Predicted Resistance at CDC

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CDC NARMS
Surveillance Epidemiologist

PulseNet/OutbreakNet West Coast Regional Meeting
Omaha, NE
March 6, 2019
Outline:

- Background on NARMS traditional, phenotypic testing
- Predicted resistance and resistance determinants
- Accuracy of predicted resistance
- The value of predicted resistance
- How predicted resistance is changing workflows
- Where to find predicted resistance information
NARMS Surveillance and AST WORKFLOW

Sick patient Stool, blood, urine, or other specimen taken, yielding isolate Public health laboratory

Sampling for CDC NARMS
- 1 in 20 nontyphoidal *Salmonella*, *Shigella*, and *E. coli* O157
- All *Salmonella Typhi/Paratyphi A&C*, *Vibrio*
- Selected *Campylobacter* (FoodNet Sites)
- Local and multistate outbreak isolates

NARMS antimicrobial susceptibility Testing (AST)
Combating Antibiotic Resistant Bacteria (CARB)

Funding to Strengthen Surveillance

- Relevant Milestone: CDC will identify resistance patterns for all *Salmonella* isolates and an increased proportion of other enteric pathogens
ANTIMICROBIAL RESISTANCE WORKFLOW with WGS

1. Sick patient
2. Stool, blood, urine, or other specimen taken, yielding isolate
3. Public health laboratory
4. Samples shipped to CDC NARMS
5. NARMS antimicrobial susceptibility Testing (AST)
6. ‘S’ ‘I’ ‘R’
7. Predicted Resistance on ALL sequenced isolates
8. Sequencing
9. Resistance determinants
Predicted resistance and resistance determinants
Predicted Resistance

The antimicrobial resistance pattern we’d expect to see phenotypically, based on the presence or absence of resistance genes and mutations...

You may hear...

- “Resistance predicted by WGS”
- “Predictive resistance pattern”
- “Genetic resistance”
- “Genotypic resistance”
RESISTANCE DETERMINANTS

- Acquired resistance genes from plasmids, bacteriophage, or free DNA
- Chromosomal mutations

Center for Genomic Epidemiology

ResFinder 3.0
- ResFinder database for acquired AMR genes
- PointFinder database for chromosomal mutations

PlasmidFinder 2.0
# RESISTANCE DETERMINANTS IN *SALMONELLA*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Example of common resistance determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td><em>bla</em>TEM-1B, <em>bla</em>CMY-2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td><em>bla</em>CMY-2, <em>bla</em>CTX-M-65</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>qnrB19, gyrA*, parC*</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>mph(A), erm(B)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>aac(3)-IVa, aph (2’’)</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>sul2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>tet(B)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>catB, floR, cmlA1</td>
</tr>
</tbody>
</table>

*Mutation

**Caveats:** variation by pathogen; challenging nomenclature; dependent on gene calls

**References:** Frye (2013) PMID 23734150; ResFinder; PointFinder, CARD database
Accuracy of predicted resistance
Whole-Genome Sequencing for Detecting Antimicrobial Resistance in Nontyphoidal Salmonella

Patrick F. McDermott, Gregory H. Tyson, Claudine Kabera, Yuansha Chen, Cong Li, Jason P. Folster, Sherry L. Ayers, Claudia Lam, Heather P. Tate, Shaohua Zhao

Antimicrob Agents Chemother 60:5515–5520

1.0% discordance

WGS accurately predicts antimicrobial resistance in Escherichia coli

Gregory H. Tyson, Patrick F. McDermott, Cong Li, Yuansha Chen, Daniel A. Tadesse, Sonya Bodeis-Jones, Claudine Kabera, Stuart A. Gaines, Guy H. Loneragan, Mary Torrence, Dayna M. Harhay and Shaohua Zhao*

J Antimicrob Chemother 2015; 70: 2763–2769

1.2% discordance

Whole-Genome Sequencing Analysis Accurately Predicts Antimicrobial Resistance Phenotypes in Campylobacter spp.

S. Zhao, G. H. Tyson, Y. Chen, C. Li, S. Mukherjee, S. Young, C. Lam, J. P. Folster, J. M. Whichard, P. F. McDermott

Appl Environ Microbiol 82:459–466

0.8% discordance
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. (%) resistant* by phenotypic testing</th>
<th>Predicted resistance sensitivity (%)</th>
<th>Predicted resistance specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>238 (10.1%)</td>
<td>99.6%</td>
<td>99.8%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>56 (2.4%)</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>101 (4.3%)</td>
<td>90.1%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>36 (1.5%)</td>
<td>94.4%</td>
<td>99.7%</td>
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<tr>
<td>Sulfisoxazole</td>
<td>237 (10.0%)</td>
<td>96.2%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>76 (3.2%)</td>
<td>97.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>273 (11.5%)</td>
<td>98.2%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

*See NARMS website for definitions. Includes intermediate susceptibility to ciprofloxacin.
RELIABILITY OF PREDICTED RESISTANCE

1. Gene or plasmid lost
2. Detection problem
3. Novel gene or mutation
4. ↓ gene expression
5. MIC shift below cut-off

RESISTANCE DETERMINANT DETECTED

Gene or Mutation

EXPRESSION

PREDICTED RESISTANCE

 Gene detected | Predicted | AST
-----------------|-----------|------
 aadA1 sul1 aac(3)-VIa | SsuGen | SsuGen
 aadA1 sul1 aac(6')Ib-cr | SsuTGen | SsuTGen
 aadA1 sul1 tet(A) aac(3)-VIa | SsuTGen | SsuTGen
 aadA1 tet(A) aac(3)-VIa | SsuTGen | SsuTGen
 aadA12 aadA2 blaTEM-18B dfrA12 dfrA14 lnu(F) QnrA1 QnrS1 strA strB sul1 sul2 tet(A) | ASuTCip(I/R)CotLinTmp ASuTCipCot

CONFINIRMED RESISTANCE

Gene or plasmid lost

Detection problem

Novel gene or mutation

WGS→R AST=‘S’
WGS→NPR AST=‘R’
WGS→R OR DS AST=‘S’

REASONS FOR DISCORDANCE
The value of predicted resistance
<table>
<thead>
<tr>
<th></th>
<th>Susceptibility testing (Routine Surveillance)</th>
<th>Susceptibility testing (outbreak)</th>
<th>Predicted resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time to results</strong></td>
<td>Up to several months</td>
<td>5–10 weeks</td>
<td>1–2 weeks</td>
</tr>
<tr>
<td><strong># of Isolates tested</strong></td>
<td>Mostly 1:20 sample</td>
<td>3–5 isolates per outbreak</td>
<td>Up to all available isolates</td>
</tr>
<tr>
<td><strong>Number of drugs tested</strong></td>
<td>Up to 14</td>
<td>Up to 14</td>
<td>Unlimited</td>
</tr>
<tr>
<td><strong>Mechanism of resistance</strong></td>
<td>Unknown</td>
<td>Unknown</td>
<td>Often identified</td>
</tr>
<tr>
<td><strong>Reliability of results</strong></td>
<td>Gold standard</td>
<td>Gold standard</td>
<td>Comparable to Phenotypic AST, with potential inaccuracies</td>
</tr>
</tbody>
</table>
VALUE OF SEQUENCING AND PREDICTED RESISTANCE cntd..

- Detect new strains
- Detect emerging strains
- Identify sources
Tracking of *mcr-1* (🔗) and *mcr-3* (🔗) in the United States
Impact of WGS: *Campylobacter jejuni* linked to puppies

- Resistance pattern identified almost one month earlier than AST results were available

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Outbreak identified

Isolate sequences available for screening (n = 2)

Screening results available

Isolates arrive at CDC for AST (n = 8)

AST results available

August 2017

- 21
- 23
- 28

September 2017

- 8
- 22

https://www.cdc.gov/campylobacter/outbreaks/puppies-9-17/index.html
Novel trimethoprim resistance gene \textit{dfrA34}

Journal of Antimicrobial Chemotherapy

Kaitlin A. Togg*, Louise Francois Watkins*, Matthew D. Moore*, Christy Bennett*, Yoo J. Joung†, Jessica C. Chen* and Jason P. Folster

*J Antimicrob Chemother 2019; 74: 38-41
doi:10.1093/jac/dky373 Advance Access publication 10 September 2018

Novel trimethoprim resistance gene \textit{dfrA34} identified in \textit{Salmonella} Heidelberg in the USA
How predicted resistance is changing workflows
PREDICTED RESISTANCE WORKFLOW (State PHL sequencing)

- CDC NARMS LAB
  - Initiates analyses using in-house programming scripts
  - Screen with ResFinder, PointFinder, PlasmidFinder

- Resistance genes and mutations imported
- Predicted resistance calculated

- CDC DATA BROKER

- Bitbucket
  - Updates to blast tools (Center for Genomic Epidemiology)

- MiSeq

- National Center for Biotechnology Information

- Computational Cluster (CDC)

- PulseNet National Databases
Where to find predicted resistance information
FINDING PREDICTED RESISTANCE

- NARMS Secure Site
  - https://wwwn.cdc.gov/NARMS/
<table>
<thead>
<tr>
<th>Log Sheet Name</th>
<th>Created By</th>
<th>Created Date</th>
<th>Date Submitted to CDC</th>
<th>Status</th>
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<td></td>
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<td>In Progress</td>
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<td>9/26/2018</td>
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<td>In Progress</td>
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</table>

**DISCLAIMER:** The information contained herein is the property of the Centers for Disease Control and Prevention and its participating partners in the National Antimicrobial Resistance Monitoring System. Any disclosure of this information is restricted to employees with a need to know and is subject to confidentiality measures to protect from unauthorized disclosure and dissemination to third parties.
<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>PulseNet Key (from PulseNet)</th>
<th>Isolate ID (site)</th>
<th>WGS ID</th>
<th>Site Genus</th>
<th>Site Species</th>
<th>Site Serotype</th>
<th>Status</th>
<th>Collection Year</th>
<th>Date Submitted</th>
<th>Source</th>
<th>Primary Purpose</th>
<th>Secondary Purpose</th>
<th>Resistance Pattern</th>
<th>Resistance Genes Detected</th>
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<tbody>
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<tr>
<td>2016AM-155</td>
<td>2016AM-155</td>
<td>Salmonella enterica</td>
<td>Infants</td>
<td>Accepted</td>
<td>2016</td>
<td>9/14/2016</td>
<td>Stool</td>
<td>76 yrs.</td>
<td>Surveillance</td>
<td>Outbreak</td>
<td>ATaAcFxFoS</td>
<td></td>
<td>AaAaCmFx</td>
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</tr>
</tbody>
</table>
### Site Isolate Information

- **Site Isolate ID**: [Redacted]
- **Other Site Isolate ID**: [Redacted]
- **Site**: Source Stool
- **County**: [Redacted]
- **Genus**: Salmonella
- **Species**: enterica
- **Serotype**: Infantis

### CDC Isolate Information

- **Purpose**: Primary Surveillance Secondary Outbreak
- **Exclude this isolate**: [Redacted]
- **CDC Received Date**: 9/16/2016
- **Final Genus**: Salmonella
- **Final Species**: enterica
- **Other information**:
  - **Source**: PulsNet Cluster Code: 1604HLJFX-1
  - **PulsNet Key**: [Redacted]

### CDC information

- **NCBI Accession #: [Redacted]**

### Resistance Pattern

- **Resistance Pattern**: ATAuCxFox
- **Predictive Resistance Pattern**: AAuCxFox
- **Resistance Genes Detected**: blaCMY-2

### Susceptibility Results:

**Note**: These test results are provided for epidemiological study purposes only and must not be returned to clinicians, referral laboratories or other medical practitioners who might place the results in the patient's medical record or use the result to manage the patient's infection. The test method and antimicrobial agents tested are for epidemiologic research and cannot be used for patient assessment or treatment.

<table>
<thead>
<tr>
<th>Agent Name</th>
<th>Plate</th>
<th>Result</th>
<th>Conclusion *</th>
<th>Upper limit of susceptible range</th>
<th>Lower limit of resistant range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-Clavulanic Acid</td>
<td>CM44AGF</td>
<td>$&gt;32$</td>
<td>R</td>
<td>8</td>
<td>32</td>
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<td>Ampicillin</td>
<td>CM44AGF</td>
<td>$&gt;32$</td>
<td>R</td>
<td>8</td>
<td>32</td>
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<td>Azithromycin</td>
<td>CM44AGF</td>
<td>$=4$</td>
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<td>$&gt;32$</td>
<td>R</td>
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<td>Nalidixic Acid</td>
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<td>$=16$</td>
<td>R</td>
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</table>
### FINDING PREDICTED RESISTANCE

- **National Outbreak Reporting System (NORS)**
  - NORS-PulseNet Integration tool
FINDING PREDICTED RESISTANCE

- SEDRIC (in development)
- SEDRIC (in development)
  - Outbreak Dashboard view
● SEDRIC (in development)
  • PulseNet Outbreak Line List Editor
- SEDRIC (in development)
  - Isolate Browser view
FINDING RESISTANCE DETERMINANTS

- NCBI Pathogen Detection Tool
<table>
<thead>
<tr>
<th>#</th>
<th>Organism Group</th>
<th>Isolate</th>
<th>Create Date</th>
<th>Location</th>
<th>Isolation Sou</th>
<th>Isolation Type</th>
<th>Host</th>
<th>SNP cluster</th>
<th>Min-sat</th>
<th>Min-diff</th>
<th>BioSample</th>
<th>Assembly</th>
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<tr>
<td>1</td>
<td>E.coli and Shigella</td>
<td>PD000432313.1</td>
<td>2019-01-09</td>
<td>United Kingdom: United Kingdom</td>
<td>human</td>
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FINDING PREDICTED RESISTANCE

- BioNumerics v7.6 – class resistance and gene calls
Summary

- Detection of resistance determinants and predicting resistance are increasingly valuable tools.

- The impact of predicted resistance depends on reliable detection of resistance determinants and correlation with susceptibility tests.

Please contact entericbacteria@cdc.gov with any queries.
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Questions?

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1-800-CDC-INFO (232-4636)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.