

Project Title:	Determination of Factors Affecting Frequency of Conjugative Transfer of Antimicrobial Resistance and Virulence
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Background

E. coli and *Salmonella* are a concern to the food production industry. Both possess combination of antimicrobial resistance and virulence genes that act in concert with undefined host factors, to gain an advantage over beneficial bacteria. In order to understand factors involved in the dissemination of multi-drug resistance (MDR) among *E. coli* and *Salmonella* it is necessary to study the relationship between antimicrobial resistance and disease-causing genes, as well as their ability to transfer between bacteria. The relationship of other host factors must also be considered and examined.

The objectives of this study were to: 1) Evaluate the association between multiple-drug resistance (MDR) and virulence in *E. coli* and *Salmonella*, 2) Evaluate compartment proximity on conjugative frequency; and 3) Effect of pH and dissociated organic acids on conjugative frequency.

Methodology

One hundred each of 1) Multiple-drug resistant *Salmonella* (MDRS); 2) Multiple-drug resistant *E. coli* (MDRE); 3) pansusceptible *Salmonella* (PSS); 4) pansusceptible *E. coli* (PSE) from beef and dairy cattle were pheno- and genotypically characterized for content of antimicrobial resistance and virulence genes. Conjugation and transfer of the resistance and virulence genes were evaluated. All MDR isolates were used as potential gene donors capable of transfer of resistance and virulence genes and all PS isolates as potential recipients of genes. To evaluate the cell proximity on the frequency of conjugative transfer, test tubes of different diameters were used in the conjugation experiments.

To investigate the effect of pH on the frequency of conjugative transfer, a donor and recipient pair were used in conjugation experiments at different pHs. This was done in a pH range similar to the bovine gut. Additionally, to evaluate dissociated organic acid toxicity found in the gastrointestinal tract the same donor and recipient pair were used in conjugation experiments with different mM concentrations of acetic acid, propionic acid and butyric acid.

Findings

First, the pansusceptible *E. coli* and *Salmonella* possessed higher numbers of virulence genes the multi-drug resistant isolates did and had a higher incidence of being conjugative (being able to receive resistance plasmids) than the MDR *E. coli* and *Salmonella* did of transferring resistance plasmids. Second, the potential diameter of the gastrointestinal tract may play a role in the ability of bacteria to conjugate and transfer resistance genes,

Implications

The use of antibiotics provides a selective advantage for MDRE and MDRS bacteria. Additionally, isolates that possess virulence genes may also have a selection advantage.

Therefore, it is possible that the PSS isolates with virulence traits may be present in the gut simultaneously with the MDR isolates. This might increase the likelihood that virulent strains may have a greater opportunity to acquire resistance than harmless commensals. The results show that a small diameter may be a benefit with regard to cattle in that they have a considerably larger intestinal tract than other species.

Table 1. Incidence of *E. coli* Virulence Genes

Virulence genes	stx1 (n=)	stx2 (n=)	eaeA (n=)	hlyA (n=)	saa (n=)
MDRE n=100	3	8	8	2	0
trMDRE n=27	0	0	0	0	0
PSE n=100	9	11	40	41	0

trPSE was the same as PSE

Table 2. Incidence of *Salmonella* Virulence Genes

Virulence genes	invA (n=)	spvA (n=)	rck (n=)
MDRS n=100	96	10	5
trMDRS n=27	0	1	1
PSS n=100	100	27	1

trPSE was the same as PSE

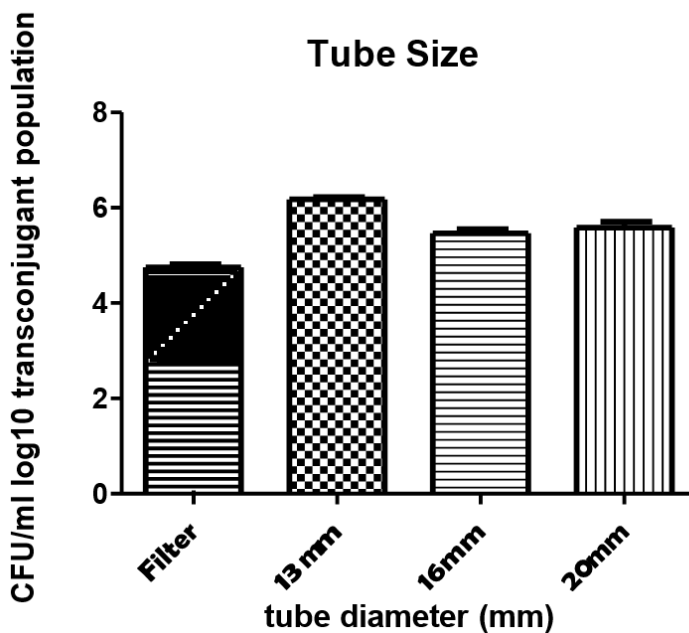


Figure 1. Tube size.