The plasmid puzzle: challenges for *Salmonella* monitoring

Kristina Feye¹, Danielle Sopovski², Javier Revollo³, Jaimie Miranda-Colon², Dereje Gudeta¹,³, Jing Han¹, Steven Foley¹*#

¹Division of Microbiology and ²Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA

²Oak Ridge Institute of Science and Education, Oak Ridge, Tennessee, USA

### Introduction

- *Salmonella enterica* is an important foodborne pathogen that is well studied and characterized.
- *Salmonella* typically harbors a consortia of plasmids across incompatibility (Inc) groups ranging in size that contribute to antibiotic resistance and virulence.
- Plasmids are capable of rearranging as well as transferring from one microorganism to another microorganism.
- Studying plasmids through Next-Generation Sequencing technologies is changing and is starting to include 4th Generation Sequencing technologies, such as PacBio Sequel II Sequencing.
- Evidence suggests that long-read sequencing technology is not necessarily capturing all of the plasmids and may demonstrate a bias.
- Incomplete sequencing and characterization of plasmids may miss important genomic features during public health crises.

### Hypothesis:

**Does PacBio Sequel II Sequencing efficiently sequence all of the plasmids from wild-type *Salmonella***?

### Results:

- **Disparities exist between sequencing platforms**
- **Low and high plasmids may not be identified completely**
- **Innovations to sequencing may likely result in more resolution**

### Research Design

- There were 9 isolates of *Salmonella enterica* cultured on Tryptic Soy Agar with 5% Sheep’s Blood (TSA-B).

- Total DNA was extracted via the Qiagen Blood and Tissue Kit.

- Log10 phase growth was confirmed prior to DNA extraction.

- Benchtop confirmation may still be important.

- Small and large plasmids may be hard to identify.

- Variations in sequencing results may result in the inability to track all genomic material that could be relevant to tracking and detection.

- New approaches and investigations will be needed.

### Table 1. PCR Confirmation of the INC families and Antibiotic Resistance Genotypes per Isolate

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Inc</th>
<th>Size (kb)</th>
<th>Multiresistant</th>
<th>PCR Replicon Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>X</td>
<td>135-150</td>
<td>Yes</td>
<td>Type A, B</td>
</tr>
<tr>
<td>002</td>
<td>Y</td>
<td>150-200</td>
<td>Yes</td>
<td>Type A, B, C</td>
</tr>
<tr>
<td>003</td>
<td>Z</td>
<td>200-250</td>
<td>No</td>
<td>Type A, B, C, D</td>
</tr>
<tr>
<td>004</td>
<td>W</td>
<td>250-300</td>
<td>Yes</td>
<td>Type A, B, C, D</td>
</tr>
<tr>
<td>005</td>
<td>V</td>
<td>&gt;300</td>
<td>Yes</td>
<td>Type A, B, C, D</td>
</tr>
</tbody>
</table>

- **Some differences between the platforms exist**

- **Benchtop confirmation may still be important**

### Conclusion and Discussion

- **Some differences between the platforms exist**

- **Benchtop confirmation may still be important**

- **Small and large plasmids may be hard to identify**

- **Variations in sequencing results may result in the inability to track all genomic material that could be relevant to tracking and detection**

- **New approaches and investigations will be needed**

### Future Work

- **Nanopore will be compared (Hybridized or alone, SQK-LSK-109 or SQK-RBK-004)**
- **Pulse Field Gel Electrophoresis will be performed**
- **Pulse Field Gel Electrophoresis bands will be sequenced on the MSeq platform and compared**
- **AMR Phenotyping via a Microwell Dilution Assay**

### With Thanks

- National Center for Toxicological Research
- The Division of Microbiology
- The Division of Genetic and Molecular Toxicology
- Oak Ridge Institute for Science and Education

The views and opinions expressed on this poster do not represent the views and opinions of the FDA.