

# Molecular Detection of Resistance in *Neisseria gonorrhoea*

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# Why develop molecular methods for resistance detection?

- Transition to NAAT diagnostic methods may limit isolate availability for resistance surveillance
- Need for resistance surveillance may limit use of NAAT diagnostics
- New technology may create new approaches to prevent the development and spread of resistance

# Current surveillance and medical management

- Presumptive treatment at point of contact
- Antibiotic choices guided by surveillance data
- Routine determination of culture susceptibility and contact tracing not feasible in many areas

# Study Goals

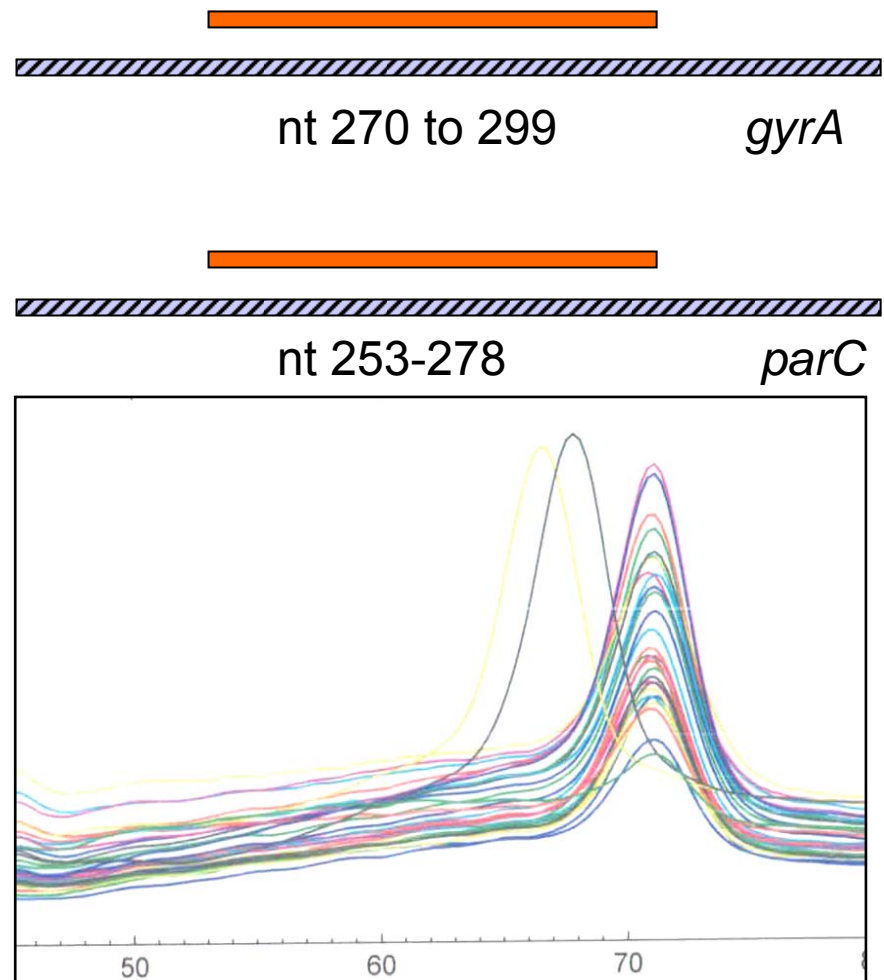
- Long term goal
  - Point of contact identification of individuals with resistant gonococcal infections
- Study objectives
  - To develop a rapid QRNG PCR based assay that detects the genetic mutations associated with ciprofloxacin resistance
    - (QRDR) of *gyrA* and *parC* genes
  - To apply this assay to in the context of clinical isolates
  - To evaluate the performance of the assay in comparison to MIC and DNA sequencing of *gyrA* and *parC*
  - To utilize strain typing to examine diversity of resistant strains

# Study design

- Screening by *gyrA* and *parC* QRDR melt curve analysis
  - All GC + cultures at two STI clinics in Baltimore
  - March to October 2005
- All QRDR mutants and of 5% of QRDR non-mutants
  - *gyrA* and *parC* sequencing
  - MIC determination
  - Additional GC NAAT testing
  - *porB* Variable Region typing
- Record review of QRDR mutants

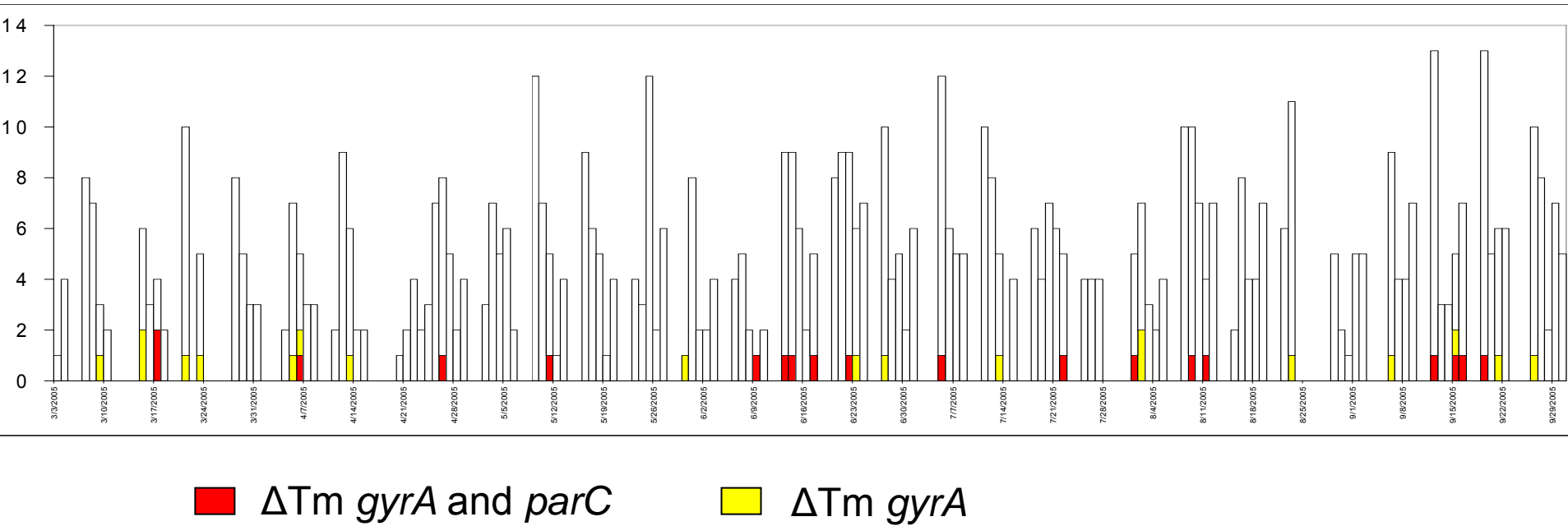
# QRDR Melt-curve Analysis

- Probes designed to regions encoding
  - aa 90 to 100 *gyrA*
  - aa 84 to 92 *parC*
- Wild type for *parC*
  - melt curve peaks at 71°C.
- *parC* mutants
  - melt curve peaks below 71°C



# Results of QRDR Melt-Curve Analysis

Number of isolates by date of clinic visit



# QRDR Sequence Analysis

- Isolates with *gyrA* and *parC* mutations:
  - All 16 GC had translated mutations at:
    - *gyrA* aa 91 and 95
    - *parC* aa 87
  - Additional mutations at *gyrA* aa 91, 92, 96, 97 in some strains
  - Three isolates were *N. meningitidis*
- Isolates with *gyrA* mutations only:
  - All Eight GC strains had mutations at aa 91
    - Four strains had a silent TCC → TCA mutation
  - Seven isolates were *N. meningitidis*
- 36 with no  $\Delta T_m$  – No *gyrA* or *parC* mutations

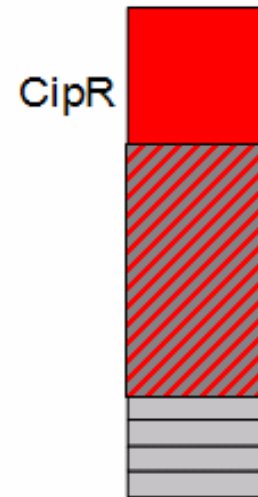
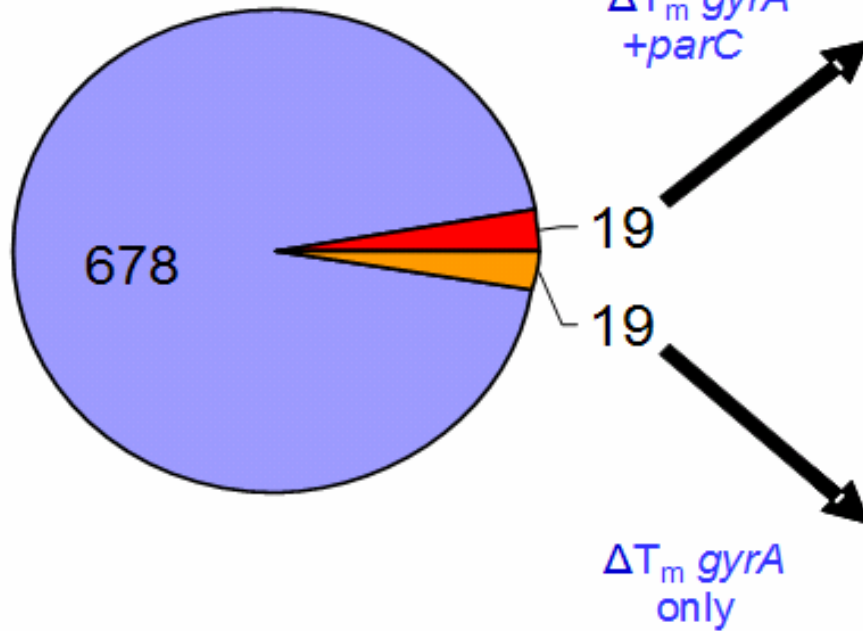
# Susceptibility Testing

- 14 of 28 *N. gonorrhoeae* isolates identified by melt-curve analysis were viable for MIC testing:
  - 6 were *cipR* (Highly resistant; MIC  $\geq 1$   $\mu\text{g/ml}$ ) with mutations in both *gyrA* and *parC*
  - 4 were *cipI* (Intermediate; MIC = 0.12-0.5  $\mu\text{g/ml}$ ) with mutations in *gyrA* only
  - 4 were *cipS* (Susceptible; MIC  $\leq 0.006$   $\mu\text{g/ml}$ ) with single base silent mutations
- 36 of 678 isolates with normal melt-curve analyses were tested for MIC:
  - All 36 were *cipS*

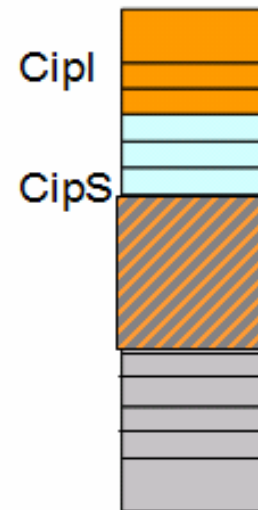
| Melt Curve                                    | MIC                    | # Strains | <i>porB</i> VR Type        |
|---|------------------------|-----------|----------------------------|
| $\Delta$ <i>gyrA</i> and $\Delta$ <i>parC</i> | CipR                   | 6         | B 2;2;7;(4);2              |
|   | Not viable             | 7         | B 2;2;7;(4);2              |
|   |                        | 2         | B 1;1;4;4;2                |
|   |                        | 1         | B 2;2;6;6;2                |
|   | <i>N. meningitidis</i> | 3         | N.m. <i>porB</i>           |
| $\Delta$ <i>gyrA</i>                          | Cipl                   | 2         | B 2;2;nt;4/6;2             |
|   |                        | 1         | B 2;2;1/2;4;2              |
|   |                        | 1         | B 2;2;nt;5;1/3             |
|   | CipS                   | 4         | Other PIB types            |
|   | Not viable             | 2         | B 2;2;nt;5;1/3             |
|   |                        | 2         | Other PIB types            |
|   | <i>N. meningitidis</i> | 7         | N.m. <i>porB</i>           |
| Normal Tm                                     | CipS                   | 36        | 20 other <i>porB</i> types |
|   | No MIC tested          | 642       |                            |

## Melt Curve Analysis

716 isolates  
2 QRDR probes



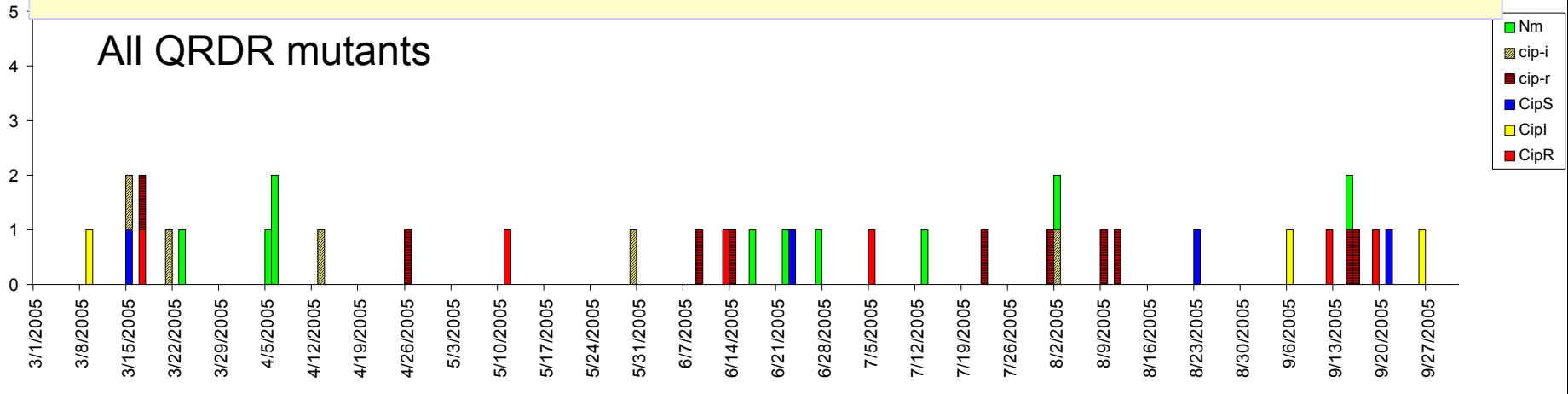
| MIC       | # | <i>porB</i> type       |
|-----------|---|------------------------|
| 2-4       | 6 | B 2;2;7;(4);2          |
| No growth | 7 | B 2;2;7;(4);2          |
|           | 3 | other                  |
| Not GC    | 3 | <i>N. meningitidis</i> |



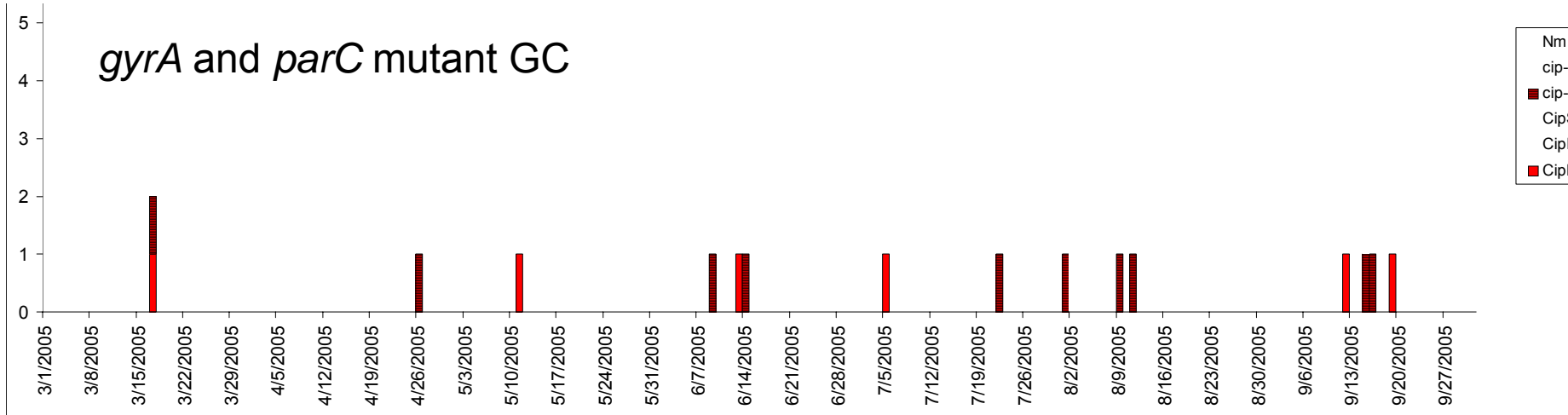
| MIC       | # | <i>porB</i> type       |
|-----------|---|------------------------|
| 0.125     | 2 | B 2;2;nt;4;2           |
|           | 2 | other                  |
| <0.002    | 4 | other                  |
| No growth | 3 | B 2;2;(3);5;1/3        |
|           | 1 | other                  |
| Not GC    | 7 | <i>N. meningitidis</i> |

# Molecular analysis of clinical isolates from Baltimore STI clinics

All QRDR mutants



*gyrA* and *parC* mutant GC



# *N. meningitidis* isolates

- 10 of 38 samples with QRDR by melt-curve analysis were determined to be *N. meningitidis*
  - *gyrA* sequence similar to *N. meningitidis*
  - GenProbe negative or indeterminant
  - Class 3 *porB* gene by VR typing
- Only 1 of 10 negative by BD NAAT
- 7 throat, 1 cervix, 1 urethral, 1 unknown

# Melt-curve analysis for QRDR mutations

- Detection of *cipR* or *cipI* GC by QRDR melt-curve analysis among 76 strains:
  - 100% sensitivity
  - 77% - 96% specificity (any vs. double mutant)
- Using the whole study population:
  - 97% - 98% Specificity

# Conclusions

- Molecular screening for resistance by melt-curve analysis for loss of QRDR wild type sequence
  - Accurate and Sensitive
- Specificity affected by:
  - Non-translated substitution mutations
  - *N. meningitidis* isolates
- Feasible screening tool
  - Identify individuals likely infected with a resistant strain
  - Allow focused interventions when resources are scarce