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

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Introduction

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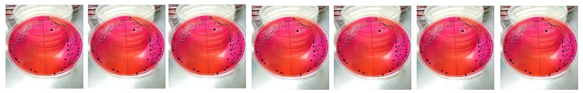
- Non-typhoidal *Salmonella enterica* a leading cause of Salmonellosis in the United States
- Previous studies have detected unexpected phenotypic resistance patterns in sequences of *Salmonella* isolates, specifically in gentamycin resistance
- While other antibiotic resistant strains were analyzed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

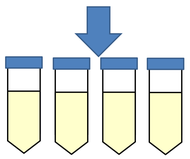
Hypothesis: Is there true variation between the phenotypic and genotypic results and is that consistent per CFU within each isolate tested?

Results:
- Variation in gentamicin resistance exists between strains and within CFU's of each strain
- MIC and MBC also vary

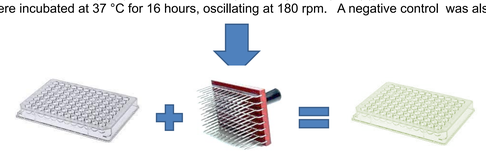
Research Design



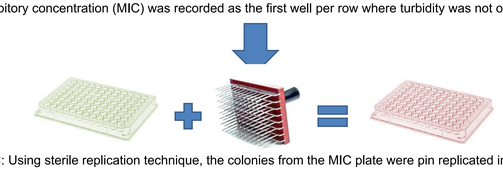
Seven serovars of *Salmonella enterica* that were previously identified as phenotypically resistant yet genotypically susceptible to gentamicin were cultured on XLD media for 24 hours



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 (CFU(s)) were cultured per isolate. The cultures were incubated at 37 °C for 16 hours, oscillating at 180 rpm. A negative control was also included.

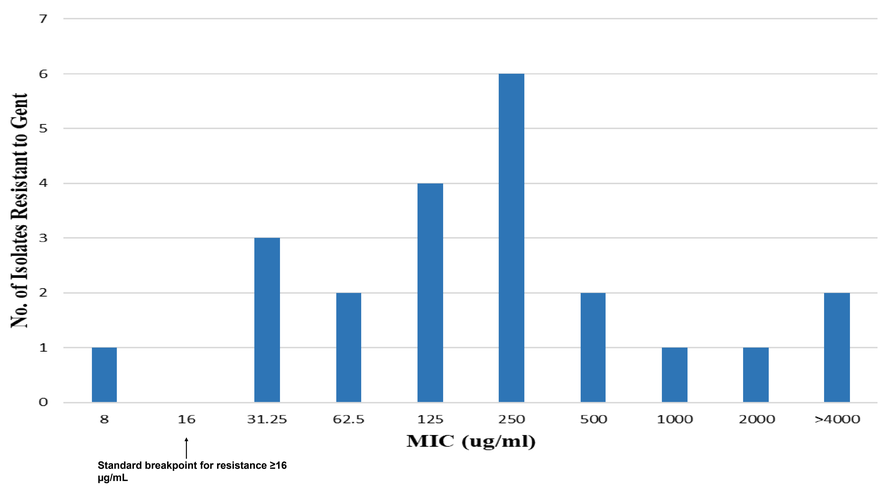


MIC: Exactly 200 µL of the overnight culture was aseptically added to each row, with one plate containing the CFUs from a single isolate. Using sterile replication technique, the colonies were pin replicated into a fresh antibiotic resistant plate containing gentamicin diluted (double) from 32,000 µg/mL to 0.0625 µg/mL in TSB in each row. The negative control (no CFU) from the first step of the experiment was included. 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 96-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.

Results



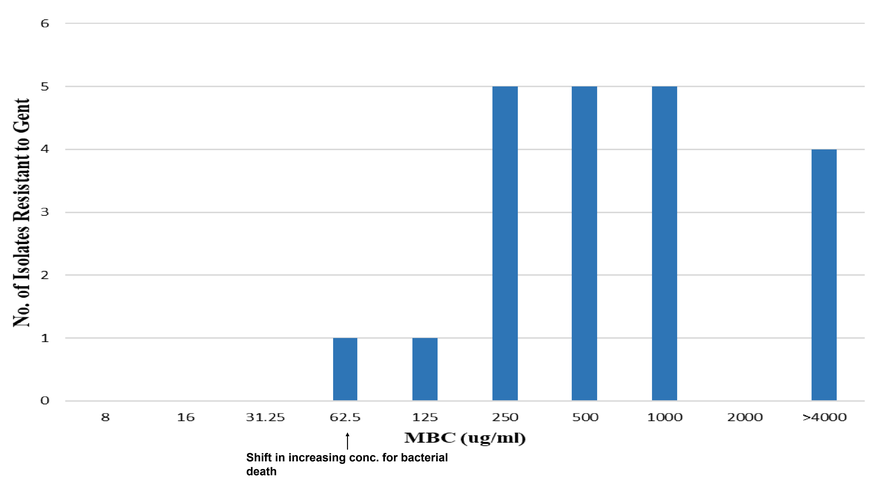


Figure 1: MIC and MBC of gentamicin resistant *Salmonella* isolates. The MIC and MBC of resistant CFUs per serovar varied. The CFUs tested were resistant, with 93% of the isolates demonstrating resistance above the breakpoint concentration. While going above 16 µg/mL is not clinically relevant, from a resistance standpoint, seeing potential changes to resistance within serovar was important for downstream analyses using fourth generation sequencing techniques.

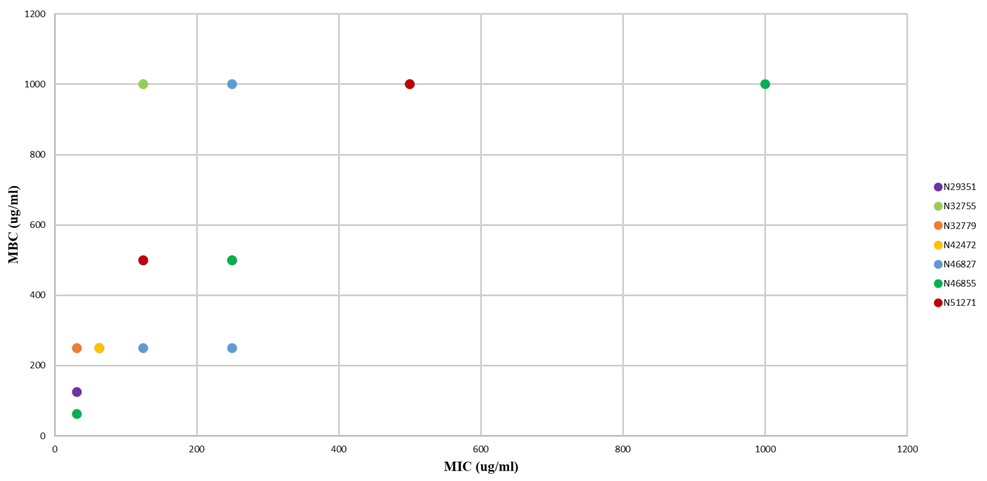


Figure 2: Differences in MIC and MBC of gentamicin resistant *Salmonella* isolates by strain. For one trial, the different strains of *Salmonella* MIC vs. MBC were compared. Within strain, there is variability. While N42472, N32779, and N3725 MIC and MBC are repeatable, other strains, like N46827 vary significantly. Therefore, it is possible that gentamicin resistance is heterogenous within an isolate.

Conclusion and Discussion

Conclusions
- 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

With Thanks
- National Center for Toxicological Research
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