Enriching National Surveillance for AMR in *N. gonorrhoeae* using WGS

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Outline

- Overview of surveillance for gonorrhea
  - Why is AR a concern for gonorrhea?
- Utility of WGS for gonococcal isolates
- Challenges to implementation
  - Development of tools for improved genomic analysis of gonococcal isolates
Gonorrhea in the US

- Second most commonly reported notifiable disease in the US
- Total of 555,608 cases reported in 2017
- Recommended treatment since 2015
  - 250 mg ceftriaxone plus 1 g azithromycin
Gonococcal Isolate Surveillance Project (GISP)

- Established in 1986 as a sentinel surveillance system
  - Male patients with urethral gonorrhea attending STD clinics
- Monitor antimicrobial susceptibility trends in *Neisseria gonorrhoeae* in the United States
  - Agar dilution method
- Establish a rational basis for the selection of gonococcal therapies

**Primary Antimicrobial Drugs Used to Treat Uncomplicated Gonorrhea**

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Year</th>
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<tbody>
<tr>
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<tr>
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<td>2015</td>
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<td>70</td>
<td>2017</td>
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</tbody>
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- Tetracyclines
- Penicillins
- Other
- Ofloxacin
- Ciprofloxacin
- Cefixime
- Ceftriaxone 250 mg
- Spectinomycin
- Ceftriaxone 125 mg
- Other Cephalosporins
- Gentamicin
Susceptibility to extended spectrum cephalosporins: GISP isolates, 2006-2017

- Elevated Cefixime MIC ($\geq 0.25 \mu g/ml$)
- Elevated Ceftriaxone MIC ($\geq 0.125 \mu g/ml$)

*Cefixime not tested
Susceptibility to azithromycin: GISP isolates, 2006-2017

Elevated Azithromycin MIC (≥ 2.0 μg/ml)
Other CDC programs that collect gonorrhea isolates

- **eGISP – enhanced GISP**
  - *N. gonorrhoeae* strains in women and in extragenital sites
  - *N. meningitidis* strains in genital and extragenital sites

- **SURRG - Strengthening the United States Response to Resistant Gonorrhea**
  - Program to establish capacity to rapidly detect resistance and implement response activities in selected jurisdictions.
  - Only program pursuing local public health action
ARLN laboratories conducting gonorrhea isolate testing

Antimicrobial sensitivity testing (7 antibiotics)

Genome sequencing

Ng AR Lab Network

Washington
Tennessee
Texas
Maryland

Program Sites
- GISP/eGISP
- GISP
- SURRG

GISP
GISP/SURRG
Added value of GC genomic sequencing

- **Enrich the characterization of US isolates**
  - Allows rapid comparison with previously collected isolates, global isolates, etc.

- **Improve detection and characterization of isolates with unique antimicrobial susceptibility patterns**
  - Understand dynamics of strain populations containing unique AR markers

- **Strengthen epidemiological investigations**
  - Analysis of potential transmission networks
What gets sequenced?

- Subset of male urethral isolates
- Isolates with high MICs
- Isolates recovered from females
- Isolates recovered from extragenital sites
- Isolate recovered during epidemiological investigation
GC Bioinformatics Workflow Used at CDC

Data Preprocessing and Assembly

- QC Raw Reads
- FastQC
- Trimming
- cutadapt
- QC Trimmed Reads
- Assembly
- SPAdes
- QC Assembly
- Quast
- Reassemble using best k
- Best kmer
- Kraken
- Kmergenie
- AMR Profiler

Core SNP Analysis

- Variant Detection and Multi-alignment
  - PatSNP
- Recombination filter
  - Gubbins
- ML phylogenetic tree
  - RasML

Analysis conducted on high performance computing cluster
Enhancing the knowledge base with WGS
Detailed genetic analysis of strains with decreased antibiotic susceptibilities (2014-2016)

- **Clade A** contains a large number of isolates with elevated MIC to AZI
  - High prevalence of isolates containing the mutations in *mtrR* and *mtrD*.

- **Clade B** contains isolates with elevated MIC to CFX
  - Clade associated with *penA* mosaic alleles and mutations in *ponA*, *mtrR*, and *porB*.

- **Mutations in the 23S rRNA genes** appear sporadically throughout the phylogeny, but contribute to the highest AZI MICs.

Diversity of GC isolates with high-level AZI (HL-AZI) MICs

- HL-AZI GC isolates (2005-2017) are diverse
  - >13 STs represented
  - Geographically-dispersed

- Isolates with highest MICs (≥ 256 µg/ml) were associated with 23S rRNA mutations
  - Isolates with these mutations were found distributed throughout phylogenetic tree
Overcoming challenges to using WGS data
Typing schemes for GC

- **Neisseria MLST**
  - Multilocus sequence Typing
  - Six alleles

- **NG-MAST**
  - *N. gonorrhoeae* Multi-Antigen Sequence Typing
  - *por* and *tbpB*

- **NG-STAR**
  - *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance
  - Targets 7 AMR determinants

- **wgMLST and cgMLST**
Analysis standardization using whole genome MLST (wgMLST)

- 2431 loci
- Allele profile computed once
- Sequencing and analysis can be conducted anywhere, stored, shared, and added to later
- Does not exclude loci subject to recombination
- Distances proportional to SNPs

![Bar chart showing distribution of allele differences among isolates with ≤10 differences (N=89 pairs)]
OAMD Portal

- Transfer data to CDC
  - Lab-specific location
- Bioinformatics workflows/tools
  - Run analyses on demand
- Visualization
  - Microreact
Visualization of WGS data - Microreact

- **Interactive tool**
  - viewed in web browser
  - Displays geography, timeline, phylogeny

- **Project requires**
  - Tree file
  - CSV file with metadata

Visualization of metadata in the context of WGS may highlight relationships among isolates not previously recognized.
Neisseria gonorrhoeae AMR Profiler and Typing Tool

- **AMR profiler**
  - Detection of 25 AR loci
  - Read coverage across each locus

- **Typing**
  - Assembly QC check
  - Detects if isolate is *N. meningitides*
  - MLST
  - NG-MAST

Current workflow as custom python script

Development of GUI app on OAMD portal
Summary

- **WGS provides an additional tool for surveillance efforts**
  - Can track genetically similar isolates within a population over time
  - Can identify isolates with unique AMR determinant patterns

- **Use of gonorrhea WGS data for rapid public health action is under development**
  - Data visualization will be a key component

- **WGS implementation for GC will require the deployment of new data analysis tools**
  - How can jurisdictions utilize WGS data to support public health action?
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