Molecular characterization of Neisseria gonorrhoeae strains in high prevalence jurisdiction exhibits clonal lineage with decreased susceptibility to Azithromycin

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BACKGROUND:
N. gonorrhoeae (GC) has emerged as a global threat with an estimated 550,000 drug resistant Gonorrhea infections per year in the U.S. DCV has developed resistance to almost all antimicrobials, including third-generation cephalosporins and azithromycin. Antibiotic-resistant N. gonorrhoeae (ARGC) are associated with key molecular traits; e.g. accepting/integrating exogenous DNA genome, acquisition of macrolide resistance from occasional Neisseria low level resistance by mutation and enzymatic modification, upregulation of Efflux pumps, and high level resistance by point mutation, thus contributes to emergence of resistance. GC strains with evidence of evolution of ARGC, antimicrobial resistant variants with elevated minimum inhibitory concentrations (MICs), and those collected from same patient at different body sites or repeat infection yields distinct strains were analyzed.

OBJECTIVES:
In this initial study, we sought to answer the following questions:

WHAT STRAINS ARE PRESENT IN THE LOCAL POPULATION?
• A subset of 100 (77 patient + 23 reference) GC isolates collected in Milwaukee jurisdiction from 10/2017 to 12/2018

ARE THERE DIFFERENT STRAINS FROM DIFFERENT CLINICS (STD vs COMMUNITY PARTNER CLINIC) OR DIFFERENT AREAS WITHIN JURISDICTION?
• Selected a minimum of 5 isolates from each clinic
• Selected 25 isolates from highest prevalence zip code with a wide range of AZ MICs

IDO STRAIN TYPES CHANGE OVER TIME?
• Selected isolates recovered from same patient during different visits
• Selected isolates collected across a 15-month period

WHAT ANTIMICROBIAL RESISTANT SIGNS OR VARIANTS ARE PRESENT IN SAMPLES WITH ELEVATED MIC?
• Selected isolates with AZ MIC ranging from 2 to 16
• Selected susceptible isolates as control subset

SELECTED ISOLATES FROM PATIENTS AND THEIR PARTNERS WHERE AVAILABLE
• Isolates with decreased susceptibility to azithromycin were associated with ST-1579 (n=12), isolates with decreased susceptibility to azithromycin belonged to ST-1579 and also expressed tet(M)penA*bla-TEM135bla-TEM1B

SELECTED ISOLATES RECOVERED FROM SAME PATIENT DURING SAME VISIT (≥ 3 months apart): 2 cases had different MLST

WHAT STRAINS ARE PRESENT IN PATIENTS AND THEIR PARTNERS WHERE AVAILABLE
• Isolates with ST-7363 are resistant to Ciprofloxacin only (MIC 16) and Cefixime only (MIC 10)
• Isolates with ST-9363 were resistant to Penicillin (MIC 10) and Ceftriaxone (MIC 5)

ISOLATES COLLECTED FROM SAME PATIENT, DIFFERENT SOURCE
• 5 cases had the same ST-7363
• 1 case had the same ST-1579 and Ceftriaxone (MIC 5)
• 2 cases had different ST-7363

METHODS:
A subset of N. gonorrhoeae clinical isolates (n=107) collected between 10/2017 and 12/2018 was selected based on phenotypic antimicrobial susceptibility (AST) profiles (30 susceptible, 15 nonsusceptible to Azithromycin), specimen source (Table 1), and high risk patient cohort (based on patient zip code, not shown). Patient demographic information shown in Table 2. Whole genome sequencing (WGS) was performed on Illumina MiSeq platform at Dept of State Health Science Lab, Texas. Paired-end reads were aligned using SPAdes following quality assessment via Check-M. Parsnp was utilized to align GC core genome assembly to study evolutionary hierarchy and genetic polymorphisms. Public/LST databases were used for molecular typing and genome dosage analysis. Average Nucleotide Identity (ANI) analysis found nucleotide-level genome similarity between the coding regions of unknown strains against reference strain. NG-MAST sequence types were interpreted using CosmosID bioinformatics pipeline.

RESULTS:
• Single nucleotide polymorphism (SNP)-based phylogenetic tree was generated upon core genome analysis grouped 97 isolates into 21 multi locus sequence types (MLSTs) consisting of 3 clusters/clades with less than 10 SNPs (Figure 1).
• The most common STs were ST-9363 (n=16), ST-1579 (n=13, 14.4%), Figure 3.
• Isolates with decreased susceptibility to azithromycin were associated with ST-1579 (n=12), ST-12093 (n=2), and ST-3563 (n=1).

Ciprofloxacin resistance was associated with ST-1579 and ST-7363. AMR markers penA, blaTEM, tetM, and 235 RNA variants were also detected (Figure 2).
• None of the study isolates had reduced susceptibility to Cefoxime or Ceftriaxone.

CONCLUSIONS:
A wide variation in sequence types was obtained in surveillance jurisdiction, however almost all isolates with decreased susceptibility to azithromycin belonged to ST-1579 and also expressed gene markers for Cipfoxacin resistance. This analysis demonstrates the necessity to incorporate advanced molecular methods like next generation sequencing (NGS) as a routine surveillance tool to aid in molecular epidemiology, evidence-based antibiotic treatment and better characterize sexually-transmitted disease clusters and/or public health outbreaks involving ARGS.