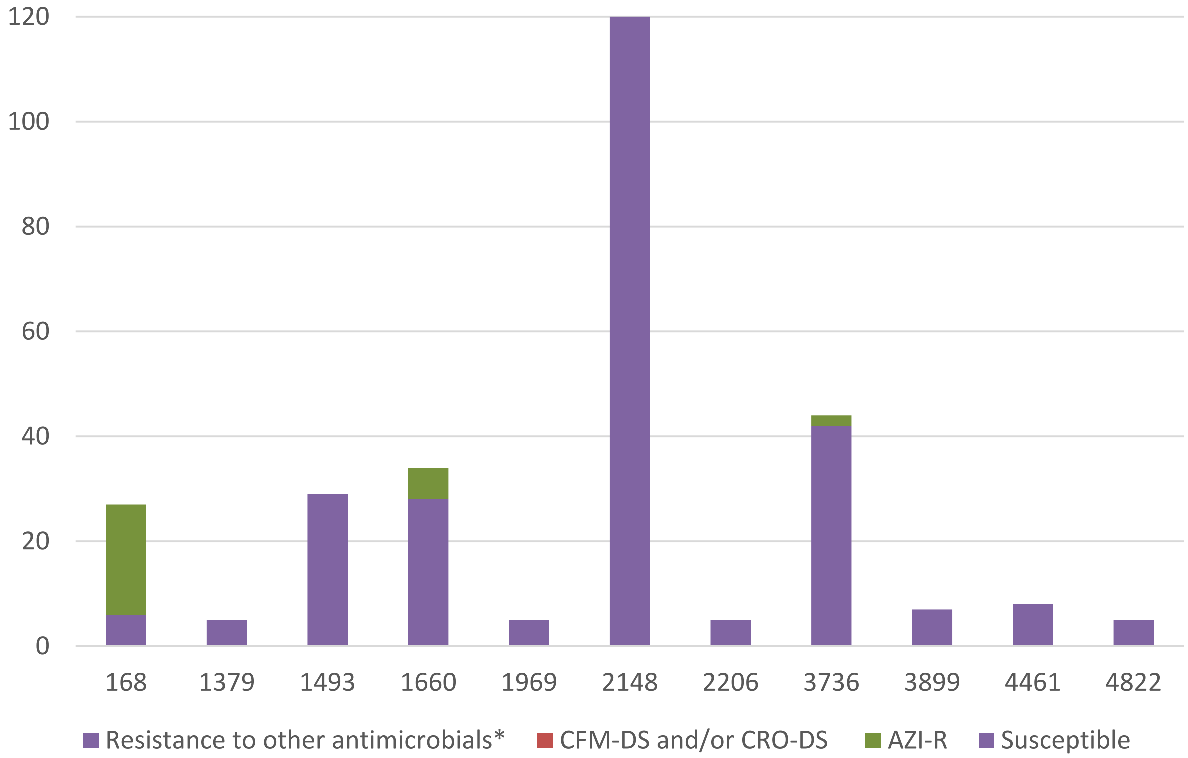


Distribution of antimicrobial resistance characterizations within *N. gonorrhoeae*-multi-antigen sequence typing sequence types (NG-MAST), 2022, n=2,544



Top 10 NG-MAST and NG-STAR STs and AMR characterization in 2022 for Ontario

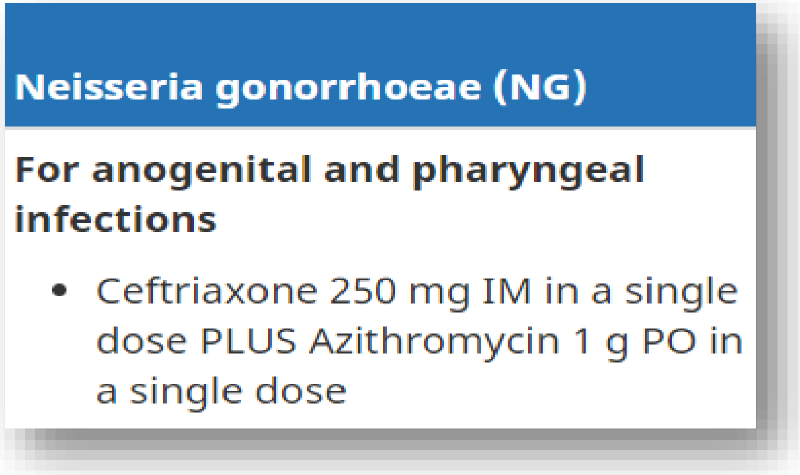




Other antimicrobials include: ciprofloxacin, erythromycin (isolates with agar dilution results), penicillin, and tetracycline.

Ceftriaxone-resistant *Neisseria gonorrhoeae*:

Threat to the current recommended therapy



Neisseria gonorrhoeae (NG)

For anogenital and pharyngeal infections

- Ceftriaxone 250 mg IM in a single dose PLUS Azithromycin 1 g PO in a single dose

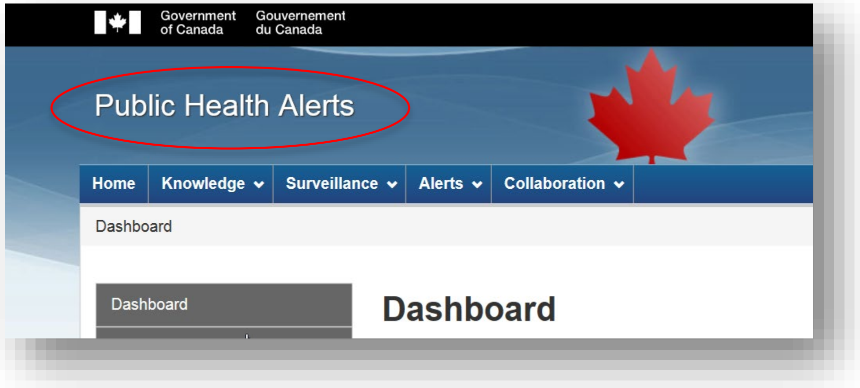
- **H041** strain: Japan 2009 *(Ohnishi et al. Emerg Infect Dis 2011)*
- **F89** strain: France 2010 & Spain 2011 *(Unemo et al. AAC 2012 & Camara et al. JAC 2012)*
- **A8806** strain: Australia 2013 *(Lahra et al. NEJM 2014)*
- **FC428** strain: Japan 2015 *(Nakayama et al. Emerg Infect Dis 2016)*

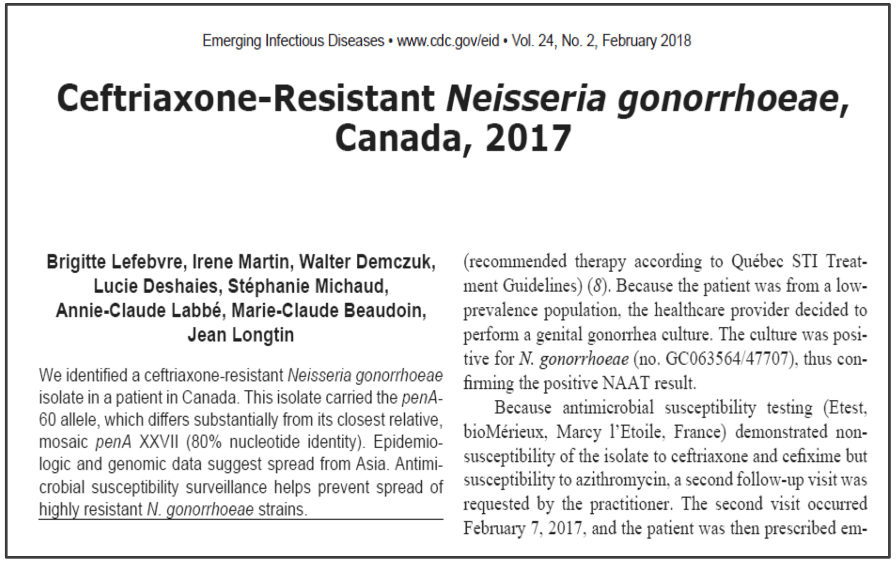
Sporadic…no further transmission due to lack of fitness and/or opportunity

First high-level ceftriaxone resistant gonorrhea identified in Canada (Quebec) – Treatment Failure

Case Characteristics

- 23 yr F
- Genital sample
- Collected: 2017
- Ceftriaxone MIC = 1 mg/L/ Cefixime MIC = 2 mg/L
- NG-MAST-1614/ MLST-1903/ NG-STAR-233
- Treated with cefixime 800 mg, PO and Azithromycin 1 g, PO
- Followed by Azithromycin 2 g, PO
- 13 days post-treatment, test-of-cure GC negative
- Partner reported having unprotected sex during a trip to Asia in the fall of 2016
  - No culture obtained from partner





Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 24, No. 2, February 2018

Ceftriaxone-Resistant *Neisseria gonorrhoeae*, Canada, 2017

Brigitte Lefebvre, Irene Martin, Walter Demczuk, Lucie Deshaies, Stéphanie Michaud, Annie-Claude Labbé, Marie-Claude Beaudoin, Jean Longtin

We identified a ceftriaxone-resistant *Neisseria gonorrhoeae* isolate in a patient in Canada. This isolate carried the *penA*-60 allele, which differs substantially from its closest relative, mosaic *penA* XXVII (80% nucleotide identity). Epidemiologic and genomic data suggest spread from Asia. Antimicrobial susceptibility surveillance helps prevent spread of highly resistant *N. gonorrhoeae* strains.

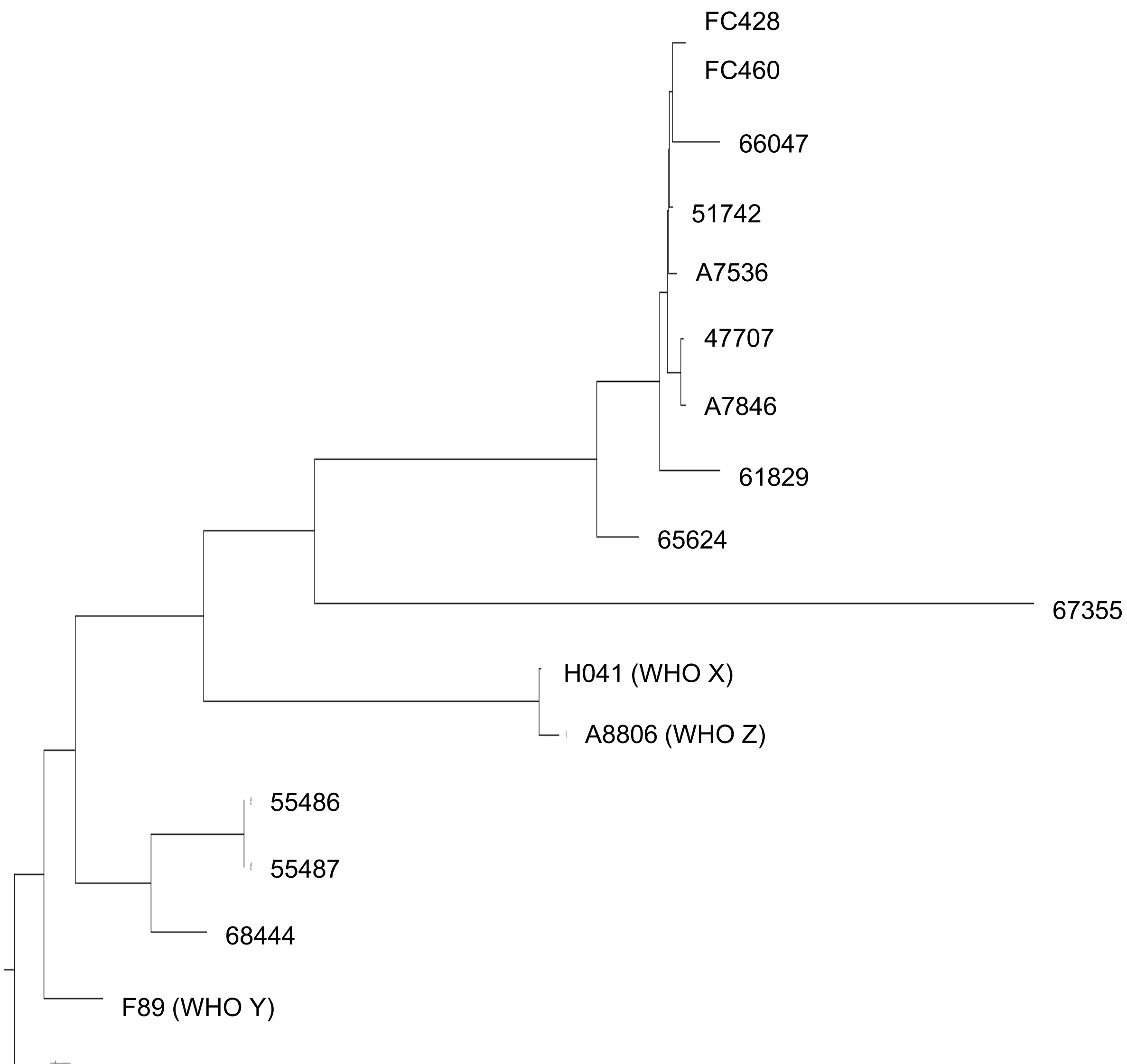
(recommended therapy according to Québec STI Treatment Guidelines) (*8*). Because the patient was from a low-prevalence population, the healthcare provider decided to perform a genital gonorrhea culture. The culture was positive for *N. gonorrhoeae* (no. GC063564/47707), thus confirming the positive NAAT result.

Because antimicrobial susceptibility testing (Etest, bioMérieux, Marcy l'Etoile, France) demonstrated nonsusceptibility of the isolate to ceftriaxone and cefixime but susceptibility to azithromycin, a second follow-up visit was requested by the practitioner. The second visit occurred February 7, 2017, and the patient was then prescribed em-

N. gonorrhoeae cultures identified in Canada with ceftriaxone MIC >=0.25 mg/L, 2017-2024

NML #	Region	Collection date	NG-MAST	Resistance Profile	MIC (ug/mL)			NG-STAR	MLST	penA	Travel History	Treatments
					CRO	CFM	AZ					
47707	Central	2017	ST-1614	CFM-DS/ CRO-DS/ Cip-R/ Ery-R/ Pen-R/ Tet-R	1	2	0.3	233	1903	60.001	Partner travelled to Asia in fall of 2016	1. CFM 800 mg, PO and AZI 1g, PO/ 2. AZI 2g, PO
51742	Western	2018	ST-3435	CFM-DS/ CRO-DS/ Cip-R/ Ery-R/ Pen-R/ Tet-R	0.5	2	0.3	233	1903	60.001	North East Asia	1. CFM 800 mg, PO and AZI 1g, PO/ 2. AZI 2g, PO
55486	Central	2018	ST-18042	CFM-DS/ CRO-DS/ Cip-R/ Ery-R/ Pen-I/ Tet-R	0.5	2	0.3	3865	1901	121.002	Travelled to South-East Asia (not verified)	1. CRO 250 mg IM and AZI 1g, PO
55487	Central	2018	ST-18042	CFM-DS/ CRO-DS/ Cip-R/ Ery-R/ Pen-R/ Tet-R	1	2	0.5	3865	1901	121.002	Travelled to South-East Asia (not verified)	1. CRO 250 mg IM + 100mg doxy BID 14 days
61829	Western	2021	ST-19937	CFM-DS/ CRO-DS/ Cip-R/ Ery-R/ Pen-R/ Tet-R	1	2	0.5	3903	7365	60.001	Travelled in Canada	1. CFM 800 mg, PO. 2. CRO 250 mg IM
66047 66872	Central	2023	ST-21711	CFM-DS, CRO-DS, Cip-R, Ery-R, Pen-R, Tet-R	1	2	0.3	233	13943	60.001	Acquired locally	1. CRO 250 mg IM and AZI 2g, PO
65624	Western	2023	ST-21730	CFM-R/ CRO-R/ Cip-R/ Ery-R/ Pen-I/ Tet-R	2*	2*	0.5	233	17523	60.001	South East Asia	1. CRO 1g IV and doxy 100 mg BID x 14 days (as well as MET 500 mg BID x 14 days)
67356	Western	2023	ST-22789	CFM-R/ CRO-R/ Cip-R/ Ery-R/ Pen-R/ Tet-I	0.25/1	2	0.5	5575	1588	237.001	International	1. AZI 1g PO, CFM 800mg PO
68444	Western	2024		CFM-R/ CRO-R/ Cip-R/ Ery-R/ Pen-R/ Tet-R	0.5	2	0.3	5852	1901	237.001	Unknown	1. AZI 1g PO, CFM 800 mg PO
68758	Central	2024	ST-23921	CFM-R/CRO-R/CIP-R/PEN-R/TET-R	0.25	2	1	6029	15809	60.001		1. AZI 1g PO, CFM 800mg PO, CRO 250mg IM
69155	Central	2024	ST-22862	AZI-R/CFM-R/CRO-R/CIP-R/PEN-R/TET-R	0.25	2	>=256	5793	16406	60.001	South East Asia	1. AZI 2g PO, CFM 800mg PO 2. CRO 250mg IM, Doxy 100mg PO BID (7 days) 3. CRO 500mg IM
69750	Central	2024	ST-22860	CFM-R/CRO-R/CIP-R/ ERY-R/PEN-R/TET-R	1	2	0.25	4837	1901	237.001	North East Asia	1. CRO 500 mg IM and AZI 1g, PO
70162	Western	2024	ST-2083	CFM-R/CRO-R/CIP-R/ PEN-R/TET-R	0.5	2	0.50	6034	7827	60.001	North East Asia	1. AZI 1g PO, CFM 800mg PO 2. CFM 800mg SD 3. AZI 1g PO, CRO 500mg IM

Comparison of National and International strains of Ceftriaxone Resistant *N. gonorrhoeae*



Isolate	Country Isolated	Year	NG-STAR	penA allele	NG-MAST	CRO MIC	AMR SNPs			
							penA	mtrR	porB	ponA
FC428	Japan	2015	ST-233	60.001	ST-3435	0.5	A311V/N513Y	-35AΔ	G120K/A121D	L421P
FC460	Japan	2015	ST-233	60.001	ST-3435	0.5	A311V/N513Y	-35AΔ	G120K/A121D	L421P
66047	Canada	2023	ST-233	60.001	ST-21711	1	A311V/N513Y	-35AΔ	G120K/A121D	L421P
51742	Canada	2018	ST-233	60.001	ST-3435	0.5	A311V/N513Y	-35AΔ	G120K/A121D	L421P
A7536	Australia	2017	ST-233	60.001	ST-15925	0.5	A311V/N513Y	-35AΔ	G120K/A121D	L421P
47707	Canada	2017	ST-233	60.001	ST-1614	1	A311V/N513Y	-35AΔ	G120K/A121D	L421P
A7846	Australia	2017	ST-233	60.001	ST-1614	0.5	A311V/N513Y	-35AΔ	G120K/A121D	L421P
61829	Canada	2021	ST-3903	60.001	ST-19937	1	A311V/N513Y	-35AΔ	G120K/A121D	L421P
65624	Canada	2023	ST-233	60.001	ST-21730	2	A311V/N513Y	-35AΔ	G120K/A121D	L421P
67356	Canada	2023	ST-5575	237.001	ST-22789	0.25	A311V/N513Y	WT	WT/WT	L421P
H041 (WHO X)	Japan	2009	ST-226	37.001	ST-4220	1	A311V/N513Y		G120K/A121D	L421P
A8806 (WHO Z)	Australia	2013	ST-227	64.001	ST-1407	1	A311V/N513Y	-35AΔ	G120K/A121D	L421P
55486	Canada	2018	ST-3865	121.002	ST-18042	0.5	A311V/A517G	-35AΔ	G120D/WT	L421P
55487	Canada	2018	ST-3865	121.002	ST-18042	1	A311V/A517G	-35AΔ	G120D/WT	L421P
68444	Canada	2024	ST-5852	237.001		0.5	A311V/N513Y	-35AΔ	G120K/A121N	L421P
F89 (WHO Y)	France	2010	ST-16	42.001	ST-4015	1	A501P/N513Y	-35AΔ	G120K/A121N	L421P

What do we do when we identify a Ceftriaxone-resistant *N. gonorrhoeae* ?

Identified by P/T lab?

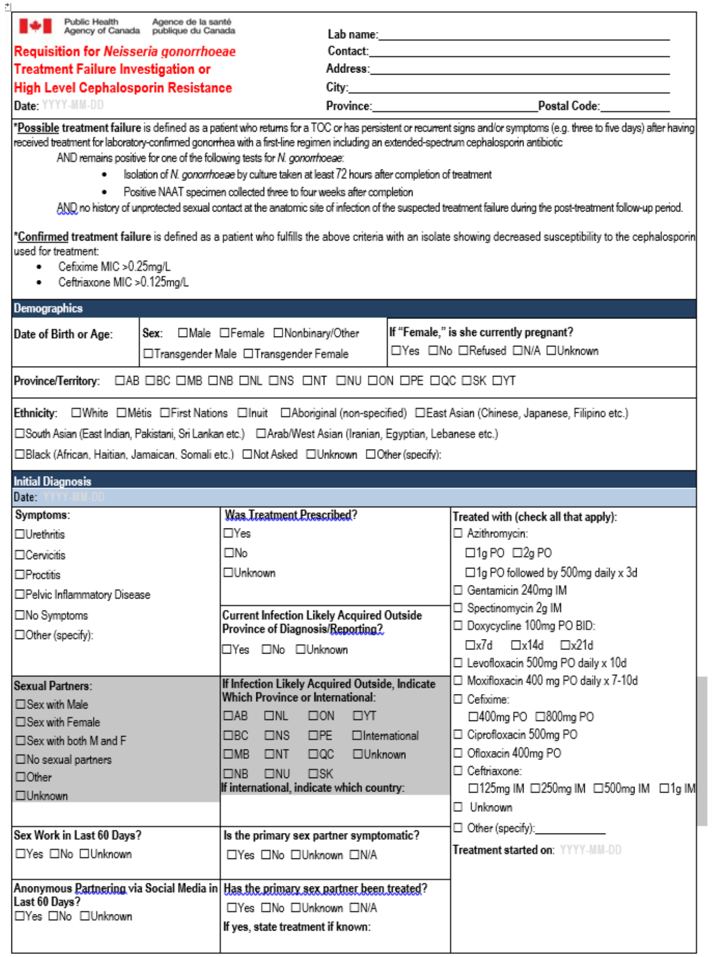
- Submit to NML and NML will prioritize phenotypic and WGS testing
- Email to notify is appreciated

Identified by NML?

- Prioritize confirmatory testing (phenotypic and WGS)
- Report back to submitting laboratory

After testing is completed

- Will send submitting laboratory the 'Requisition for *Neisseria gonorrhoeae* Treatment Failure Investigation or High Level Cephalosporin Resistance'
- Will notify CCDIC
- Discussions on additional enhanced surveillance?
- Possible CNPHI alert?



Public Health Agency of Canada / Agence de la santé publique du Canada

Requisition for *Neisseria gonorrhoeae* Treatment Failure Investigation or High Level Cephalosporin Resistance

Date: YYYY-MM-DD

Lab name:__________
Contact:__________
Address:__________
City:__________
Province:__________ Postal Code:__________

***Possible treatment failure** is defined as a patient who returns for a TOC or has persistent or recurrent signs and/or symptoms (e.g. three to five days) after having received treatment for laboratory-confirmed gonorrhea with a first-line regimen including an extended-spectrum cephalosporin antibiotic

AND remains positive for one of the following tests for *N. gonorrhoeae*:

- Isolation of *N. gonorrhoeae* by culture taken at least 72 hours after completion of treatment
- Positive NAAT specimen collected three to four weeks after completion

AND no history of unprotected sexual contact at the anatomic site of infection of the suspected treatment failure during the post-treatment follow-up period.

***Confirmed treatment failure** is defined as a patient who fulfills the above criteria with an isolate showing decreased susceptibility to the cephalosporin used for treatment:

- Cefixime MIC >0.25mg/L
- Ceftriaxone MIC >0.125mg/L

Demographics

Date of Birth or Age:	**Sex:** ☐Male ☐Female ☐Nonbinary/Other ☐Transgender Male ☐Transgender Female	**If "Female," is she currently pregnant?** ☐Yes ☐No ☐Refused ☐N/A ☐Unknown

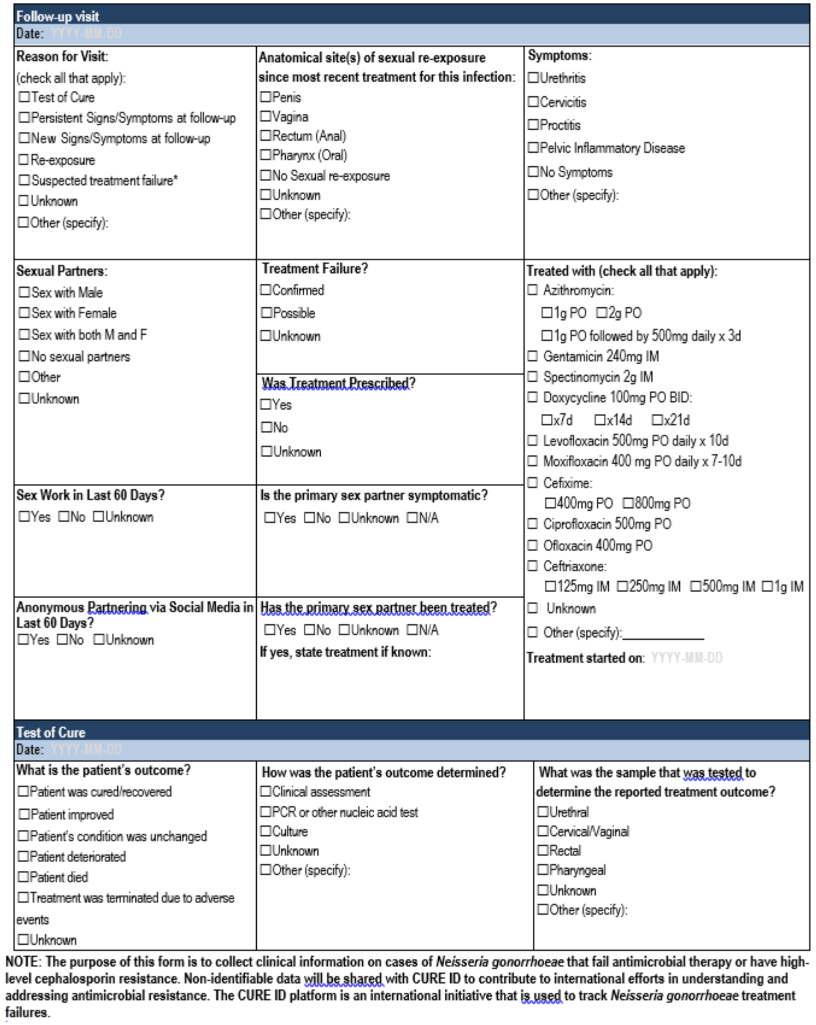
Province/Territory: ☐AB ☐BC ☐MB ☐NB ☐NL ☐NS ☐NT ☐NU ☐ON ☐PE ☐QC ☐SK ☐YT

Ethnicity: ☐White ☐Métis ☐First Nations ☐Inuit ☐Aboriginal (non-specified) ☐East Asian (Chinese, Japanese, Filipino etc.) ☐South Asian (East Indian, Pakistani, Sri Lankan etc.) ☐Arab/West Asian (Iranian, Egyptian, Lebanese etc.) ☐Black (African, Haitian, Jamaican, Somali etc.) ☐Not Asked ☐Unknown ☐Other (specify):

Initial Diagnosis

Date: YYYY-MM-DD

Symptoms: ☐Urethritis ☐Cervicitis ☐Proctitis ☐Pelvic Inflammatory Disease ☐No Symptoms ☐Other (specify):	**Was Treatment Prescribed?** ☐Yes ☐No ☐Unknown	**Treated with (check all that apply):** ☐ Azithromycin: ☐1g PO ☐2g PO ☐1g PO followed by 500mg daily x 3d ☐ Gentamicin 240mg IM ☐ Spectinomycin 2g IM ☐ Doxycycline 100mg PO BID: ☐x7d ☐x14d ☐x21d ☐ Levofloxacin 500mg PO daily x 10d ☐ Moxifloxacin 400 mg PO daily x 7-10d ☐ Cefixime: ☐400mg PO ☐800mg PO ☐ Ciprofloxacin 500mg PO ☐ Ofloxacin 400mg PO ☐ Ceftriaxone: ☐125mg IM ☐250mg IM ☐500mg IM ☐1g IM ☐ Unknown ☐ Other (specify):______ **Treatment started on:** YYYY-MM-DD
	Current Infection Likely Acquired Outside Province of Diagnosis/Reporting? ☐Yes ☐No ☐Unknown	
Sexual Partners: ☐Sex with Male ☐Sex with Female ☐Sex with both M and F ☐No sexual partners ☐Other ☐Unknown	**If Infection Likely Acquired Outside, Indicate Which Province or International:** ☐AB ☐NL ☐ON ☐YT ☐BC ☐NS ☐PE ☐International ☐MB ☐NT ☐QC ☐Unknown ☐NB ☐NU ☐SK **If international, indicate which country:**	
Sex Work in Last 60 Days? ☐Yes ☐No ☐Unknown	**Is the primary sex partner symptomatic?** ☐Yes ☐No ☐Unknown ☐N/A	
Anonymous Partnering via Social Media in Last 60 Days? ☐Yes ☐No ☐Unknown	**Has the primary sex partner been treated?** ☐Yes ☐No ☐Unknown ☐N/A **If yes, state treatment if known:**	



Follow-up visit

Date: YYYY-MM-DD

Reason for Visit: (check all that apply): ☐Test of Cure ☐Persistent Signs/Symptoms at follow-up ☐New Signs/Symptoms at follow-up ☐Re-exposure ☐Suspected treatment failure* ☐Unknown ☐Other (specify):	**Anatomical site(s) of sexual re-exposure since most recent treatment for this infection:** ☐Penis ☐Vagina ☐Rectum (Anal) ☐Pharynx (Oral) ☐No Sexual re-exposure ☐Unknown ☐Other (specify):	**Symptoms:** ☐Urethritis ☐Cervicitis ☐Proctitis ☐Pelvic Inflammatory Disease ☐No Symptoms ☐Other (specify):
Sexual Partners: ☐Sex with Male ☐Sex with Female ☐Sex with both M and F ☐No sexual partners ☐Other ☐Unknown	**Treatment Failure?** ☐Confirmed ☐Possible ☐Unknown	**Treated with (check all that apply):** ☐ Azithromycin: ☐1g PO ☐2g PO ☐1g PO followed by 500mg daily x 3d ☐ Gentamicin 240mg IM ☐ Spectinomycin 2g IM ☐ Doxycycline 100mg PO BID: ☐x7d ☐x14d ☐x21d ☐ Levofloxacin 500mg PO daily x 10d ☐ Moxifloxacin 400 mg PO daily x 7-10d ☐ Cefixime: ☐400mg PO ☐800mg PO ☐ Ciprofloxacin 500mg PO ☐ Ofloxacin 400mg PO ☐ Ceftriaxone: ☐125mg IM ☐250mg IM ☐500mg IM ☐1g IM ☐ Unknown ☐ Other (specify):______ **Treatment started on:** YYYY-MM-DD
	Was Treatment Prescribed? ☐Yes ☐No ☐Unknown	
Sex Work in Last 60 Days? ☐Yes ☐No ☐Unknown	**Is the primary sex partner symptomatic?** ☐Yes ☐No ☐Unknown ☐N/A	
Anonymous Partnering via Social Media in Last 60 Days? ☐Yes ☐No ☐Unknown	**Has the primary sex partner been treated?** ☐Yes ☐No ☐Unknown ☐N/A **If yes, state treatment if known:**	

Test of Cure

Date: YYYY-MM-DD

What is the patient's outcome? ☐Patient was cured/recovered ☐Patient improved ☐Patient's condition was unchanged ☐Patient deteriorated ☐Patient died ☐Treatment was terminated due to adverse events ☐Unknown	**How was the patient's outcome determined?** ☐Clinical assessment ☐PCR or other nucleic acid test ☐Culture ☐Unknown ☐Other (specify):	**What was the sample that was tested to determine the reported treatment outcome?** ☐Urethral ☐Cervical/Vaginal ☐Rectal ☐Pharyngeal ☐Unknown ☐Other (specify):

NOTE: The purpose of this form is to collect clinical information on cases of *Neisseria gonorrhoeae* that fail antimicrobial therapy or have high-level cephalosporin resistance. Non-identifiable data will be shared with CURE ID to contribute to international efforts in understanding and addressing antimicrobial resistance. The CURE ID platform is an international initiative that is used to track *Neisseria gonorrhoeae* treatment failures.

Questions?
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Acknowledgements

Canadian Provincial Public Health Laboratories

- British Columbia Centre for Disease Control
- Provincial Laboratory of Public Health Alberta
- Roy Romanow Provincial Laboratory, Saskatchewan
- Cadham Provincial Laboratory, Manitoba
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- New Brunswick Regional Hospitals
- Newfoundland and Labrador Public Health Laboratory
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