The plasmid puzzle: challenges for *Salmonella* monitoring

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Introduction

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- *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

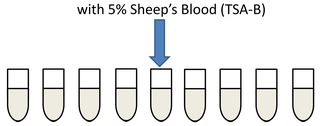
Hypothesis: Does PacBio Sequel II Sequencing efficiently sequence all of the plasmids from wild-type *Salmonella*?

Results:

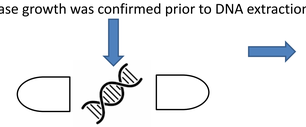
- Disparities exist between sequencing platforms
- Low and high plasmids may not be identified completely
- Innovations to sequencing may likely result in more resolution

Research Design

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



1 individual CFU per isolate was grown overnight at 37 °C at 150 RPM and log10 phase growth was confirmed prior to DNA extraction.



Total DNA was extracted via the Qiagen Blood and Tissue Kit

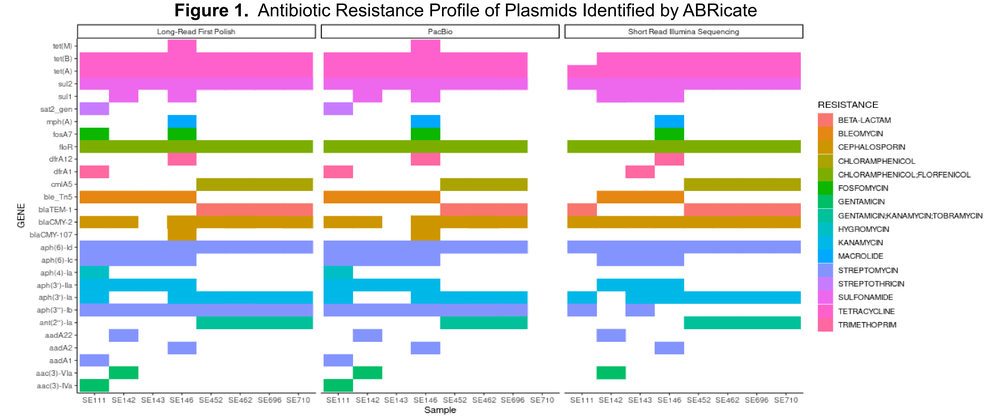
Results

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

Isolate	Serotype	Source	Year	Location	Plasmids (in KB)	Replicon (INC) Types by PCR	Transconjugant Replicons
111	Heidelberg	Cattle	2001	OH	>100 (x2),75,5		
142	Heidelberg	Swine	2002	IN	>100,10,4	A/C, I1, HI2	I1 (3/4)
143	Heidelberg	Swine	2002	MN	>165 (x2), 92,34	A/C, I1, HI1, HI2	A/C (4/4), I1 (4/4)
146	Heidelberg	Swine	2002	MN	>165, 70,40	A/C, I1	I1 (4/4)
163	Typhimurium	Turkey	2002	OH	135, 120, 34, 6, 4	A/C, FIB	A/C (4/4), FIB (4/4)
452	Typhimurium	Turkey	1999	UNK	124, 4, 1	A/C, FIB, FIA	FIA (3/4), FIB(3/4), NT(1/4)
462	Typhimurium	Turkey	1999	UNK	>150, 6, 4, 2		
696	Typhimurium	Turkey	2000	UNK	120, 34, 6, 4	A/C, FIB	FIB (2/4)
710	Heidelberg	Turkey	1992	ND	>120, 34, 8, 3, 2	A/C, FIA, FIB	FIA (2/4), FIB (2/4),

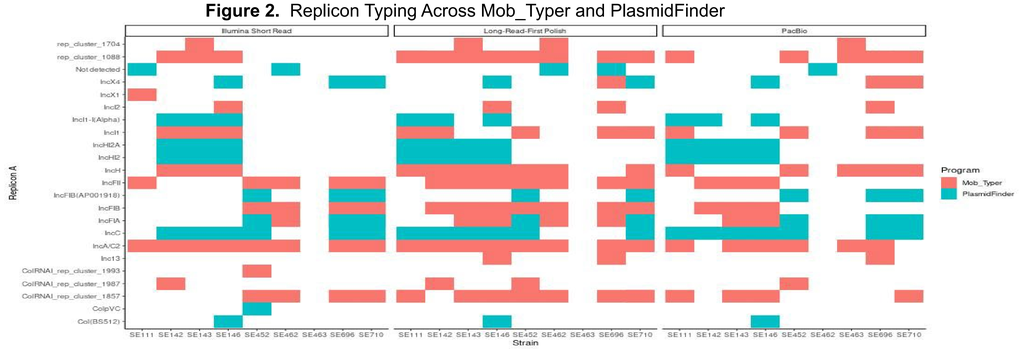
The table represents the isolate ID, the original source of the isolate, and a basic benchtop confirmation of the replicons, plasmids, and the transconjugant replicons. Plasmid size varied (data not shown) between the assemblies and across programs.

Figure 1. Antibiotic Resistance Profile of Plasmids Identified by ABRicate

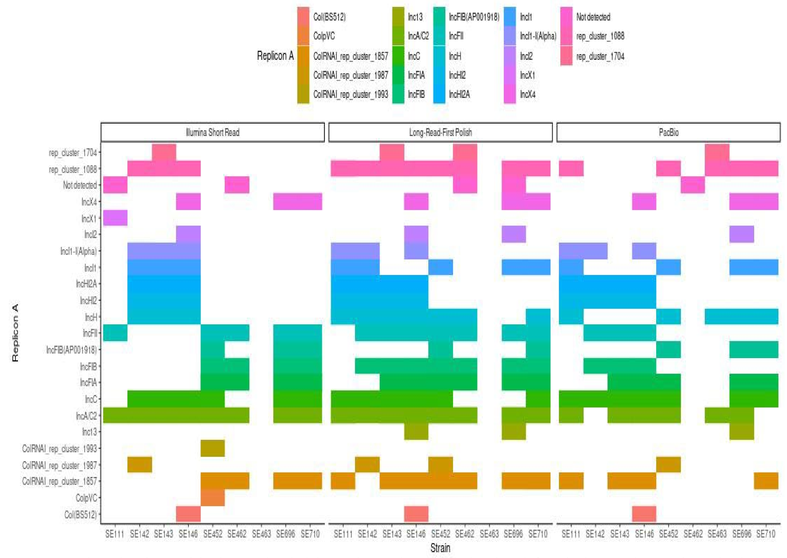


This figure represents the AMR genotypes identified by Mob_Recon . There is variability between the platforms, with the Long-Read First Polish and the PacBio results being nearly identical. Similar features between the Long-Read First Polish may be due to pre-assembly conditions from the PacBio long-reads and will be investigated. In short, the plasmids identified via PacBio may only be what has not been filtered. Future investigations will compare this data, along with PacBio nodes, to Nanopore. Likely, the short-read data may be more accurate as it captures a broader range of plasmids.

Figure 2. Replicon Typing Across Mob_Typer and PlasmidFinder



Using Mob Suite and Staramr, the assemblies were fed through the respective programs and the replicon features are visualized above. There is diversity in the plasmid calling between platforms, which the PacBio and Long Read First polishing having the highest number of called replicons. However, the individual replicon called seems to vary between platforms. Further investigations will include rescuing plasmids and confirming their individual sequence and ID on the bench. There is a greater diversity in the observed sequences compared to previous benchtop validation efforts.



The Replicon (Inc types) distribution is described above and is a combination of both analysis platforms (Mob Suite and Staramar). Visual evaluation of the images indicates that there may be increased annotation with the Long-Read First polish method. However, additional benchwork is required to validate this theory.

Conclusion and Discussion

Conclusions

- 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 MiSeq 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.