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

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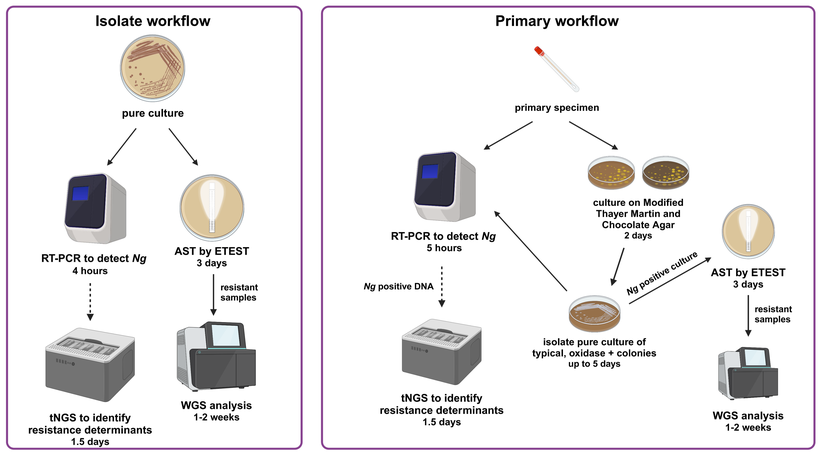
Background

- Drug-resistant *Neisseria gonorrhoeae* (*Ng*) is considered an urgent threat by the CDC (1).
- The current gold standard for diagnosis of *Ng* infections is culture-independent nucleic acid amplification testing (NAAT), and clinical laboratories no longer routinely isolate *Ng* cultures, precluding traditional antimicrobial susceptibility testing (AST).
- There are currently no FDA approved NAAT assays for drug resistance in *Ng*.
- Clinically relevant resistance mechanisms in *Ng* include mosaic versions of the *penA* gene associated with cephalosporin resistance, point mutations in the 23S rRNA gene that cause resistance to azithromycin, and substitutions in *gyrA* and *parC* genes conferring resistance to ciprofloxacin (2).
- Wadsworth Center (WC) has developed a targeted next generation sequencing (tNGS) assay to detect drug resistant *Ng* from cultured isolates and primary specimens.
- AST for ceftriaxone (TX), cefixime (IX), azithromycin (AZ), and ciprofloxacin (CI) have been performed on all viable *Ng* samples received since 2018. tNGS and Whole Genome Sequencing (WGS) have been performed on samples to identify resistance determinants.

Ng sample processing algorithm



The current Wadsworth Center algorithm requires a live, pure culture of *Ng* to determine antibiotic susceptibility.

WC frequently receives primary specimens which are positive for *Ng* DNA by real-time PCR, but are non-viable in culture, precluding any further characterization.

The addition of a tNGS assay identifying *Ng* resistance determinants directly from extracted DNA will accelerate and expand *Ng* testing capabilities.

References

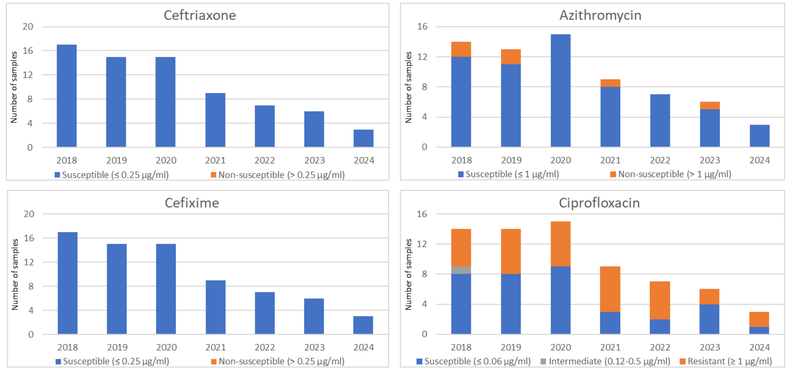
(1) CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019 .

(2) W. Demczuk, et al. *Neisseria gonorrhoeae* Sequence Typing for Antimicrobial Resistance, a Novel Antimicrobial Resistance Multilocus Typing Scheme for Tracking Global Dissemination of *N. gonorrhoeae* Strains. J Clin Microbiol 2017.

(3) CLSI. Performance Stands for Antimicrobial Susceptibility Testing, 34th ed. CLSI M100. Clinical and Laboratory Standards Institute; 2024.

Flow charts created with BioRender.com

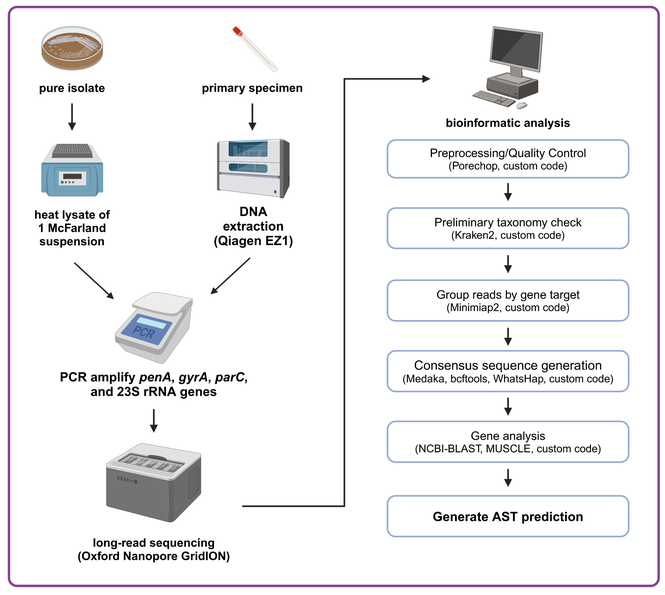
Ng antimicrobial susceptibility 2018-2024



2024 data represents samples received between January and May.

Minimum inhibitory concentrations (MIC) were determined by ETEST (bioMérieux) following manufacturer guidelines and CLSI M100 (3). AST for cephalosporins was performed by disk diffusion prior to 2021.

Nanopore tNGS assay for *Neisseria* drug resistance



Four target genes known to be antibiotic resistance determinants are PCR-amplified from *Ng* DNA and sequenced on a GridION instrument (Oxford Nanopore Technologies).

A WC-developed pipeline reports high confidence (known to confer resistance) and other variants in the target genes. This allows monitoring for new potentially significant mutations in the target genes, as well as producing an AST prediction based on high confidence variants.

tNGS results

sample	county	AST results				high confidence variants				tNGS predicted resistance
		TX	IX	AZ	CI	*penA*	23S	*gyrA*	*parC*	
NYS20-121	Steuben	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS20-3875	Bronx	S	S	S	R	non-mosaic	-	S91F/D95G	E91G	CI
NYS20-3871	Kings	S	S	S	R	non-mosaic	-	S91F/D95G	S87R	CI
NYS20-28361	Albany	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS20-38615	Albany	S	S	S	S	non-mosaic	-	-	-	none
NYS20-170174	Nassau	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS20-173943	Nassau	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS20-190755	Albany	S	S	S	S	non-mosaic	-	-	-	none
NYS20-222634	Schenectady	S	S	S	S	non-mosaic	-	-	-	none
NYS20-223159	Montgomery	S	S	S	S	non-mosaic	-	-	-	none
NYS20-224050	Chautauqua	S	S	S	S	non-mosaic	-	-	-	none
NYS20-237439	Albany	S	S	S	S	non-mosaic	-	-	-	none
NYS20-237444	Columbia	S	S	S	S	non-mosaic	-	-	-	none
NYS20-247415	OOS: NC	S	S	S	S	non-mosaic	-	-	-	none
NYS21-22086	Schenectady	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS21-27151	Monroe	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS21-71234	Lewis	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS21-78505	Westchester	S	S	S	R	non-mosaic	-	S91F/D95A	-	CI
NYS21-84471	Erie	S	S	S	S	non-mosaic	-	-	-	none
NYS21-85169	Westchester	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS21-93534	Schenectady	S	S	NS (2.0)	S	non-mosaic	-	-	-	none*
NYS21-96463	Westchester	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS21-98855	Columbia	S	S	S	S	non-mosaic	-	-	-	none
NYS22-14175	Westchester	S	S	S	R	non-mosaic	-	S91F/D95A	ND	CI
NYS22-15012	Westchester	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS22-19724	Albany	S	S	S	S	non-mosaic	-	-	-	none
NYS22-36561	Erie	S	S	S	ND	non-mosaic	-	-	-	none
NYS22-38542	Queens	S	S	S	R	non-mosaic	-	S91F/D95A	ND	CI
NYS22-41094	Kings	S	S	S	S	non-mosaic	-	-	-	none
NYS22-57725	Erie	S	S	S	R	non-mosaic	-	S91F/D95A	-	CI
NYS22-63372	Westchester	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS23-5985	Schenectady	S	S	S	S	non-mosaic	-	-	-	none
NYS23-27005	Fulton	S	S	S	R	non-mosaic	-	S91F/D95A	-	CI
NYS23-29388	Rensselaer	S	S	S	S	non-mosaic	-	-	ND	none
NYS23-39140	Kings	S	S	NS (>256)	R	non-mosaic	A2047G	S91F/D95G	E91G	CI, AZ
NYS23-44326	Albany	S	S	S	S	non-mosaic	-	-	ND	none
NYS23-52843	Oneida	S	S	S	S	non-mosaic	-	-	-	none
NYS24-11514	Monroe	S	S	S	R	non-mosaic	-	S91F/D95A	S87R	CI
NYS24-17615	Monroe	S	S	S	R	non-mosaic	-	S91F/D95A	D86N	CI

tNGS accurately predicted *Ng* drug susceptibility for 38 of 39 samples tested.

* indicates incorrect prediction for low-level azithromycin resistance.

TX: ceftriaxone, IX: cefixime, AZ: azithromycin, CI: ciprofloxacin, S: susceptible, NS: non-susceptible, R: resistant, ND: not determined, -: no high confidence resistance variants detected

Conclusions

- Ciprofloxacin resistance is common (~50%) in *Ng* samples received from 25 counties across NYS. Substitutions in *gyrA* and/or *parC* genes known to confer resistance were detected by tNGS in all 21 ciprofloxacin-resistant samples that have been sequenced.
- All WC *Ng* samples tested to date have been susceptible to both ceftriaxone and cefixime. This was accurately predicted by tNGS, which found every clinical sample sequenced lacks the mosaic version of the *penA* gene associated with cephalosporin resistance.
- Six *Ng* samples since 2018 were non-susceptible to azithromycin. Two of these have had tNGS performed: the highly resistant sample (MIC >256 µg/ml) has a known azithromycin-resistance mutation in the 23S rRNA gene, while the sample with a lower MIC (2 µg/ml) has no 23S resistance determinants. The WGS of the latter sample is undergoing analysis to determine the mechanism for its azithromycin resistance. Additional target(s) may be added to the tNGS panel as needed.
- The WC tNGS assay will provide timely and accurate drug susceptibility predictions for *Ng* samples, including those which cannot be isolated or grown in pure culture.