Evaluation of gentamycin resistance phenotypes in genotypically susceptible *Salmonella enterica* isolates

Ashraf Khan1, Sarah Xie1,2, Danielle Spropovski1, Steven Foley1, Kristina Feye1

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

1Oak Ridge Institute of Science and Education, Oak Ridge, Tennessee, USA

# Corresponding Author: Kristina Feye, Kristina.Feye@fda.hhs.gov

Introduction

- Non-typhoidal *Salmonella enterica* a leading cause of *Salmonellosis* in the United States
- Previous studies have detected unexpected phenotypic resistance patterns in sequences of *Salmonella* isolates, specifically in gentamycin resistance
- While other antibiotic resistant strains were analyzed in that study, gentamicin resistance seemed to be consistently inconsistent between genotyping and phenotypic analyses

Hypothesis: Is there true variation between the phenotypic and genotypic results and is that consistent per CFU within each isolate tested?

Results:

- Variation in gentamicin resistance exists between strains and within CFUs of each strain
- MIC and MBC also vary

Culture: Per serovar of *Salmonella enterica*, a single isolate was cultured in 15 mL conical tubes containing 10 mL of Trypticase Soy Broth (TSB). A total of 7 Colony Forming Units (CFUs) were cultured per isolate. The cultures were incubated at 37 °C for 16 hours, oscillating at 180 rpm. A negative control was also included.

MIC: Exactly 200 µL of the overnight culture was aseptically added to each row, with one plate containing the CFUs from a single isolate. Using sterile replication technique, the colonies were pin-replicated into a fresh antibiotic-resistant plate containing gentamicin diluted (double) from 32,000 µg/mL to 0.0625 µg/mL in TSB in each row. The positive control consisted of an overnight culture of an *E. coli* strain resistant to >1,000 µg/mL of gentamicin. The minimum inhibitory concentration (MIC) was recorded as the first well per row where turbidity was not observed.

MBC: Using sterile replication technique, the colonies from the MIC plate were pin-replicated into a fresh TSB well plate containing TSB. The negative control (no CFU) from the first step of the experiment was included. The minimum bactericidal concentration (MBC) was recorded as the first well per row where turbidity was not observed.

Conclusion and Discussion

- Resistance is diverse between the serovars
- Differences between CFUs within a single isolate seems to exist
- MIC is variable and above breakpoint concentrations
- MBC seems to be very high, which means that true bactericidal activity may exist beyond clinically relevant ranges
- There is not a known gene associated with this resistance pattern
- New approaches and investigations will be needed to determine the underlying mechanisms of resistance

Future Work

- ONT sequencing to evaluate methylation patterns and resistance markers that could include:
  - Novel gene
  - Novel plasmid or mobile genetic element
  - SNP driven antibiotic resistance

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