Development of the First Automated Antibiotic Susceptibility Test System for *Neisseria Gonorrhoeae*

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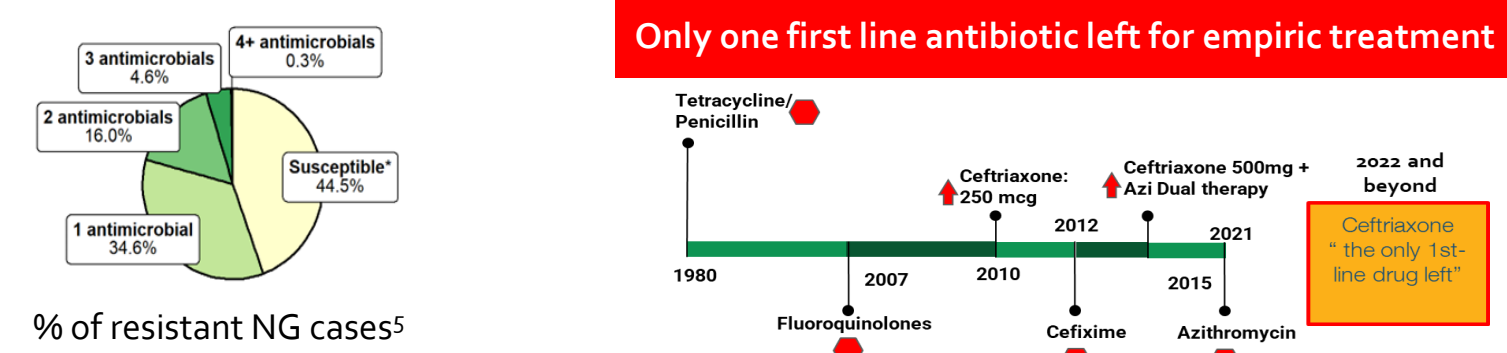
Poster #2   Booth #204

BACKGROUND

The Problem

Gonorrhea is the second most commonly reported bacterial infection in the United States. Simultaneously, it represents a critically urgent threat due to its hypermutability leading to pronounced AMR tendencies. **Of the 1.5 million estimated cases annually [1], ~ 50% are antibiotic resistant.** CDC has repeatedly called for action on NG AMR (in both 2013 [2] and 2019 [3] AR threat reports), but to date there is limited effort towards development of rapid, automated, phenotypic NG AST: the critical solution to the AMR problem, as emphasized below

2017 Harvard Modeling Study [4]
- Modeled 3 Antibiotics (treatment)
- Prediction: within 15 YRS NONE remain efficacious
- Conclusion: AST on 40% of cases ensures 40YRS efficacy
  * Azithromycin suspended by the CDC 2020

Only one first line antibiotic left for empiric treatment

% of resistant NG cases[5]

- 3 antimicrobials 4.6%
- 4+ antimicrobials 0.3%
- 2 antimicrobials 16.0%
- Susceptible 44.5%
- 1 antimicrobial 34.6%

Ceftriaxone "the only 1st-line drug left"

METHODS

Our technical approach consisted in four (4) key milestones:

1. Analysis of current workflows using CDC's GISP protocol
2. Demonstration of feasibility using broth dilution AST of *N. gonorrhoeae*.
3. Iterative development of consumables and readers controlled by in-house developed API
4. Testing of prototype devices demonstrating results that successfully call MIC for QC and AR Isolate Bank challenge strains of NG

Our System:

- Proprietary media allowing for broth culture of NG
- Vital environmental controls for optimum NG growth
- Automated measurement across individually addressed wells
- Customizable reports for MIC and S/ I/ R interpretation
- Etest® used in parallel for confirmatory testing

Vast prevalence of antimicrobial resistance in NG and its mutagenicity makes NAAT a poor substitute for phenotypic AST

- NAATs are especially adept for fast & sensitive ID needs
- Can be adopted at Point-of-Care
- Inadequate to inform best course of treatment
- Can't yield MICs or give AST profiles
- **ESC resistance involves mosaic allele mutations creating major challenges for NAAT**
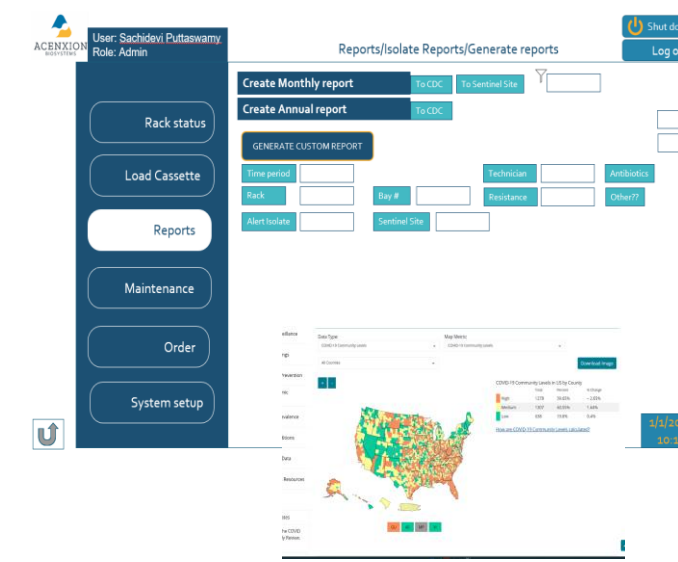
THE SYSTEM

Consumable

- Handheld
- Inexpensive, Room Temp Stable
- Single use, disposable
- 30-well customizable antibiotic cassettes

Instrument

- Benchtop (Small footprint)
- Automated Phenotypic AST results in hrs
- Seamless clinical workflow integration
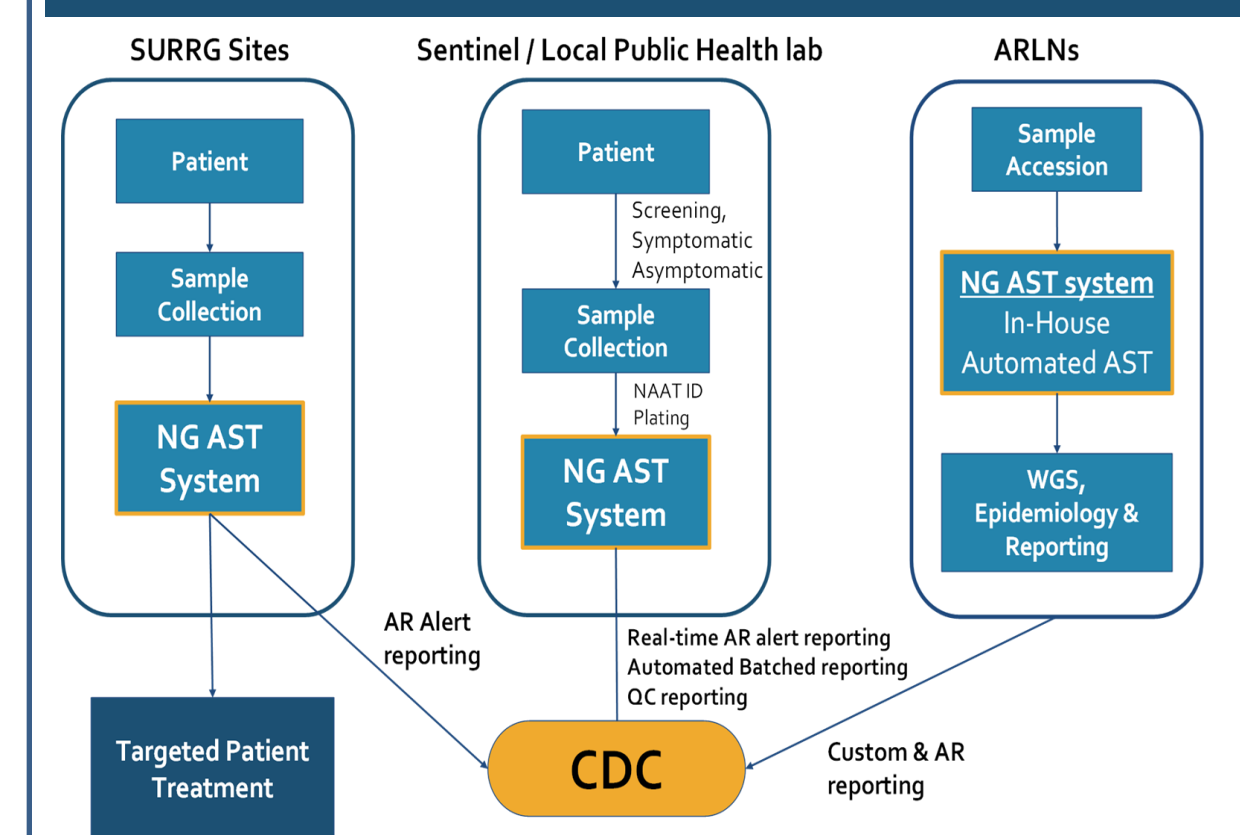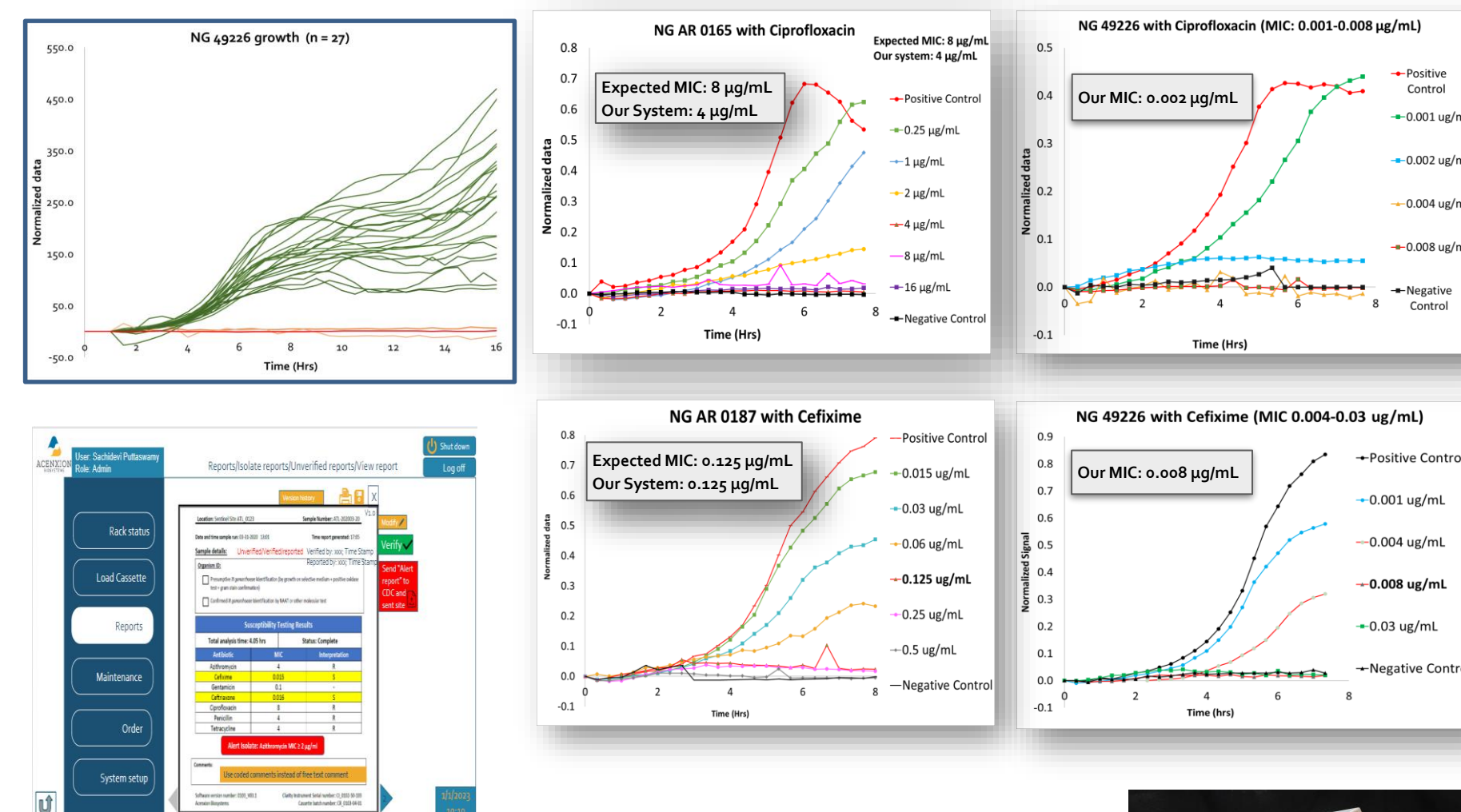- Scalable/Stackable (low to high) throughputs

Software

- Advanced Streamlined Software
- Key data acquired and analyzed automatically
- Custom formatted auto report generation
- Easy integration into IT secure communication

RAPID NG AST SYSTEM

Rapid and actionable results

Reduce incidences of antibiotic resistance among NG

Curbs Antibiotic overuse

Enable directed patient treatment within hours

Reduce NG prevalence

RESULTS

All Graphical Results are Raw, Unprocessed System Data

NG 49226 growth (n = 27)

NG AR 0165 with Ciprofloxacin — Expected MIC: 8 µg/mL Our system: 4 µg/mL

NG 49226 with Ciprofloxacin (MIC: 0.001-0.008 µg/mL) — Our MIC: 0.002 µg/mL

NG AR 0187 with Cefixime — Expected MIC: 0.125 µg/mL Our System: 0.125 µg/mL

NG 49226 with Cefixime (MIC: 0.004-0.03 ug/mL) — Our MIC: 0.008 µg/mL

Results Summary

- Extensive Antibiotic susceptibility testing (AST) of NG strains (10+) including challenge AR isolates from CDC & FDA AR bank across multiple classes of antibiotics
- Yields quantitative results: both MIC and S/I/R
- Average time to call is 6.5 hrs (using simple algorithm)
- Time-to-call Min - 2.33 hrs and Max - 15.67 hrs

Current and Future studies

- Reproducibility testing underway
- Evaluating lyophilized antibiotics
- Azithromycin antibiotic testing currently being investigated
- Test panel expansion to 7 antibiotics across 5-8 concentrations each
- Beginning evaluation of machine learning algorithms to make accurate MIC calls

Antibiotics	Essential Agreement (EA) vs Agar dilution	Essential Agreement (EA) vs BMD method	Categorical Agreement (CA)
Cefixime	89%	100%	100%
Ciprofloxacin	66.7%	100%	100%
Tetracycline	88.9%	77.8%	77.8%

Beta concept Model

A STREAMLINED WORKFLOW (Decentralized

Promotes "Enhanced Surveillance" and "Test-to-Treat"

- Fully automated phenotypic AST results for gonorrhea
- Reduced technician time and labor
- Enables expanded surveillance testing due to ease of use
- Real-time alert isolate information
- Move from empirical to informed patient treatment
- Reduce AMR spread in NG
- Limit sample losses from cryo and shipment exchanges
- Streamlined result, alert isolate and batched reporting across network

CLINICAL WORKFLOW

Collection — Analysis

Reporting

Sample — NAAT ID — Isolate

Manual sample handling — Long wait times — Labor intensive work — Documentation and tracking

Time and Effort Saved

Average results: < 6 hrs

SUMMARY

Our system & method is the world's first fully automated, phenotypic AST for *N. gonorrhoeae*. This product addresses the current clinical lab workflows established by the CDC and can be used by public health laboratories at the local, state, and regional levels. Composed of hand-held, single-use consumable; small footprint, benchtop instrument; and intuitive and user-friendly graphical-interface; **we show rapid, phenotypic, actionable results with quality-control and challenge strains of NG.**

With the threat of NG developing resistance to all known antibiotics now a reality, **the need to develop targeted, informed treatment regimens has become imperative.** Such regimen is only possible with a testing suite encompassing automated, phenotypic testing which to date has not been shown to be feasible.

We employ patent-pending and proprietary intellectual property and incorporate key elements of existing workflows **to demonstrate feasibility for implementation across multiple areas of Public Health interest**

For More Information

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References

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4. Tuite, Ashleigh R., et al. "Impact of rapid susceptibility testing and antibiotic selection strategy on the emergence and spread of antibiotic resistance in gonorrhea." *The Journal of infectious diseases* 216.9 (2017): 1141-1149.
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Acronyms:
NG - Neisseria gonorrhoeae; AMR - Antimicrobial resistance; GISP - Gonococcal Isolate Surveillance Project; MIC - Minimum Inhibitory Concentrations; S/I/R - Susceptible/Intermediate/Resistant; NAAT - Nucleic Acid Amplification Test; ESC - Extended Spectrum Cephalosporins

THE ACENXION TEAM

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