#5270
Friday, June 16, 2023
AAR-FRIDAY-432

Evaluating *Salmonella* serovars for antibiotic resistance genotypes and virulence factors

Danielle Sopovski[1], Kristina Feye[1], Dereje Gudeta[1,2], Jing Han[1], Shaohua Zhao[3], Steven Foley[1*#]
[1]Division of Microbiology and [2]Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA
[3]Oak Ridge Institute of Science and Education, Oak Ridge, Tennessee, USA
[3] College of Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA

Introduction

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, antibiotic resistance, and virulence
- The chromosomes can also harbor antibiotic resistance elements and virulence factors as well
- Studying plasmid transfer factors 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 information, specifically with plasmids
- Phenotypic data like plasmid transfer efficiency is important

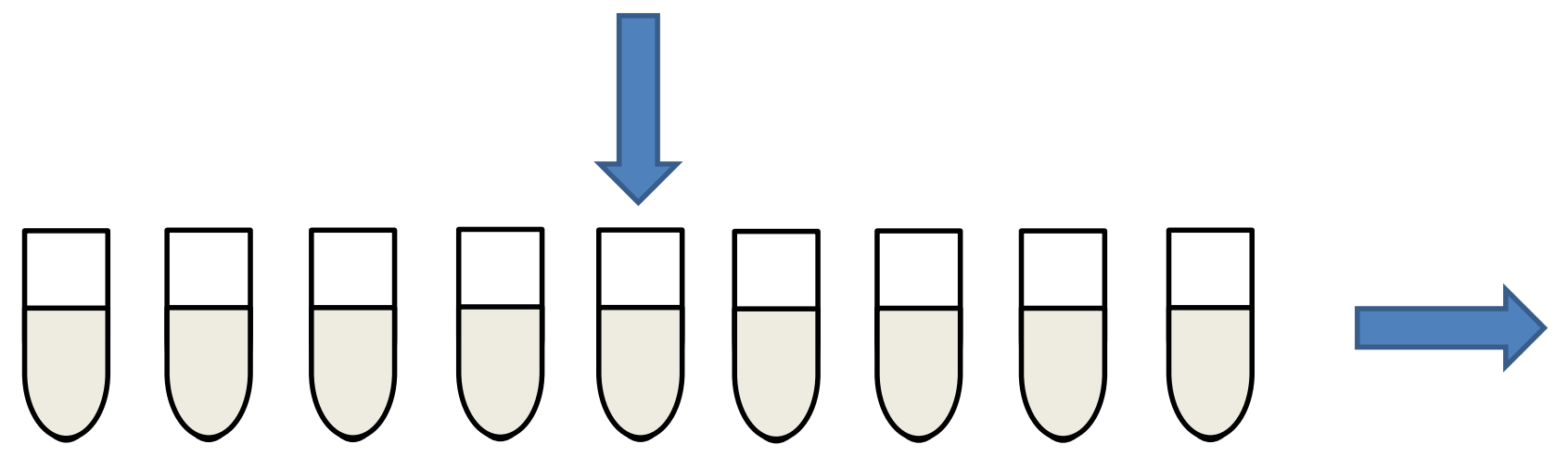
Hypothesis: Does PacBio Sequel II , Nanopore, and Illumina short read sequencing compare when it comes to detecting plasmid transfer factors?

Results:

- Disparities exist between sequencing platforms
- Hybrid approaches may be necessary
- With improved bioinformatics comes improved results

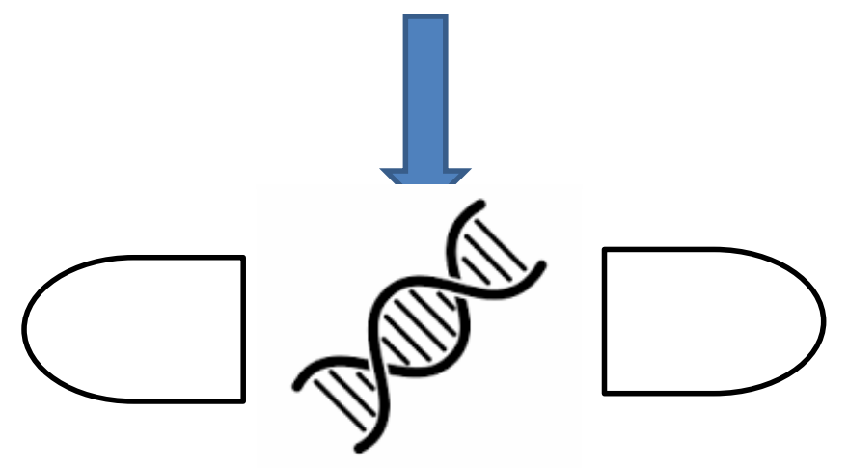
Research Design

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

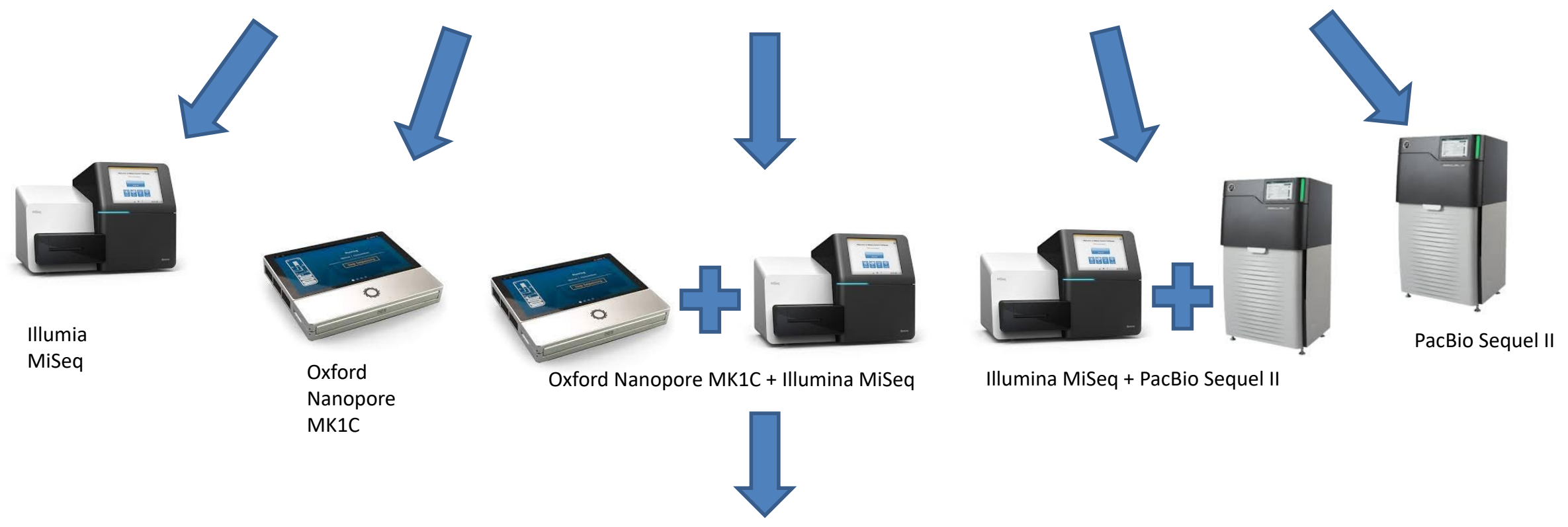


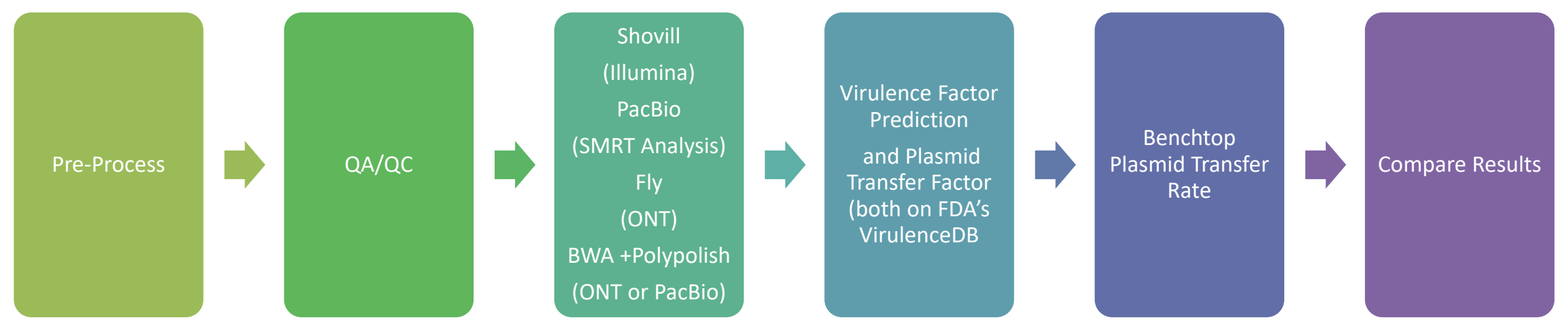
Benchtop Verification

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

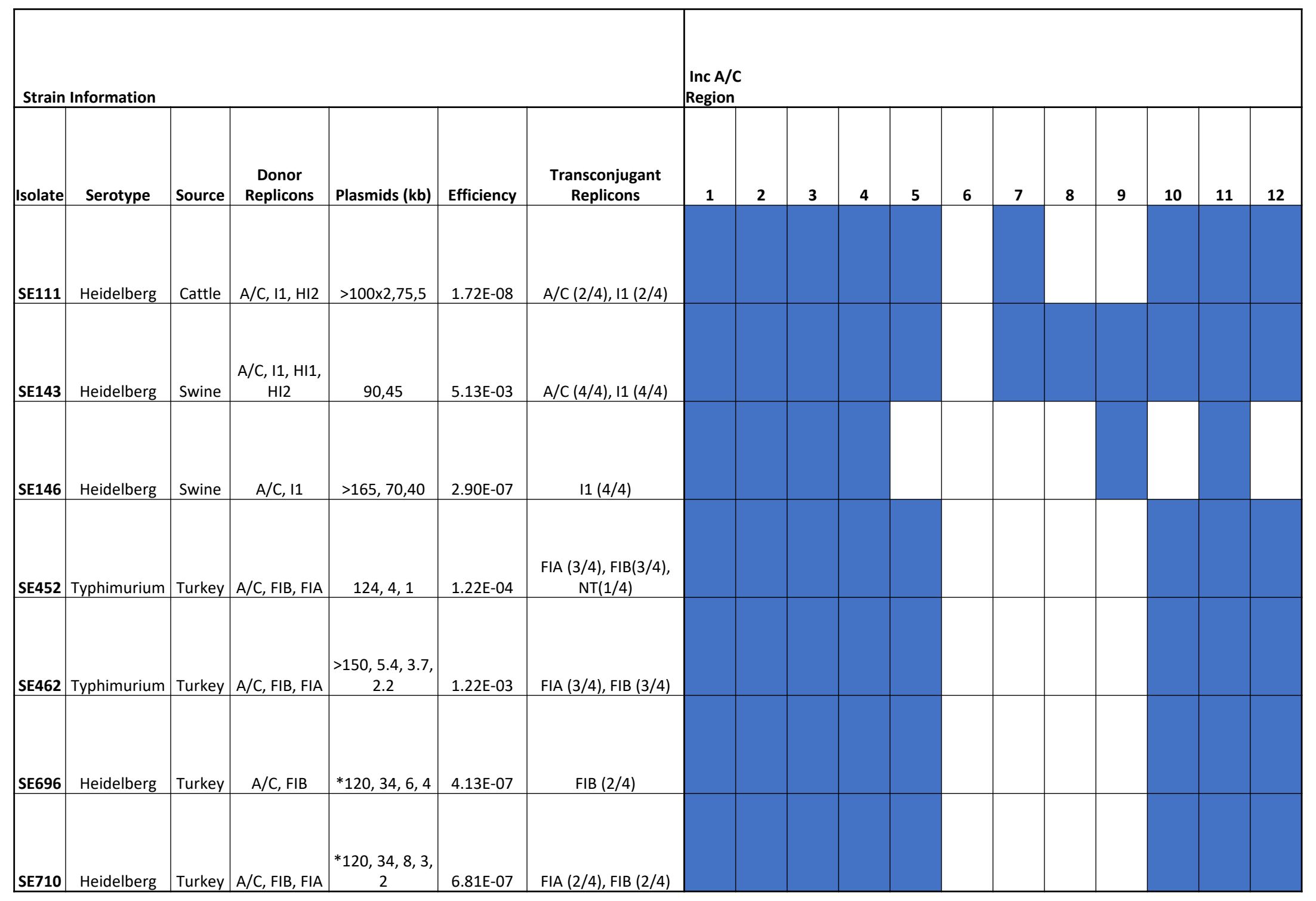


Total DNA was extracted via the Qiagen Blood and Tissue Kit





Results



Strain Information							Inc A/C Region											
Isolate	Serotype	Source	Donor Replicons	Plasmids (kb)	Efficiency	Transconjugant Replicons	1	2	3	4	5	6	7	8	9	10	11	12
SE111	Heidelberg	Cattle	A/C, I1, HI2	>100x2,75,5	1.72E-08	A/C (2/4), I1 (2/4)	■	■	■	■	■		■			■	■	■
SE143	Heidelberg	Swine	A/C, I1, HI1, HI2	90,45	5.13E-03	A/C (4/4), I1 (4/4)	■	■	■	■	■		■	■	■	■	■	■
SE146	Heidelberg	Swine	A/C, I1	>165, 70,40	2.90E-07	I1 (4/4)	■	■	■	■					■		■	
SE452	Typhimurium	Turkey	A/C, FIB, FIA	124, 4, 1	1.22E-04	FIA (3/4), FIB(3/4), NT(1/4)	■	■	■	■	■					■	■	■
SE462	Typhimurium	Turkey	A/C, FIB, FIA	>150, 5.4, 3.7, 2.2	1.22E-03	FIA (3/4), FIB (3/4)	■	■	■	■	■					■	■	■
SE696	Heidelberg	Turkey	A/C, FIB	*120, 34, 6, 4	4.13E-07	FIB (2/4)	■	■	■	■	■					■	■	■
SE710	Heidelberg	Turkey	A/C, FIB, FIA	*120, 34, 8, 3, 2	6.81E-07	FIA (2/4), FIB (2/4)	■	■	■	■	■					■	■	■

TABLE 1. 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. Additionally, data focuses on the IncA/C region present with each strain. Strain SE142 is not shown as the data was not available at the time of this poster. The regions that are positive are in blue.

FIGURE 1. AMR By Platform. Annotated antibiotic resistance varied by sequencing. Using ResFinder, the assemblies were analyzed and there is variation between the sequencing platforms regarding AMR. The MDRO designation refers to resistance to at least seven antibiotics (likely ESBL). Variation exists between platform and between assembly strategy.

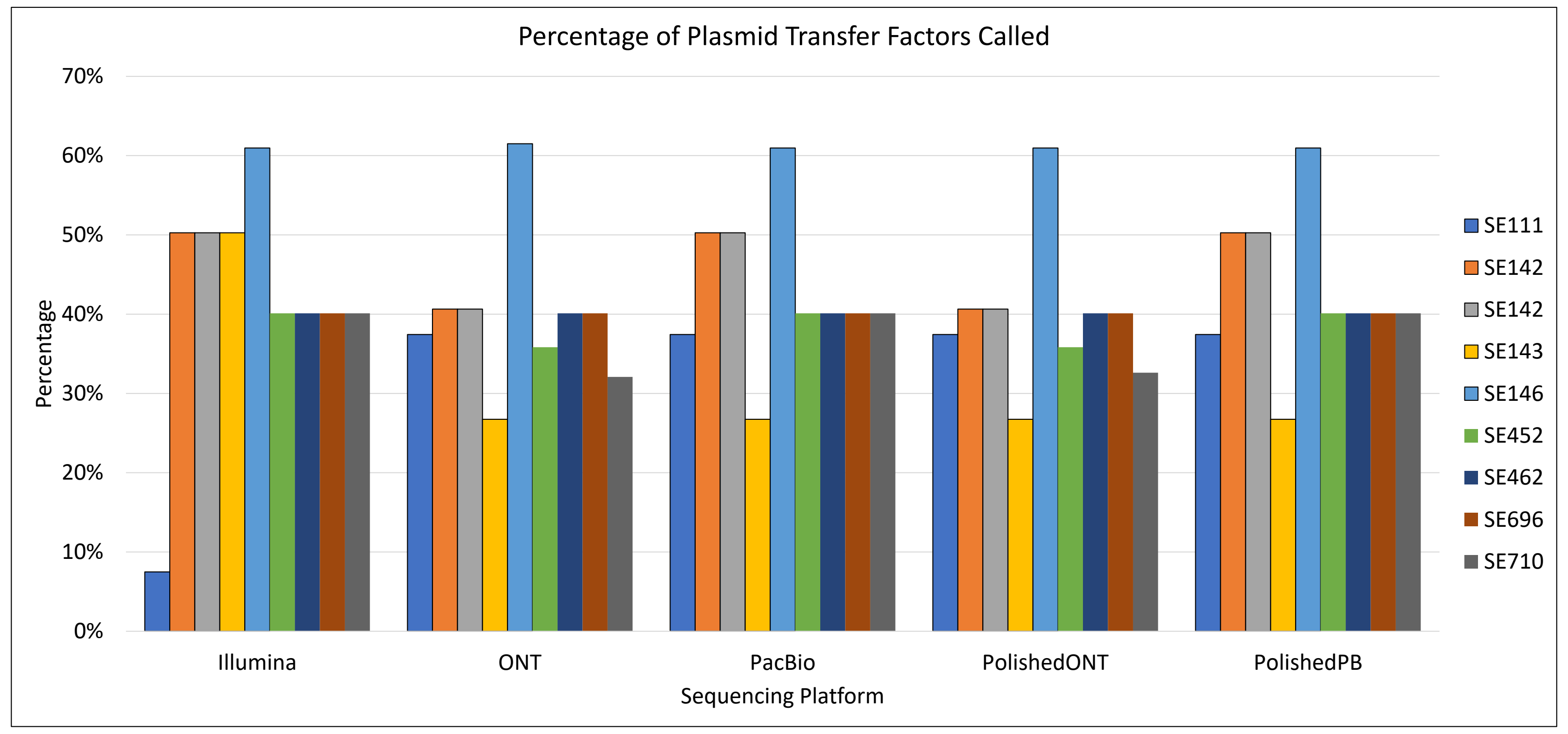


FIGURE 3. The total number of positive transfer factors were divided by the total possible transfer factors (187). Variation was noted in every serovar except SE462 and SE696. Variation between the sequencing platforms remained consistently inconsistent, which points to potential challenges in monitoring plasmid transfer factors between platforms. While not definitive, this may be influenced by the serovar.

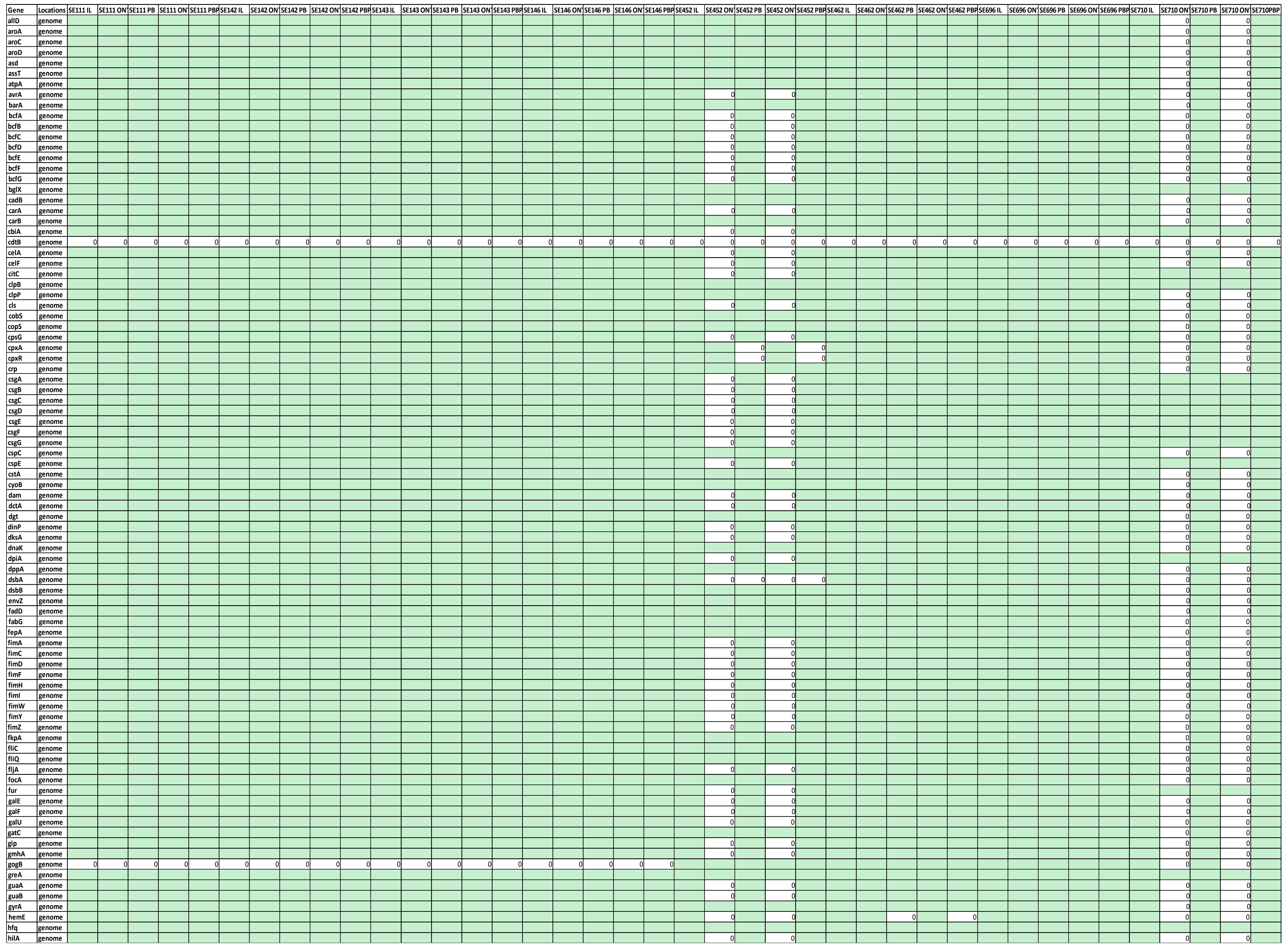


FIGURE 2. Subsample of Virulence Factors. The assemblies were loaded into the Salmonella Virulence Factor DB (FDA) and compared. The first 154 out of 594 genes were selected for the figure. Overall, there appears to be decent agreement between ONT and PacBio. If a gene is picked up by PacBio, the polished assembly also carries that gene. The same, and even the opposite, is true for ONT. There seems to be some consistency between which genes are lost with ONT and the ONT Polished Assemblies (Data not Shown).

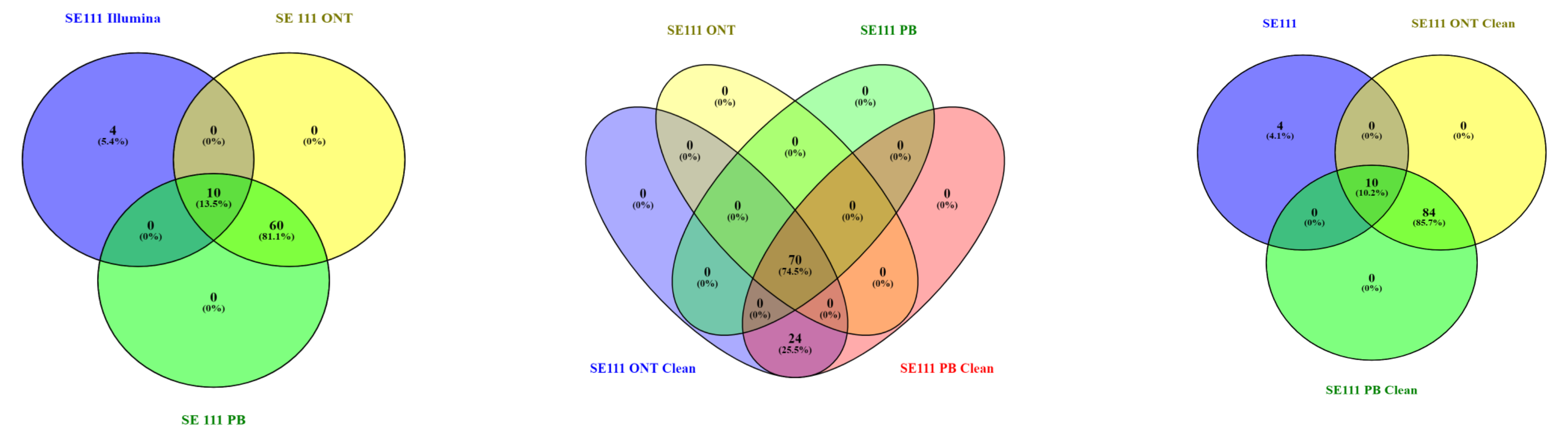


FIGURE 4. Using SE111 as an example, the plasmid factors were loaded into Vinny. With just SE111, it appears that the genes identified by the two polished/clean methods (ONT and PacBio(PB)) seem to capture similar features. However, taken with the information in the previous figure, that may not be consistent between serovar.

Conclusion and Discussion

Conclusions

- Differences between platforms exist for INC identification and AMR
- Serovar annotation is not consistent between platforms
- ONT and PacBio can vary, but it is consistent
- Benchtop confirmation may still be important
- 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

- 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.