MULTI DRUG RESISTANT PLASMID TRANSFER BETWEEN *SALMONELLA* AND TWO *E. COLI* STRAINS

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Background

Multiple drug resistance is becoming increasingly problematic in the U.S., where antibiotics are overused both in agriculture and healthcare. One way bacteria can develop this resistance is through acquisition of antibiotic resistance genes either on their chromosomes or on plasmids. A plasmid is an autonomous extrachromosomal DNA structure that replicates independently of the bacterial chromosome. Plasmids can be horizontally spread across different bacterial species through conjugation, transformation or transduction allowing multiple bacteria the ability to select for a resistant, advantageous phenotype. *Salmonella* infects around 400,000 people a year. Children, elderly, and the immunocompromised are the most at risk of severe infection and complications. Recent *Salmonella and Escherichia coli* clinical isolates have been found to carry plasmids with multiple antibiotic resistance genes. This limits treatment options even for healthy individuals. *Salmonella*, like other members of the *Enterobacteriaceae* family, have been found to carry more than one type of beta-lactamase genes, such as the bla_{CMY2} gene or $bla_{CTX\text{-}M}$ gene. These genes are expressed on several multidrug resistant plasmids.

Objective

- To determine if horizontal transfer of multiple-drug resistant plasmids is possible in a laboratory setting, two different *E. coli* lab isolates were transformed with plasmids from *Salmonella* clinical isolates.
- The goal of the current project is to isolate these plasmids and analyze them for their incompatibility groups and their antibiotic resistance profiles. Using an extraction kit specifically designed for larger plasmids, isolates were analyzed in order to form a more complete picture of the Inc plasmids found in the *E. coli* strains.
- Moving forward these plasmids can now be further analyzed to determine what specific antibiotic resistance gene each plasmid carries. This study will advance our knowledge concerning the development and dissemination of multiple antimicrobial resistance among *Salmonella* spp. and other enteric bacteria in nature.

PCR Program

- *95˚C → 5 min*
- *95˚C → 1 min ----*
- *60˚C → 1 min X35*
- *72˚C → 2 min ----*
- *4 ˚C → Hold*

Results

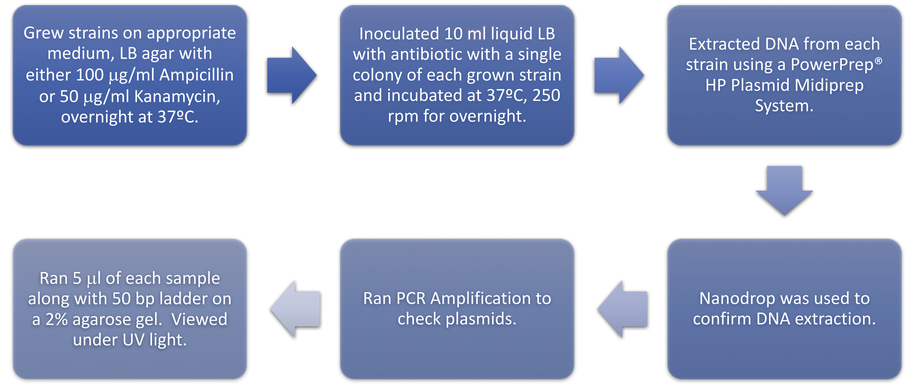
Table 1 Plasmid comparison of *E. coli* strain DH10B

Strain Number	Parent Strain Plasmids	A/C	FIB	FII	HI2	I1	P
328	A/C2, FIB, FII	X	X				
329	FII						
337	A/C2, FIB, FII	X					
338	A/C2, HI2, I1						
441	A/C2, HI2, I1	X			X	X	
442	A/C2, FII, I1, HI2	X		X	X	X	
443	A/C2	X					
444	A/C2, I2, FIB, FII, P	X	X	X		X	X
445	A/C2, HI2, I1	X					
447	A/C2, FIB, FII	X					
448	A/C2, I1	X					
449	A/C2, FIB, FII	X	X	X			
450	A/C2, FIB, FII	X	X	X			
451	I1					X	
452	A/C2, I1	X				X	
483	A/C2, HI2, I1	X			X		
484	A/C2, HI2, I1	X					
486	A/C2, HI2, I2	X					
487	A/C2, FIB, FII	X	X	X			
488	A/C2, FIB, FII	X					X
489	A/C2, HI2, I1	X			X		
490	A/C2, HI2, I1	X			X		
491	A/C2, HI2, I1	X			X		
492	A/C2, FIB, FII						
493	A/C2, FIB, FII	X	X	X			
494	A/C2, FIB, FII						
495	A/C2, HI2, I1	X			X	X	
496	A/C2, FIB, FII						
497	A/C2, I1	X				X	
498	A/C2, I2	X			X		
500	A/C2, FII, I1, HI2	X		X	X	X	

Table 2 Plasmid comparison of *E. coli* strain DH5α

Strain Number	Parent Strain Plasmids	A/C	FIB	FII	HI2	I1	P
330	FII						
331	A/C2, FIB, FII	X	X				
499	A/C2,HI2, I1	X				X	
501	A/C2, FIB FII, P	X	X	X			X
502	A/C2,HI2, I1	X					
503	A/C2, FIB, FII	X					
504	A/C2, I1	X					
505	A/C2, FIb, FII	X					
506	A/C2, FIb, FII	X	X				
507	I1					X	
508	A/C2, I1	X				X	
510	A/C2, FIB, FII						
511	A/C2, I1	X					
512	A/C2, FIB, FII	X					
513	A/C, FIB, P, I2, FII	X	X	X			

Methods and Materials



Conclusion and Discussion

1. Two former students using Qiagen® plasmid extraction kit designed for normal size plasmids had previously isolated a few Inc plasmids from *E. coli* DH10B and DH5α strains. In this study, we decided to use a plasmid purification kit designed for larger plasmids. For the DH10B strain, 15/31 strains were found to have additional plasmids. For the DH5α strain, 5/15 strains were found to have additional plasmids. Now we have a more complete picture of the Inc plasmids that were transferred from *Salmonella* clinical isolates to two laboratory *E. coli* strains via transformation.
2. Bacteria require a lot of energy to take up and keep a large plasmid. If the plasmid is not needed it will either not be taken up or be discarded. Thus, the *E. coli* strains that only picked up a few of the original *Salmonella* plasmids were not under enough of a selective pressure to maintain all of the plasmids.
3. Moving forward these plasmids can be further classified to determine the specific resistance genes carried. In the future, further conjugation studies can help advance our understanding of horizontal transfer between multiple species of bacteria. This is important as antibiotic use in agriculture and hospital environments could create opportunities in the soil and gut for the transfer of multidrug resistant plasmids.

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