Surveillance of *Neisseria meningitidis* drug resistance in New York State

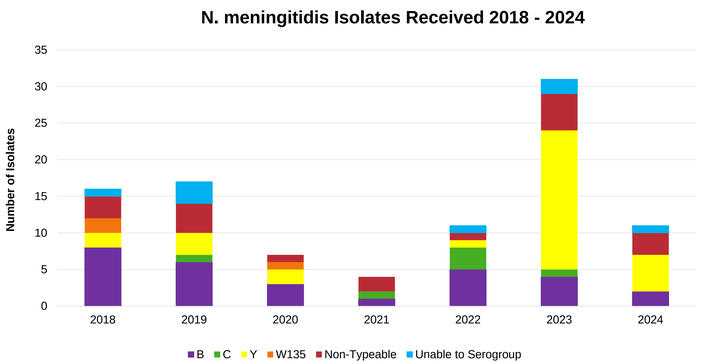
Andrew Peifer, Elizabeth Owuor, Krithivasan Sankaranarayanan, Anna Kidney, Lori Tyler, Lynn Leach, Geetha Nattanmai, Linnell Randall, Kate Wahl, Kimberlee Musser, Kara Mitchell

NEW YORK STATE | Department of Health | Wadsworth Center

Background

- *Neisseria meningitidis* is the causative agent of invasive meningococcal disease (IMD).
- *N. meningitidis* has 12 serogroups differentiated by their capsular polysaccharides.
  - IMD is predominantly caused by serogroups B, C, and Y.
  - Serogroups A, B, C, Y, and W are vaccine preventable.
- Prompt antibiotic treatment is critical due to the severity of IMD.
  - Meningococcal disease is fatal in 10-15% of patients.
  - 20% of patients suffer from other long term neurological sequelae.
- Meningococcal disease is typically treated with penicillin, ampicillin, cefotaxime, or ceftriaxone.
- Antibiotic resistance in *N. meningitidis* in the past has been uncommon.
  - Recently in the US there has been an increase of ciprofloxacin and penicillin-resistant, β-lactamase-producing *N. meningitidis* serogroup Y.
  - A Centers for Disease Control (CDC) report indicated that 33 cases of *N. meningitidis* serogroup Y reported between 2013-2020 contained the ROB-1 β-lactamase enzyme gene conferring resistance to penicillins[1].
- Updated guidelines suggest healthcare providers should ascertain susceptibility of meningococcal isolates to penicillin to ensure appropriate treatment.
  - To support management of *N. meningitidis* treatment the Wadsworth Center Bacteriology Lab performs antibiotic susceptibility testing (AST) by gradient strip (ETEST™) on all *N. meningitidis* isolates received. Isolates are additionally screened for bla_{ROB-1} by real-time PCR. The data presented was collected from 2018 up to April of 2024.
  - All viable isolates received in 2022 and 2023 underwent whole genome sequencing (WGS) for further characterization.

N. meningitidis in New York State

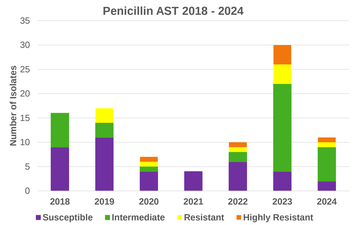


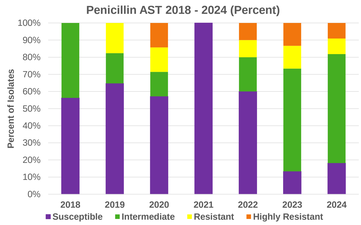
- All *N. meningitidis* isolated from sterile sites are required to be shipped to the Wadsworth Center for confirmation and additional characterization.
- Real-time PCR is used to serotype *N. meningitidis* (A, B,C, W135, Y) and screen for the bla_{ROB-1} gene.
- AST for penicillin, ciprofloxacin, and ceftriaxone is performed on all isolates.
- Primary samples which are culture negative may still be submitted for identification, serotyping, and bla_{ROB-1} testing.

Antibiotic Sensitivity Characterization

Penicillin Sensitivity

- *N. meningitidis* isolates received between 2018 and April 2024 were tested for penicillin sensitivity by ETEST™.
- Based on Clinical Laboratory Standards Institutes (CLSI) guidelines for penicillin, susceptible (S) is a minimum inhibitory concentration (MIC) of ≤0.06µg/mL, intermediate resistance (I) is MIC 0.12 to 0.25µg/mL, and resistant (R) is MIC ≥0.5µg/mL. Isolates with MICs >0.25µg/mL but <0.5µg/mL were also scored as resistant[2]. For clarity we define isolates with MIC ≥4.0µg/mL as highly resistant (R+).

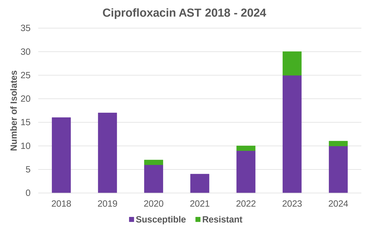


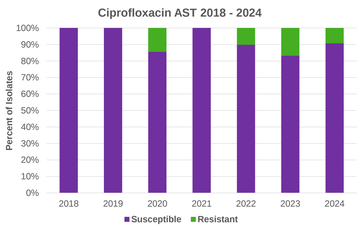


Interpretation: Penicillin insensitivity is on the rise in New York State. Of the isolates tested between 2018 and 2022, 62.96% (34) were penicillin susceptible, 24.07% (13) were intermediate resistant, 9.26% (5) were resistant, and 3.7% (2) were highly resistant. In 2023 and early 2024, however, only 14.63% (6) of the isolates tested were sensitive to penicillin. Most isolates (60.98%, or 25) in 2023 and early 2024 tested as having an intermediate resistance to penicillin.

Ciprofloxacin Sensitivity

- *N. meningitidis* isolates received between 2018 and April 2024 were tested for ciprofloxacin sensitivity by ETEST™.
- Based on CLSI guidelines for ciprofloxacin, susceptible (S) is MIC of ≤0.03µg/mL and resistant (R) is MIC of ≥0.12µg/mL[2].

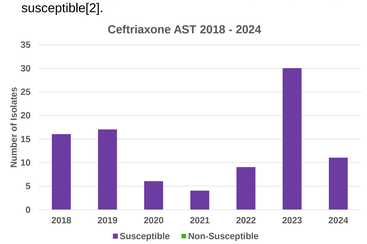


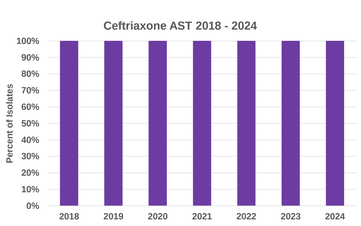


Interpretation: Except for 2021, ~10 to 15% of isolates received have been ciprofloxacin resistant since 2020. While this rate is elevated compared to 2018 and 2019, ciprofloxacin resistance among isolates tested has been consistent over the last several years.

Ceftriaxone Sensitivity

- *N. meningitidis* isolates received between 2018 and April 2024 were tested for ceftriaxone sensitivity by ETEST™.
- Based on CLSI guidelines for ceftriaxone, susceptible (S) is MIC of ≤0.12µg/mL[2]. Anything exceeding 0.12µg/mL is non-susceptible[2].

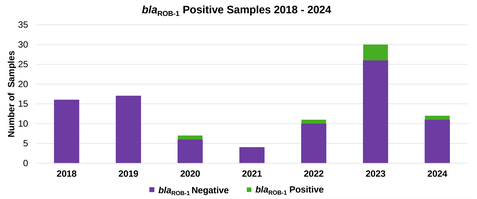




Interpretation: Ceftriaxone resistance is rare in *N. meningitidis*. To our knowledge, no ceftriaxone non-susceptible isolate has been seen in New York State.

bla_{ROB-1} Testing

- Bacterial suspensions measuring the equivalent of a 1.0 McFarland standard were tested using a lab developed bla_{ROB-1} real-time PCR assay.
- All bla_{ROB-1} positive strains of *N. meningitidis* identified by the CDC's study were serogroup Y[1]. Most were additionally ciprofloxacin resistant[1].



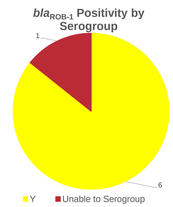


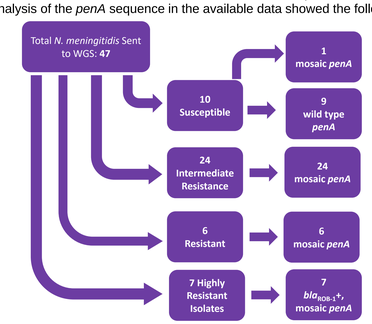
Table 1: Antibiotic Susceptibility of bla_{ROB-1} Positive Isolates

Sample	Minimum Inhibitory Concentration (µg/mL)		
	Ciprofloxacin	Penicillin	Ceftriaxone
NYSDOH-2076987	0.125	16	0.002
NYSDOH-2204656	0.125	8	0.002
NYSDOH-2301912	0.12	12	0.002
NYSDOH-2317702	0.125	4	0.002
NYSDOH-2336542	0.19	6	0.002
NYSDOH-2362890	0.125	12	0.002
NYSDOH-2420395	0.125	6	0.002

Interpretation: A majority of bla_{ROB-1} positive isolates were serogroup Y. All isolates received were ciprofloxacin resistant and account for all the samples with a penicillin MIC of ≥4.0µg/mL. Subsequent WGS found that the single non-Y serogroup isolate is missing the capsule gene targeted by our serogrouping real-time PCR. All 7 isolates belong to multilocus sequence type 3587, suggesting that the lone unable to serogroup isolate could have once been serogroup Y. The rate of bla_{ROB-1} positivity is low, but somewhat consistent from 2020 to 2024.

Penicillin Resistance

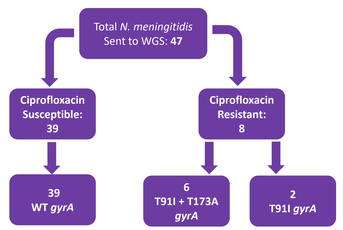
- Isolates harboring bla_{ROB-1} only account for 7 out of the 56 penicillin non-susceptible (12.5%) samples received. WGS was performed on all viable isolates (36/41) from 2022 and 2023 to help identify other potential causes of penicillin resistance. WGS data was available for an additional 9 isolates from other years.
- Sequence mosaicism in the *penA* gene (also known as penicillin binding protein 2) has been shown to be correlated with decreased penicillin susceptibility[3]. Analysis of the *penA* sequence in the available data showed the following:



Interpretation: The reduction in penicillin sensitivity observed in the non-susceptible, β-lactamase isolates can be potentially explained by these strains harboring a mosaic *penA* gene variant. All mosaic *penA* variants had the same 5 amino substitutions (F504L, A510V, I515V, H541N, I566V) that have been previously correlated with reduced penicillin sensitivity in *N. meningitidis*[3]. Only 1 specimen was identified where *penA* sequence mosaicism did not correspond with penicillin susceptibility.

Ciprofloxacin Resistance

- All 8 ciprofloxacin resistant isolates were included in the WGS sample set.
- Ciprofloxacin resistance in *N. meningitidis* is known to be caused by mutations in *gyrA*. The T91I and T173A amino acid substitutions are specifically correlated with reduced ciprofloxacin susceptibility[1].



Interpretation: All ciprofloxacin resistant isolates carried *gyrA* mutations correlated with reduced susceptibility. 6 of the 7 positive bla_{ROB-1} isolates carried both T91I and T173A *gyrA* mutations known to be present in other specimens of ROB-1 positive strains[1].

Future Direction – MinION Panel

- A minION assay sequencing the *penA* and *gyrA*, *parC*, and 23S genes has been developed to supplement WGS for screening *N. meningitidis*.
- Of the 28 samples submitted for a preliminary screen of *penA* and *gyrA*, the minION sequencing results for all 28 are concordant with AST results and the available WGS data.

Conclusion

- Penicillin resistance amongst *N. meningitidis* isolates is becoming more common in New York State.
  - Where most isolates were previously susceptible, a majority now show at least intermediate resistance to penicillin.
- While some penicillin resistant isolates harbor a known β-lactamase enzyme, the majority of resistant isolates only carry a *penA* allele correlated with reduced sensitivity.
  - The same 5 amino acid substitutions (F504L, A510V, I515V, H541N, I566V) correlated with reduced penicillin susceptibility are found in the *penA* allele of every penicillin resistant isolate.
- Ciprofloxacin resistance has not dramatically changed amongst isolates in New York, but 7 out of 8 ciprofloxacin resistant isolates were also highly resistant to penicillin due to the presence of bla_{ROB-1}.
- Ciprofloxacin resistant isolates harbor *gyrA* alleles with T91I and T173A amino acid substitutions, which are correlated with reduced susceptibility.
- No isolates were found to be non-susceptible to ceftriaxone.
- A minION based sequencing panel shows potential in supplementing future antibiotic susceptibility testing for β-lactamase negative and ciprofloxacin resistant samples.

References

1. Potts, C. C. *et al.* Acquisition of Ciprofloxacin Resistance Among an Expanding Clade of β-Lactamase–Positive, Serogroup Y *Neisseria meningitidis* in the United States. *Clin. Infect. Dis.* **73**, 1185–1193 (2021).

2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. (Clinical and Laboratory Standards Institute, Wayne, PA, 2024).

3. Thulin, S., Olcén, P., Fredlund, H. & Unemo, M. Total Variation in the *penA* Gene of *Neisseria meningitidis* : Correlation between Susceptibility to β-Lactam Antibiotics and *penA* Gene Heterogeneity. *Antimicrob. Agents Chemother.* **50**, 3317–3324 (2006).