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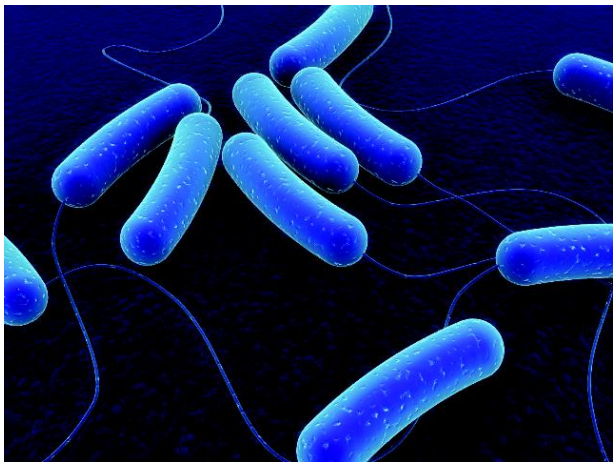
Persistence and control of pathogenic *Escherichia coli* in the primary production environment



Key external stakeholders:

Farmers, public health professionals, regulators and policy makers

Practical implications for stakeholders:

- *E. coli,* including Shiga toxin producing *E. coli* (STEC), can produce biofilm which may provide protection and support longer term survival in the environment.
- Phenotypic traits such as biofilm formation were observed to be strain dependent. This needs to be considered to ensure representative strain sets are utilised in experimental studies.
- Whole genome sequencing was able to identify clusters of STEC O157 strains, providing the highest resolution in discriminating even highly related strains.

Main results:

- Whilst the greatest level of biofilm formation was observed in soil derived *E. coli,* in general biofilm formation potential was strain dependent and not linked to pathogroup.
- The *E. coli* strain banks tested showed increased adhesion to surfaces in the presence of collagen-I.
- Genomic differences were observed between STEC strains but were not sufficient to differentiate isolates from low-shedding or super-shedding cattle.

Opportunity / Benefit: T

he scientific knowledge and information generated by this project provides a much greater understanding of the phenotypic and genotypic diversity of *E. coli* and STEC in particular. This can be employed in molecular risk assessments and in the development of targeted interventions.

Collaborating Institutions:

University College Dublin, INRA

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1. Project background

E. coli are a highly diverse group of bacteria both genotypically and phenotypically; ranging from commensal strains which colonise the human intestine, environmental *E. coli* that can be isolated from soil to Shiga toxin producing *E. coli* (STEC). STEC, the most well-known of which is *E. coli* O157 is a group of *E. coli* which can cause severe foodborne disease. It is known that some strains of *E. coli*, including STEC, can persist long term in the environment, sometimes for years. However, little is known about the phenotypic factors which influence this persistence and quite often phenotypic studies are only done on a limited strain set.

Cattle are the predominant reservoir for STEC and shed it in their faeces. Some cattle shed very high levels of the pathogen (>10^4 CFU/ g faeces) and are termed super shedders. Such animals can lead to increased transmission of the pathogen on farm and into the food chain. However there is limited understanding of the factors responsible for super-shedding, with one element thought to be genotypic traits of the strain itself.

A greater understanding of the factors which contribute to *E. coli* persistence in the primary production environment and to the super-shedding phenomenon would support the development of strategic measures for reducing STEC transmission in the farm to fork chain.

2. Questions addressed by the project:

- Do *E. coli* from various pathogroups have different phenotypic traits which may contribute to extended survival in the primary production environment?
- Do STEC isolates obtained from low and high shedding cattle have different phenotypic traits which may influence shedding rates?
- Using whole genome sequencing can differences be identified between STEC isolates from super-shedding and low-shedding cattle?

3. The experimental studies:

- In order to assess phenotypic variation between different *E. coli* pathogroups a bank of strains consisted of isolates from four different backgrounds; soil persistent *E. coli*, clinical STEC, bovine STEC and a set of antimicrobial resistant strains isolated from a food production setting were assessed. This bank of strains was examined for various phenotypic traits that have been associated with biofilm formation and adhesion and which may contribute to longer term survival. Congo red agar was used to test for the ability to produce curli and cellulose and the ability to form a pellicle at the air/broth interface was also assessed. Biofilm formation on biotic and abiotic surfaces was also examined.
- A bank of *E. coli* O157 isolates obtained from super-shedding and low-shedding animals were compared were examined for phenotypic traits associated with biofilm formation and adhesion. The isolates were also compared by whole genome sequencing.

Main results:

- Curli production was much more prominent in the soil *E. coli* and AMR strains whereas little or no curli production was seen in the STEC strains. The amount of biofilm produced by soil *E. coli* strains

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was significantly greater at 20°C than 37°C, and the soil *E. coli* strains produced greater quantities of biofilm than the other groups at 20°C.

- Bacterial adhesion to collagen-I varied from strain to strain but in general the bacteria adhered at a greater rate to the collagen-I coated wells when compared with adhesion to the polystyrene wells.

- In the shedding isolates study curli production was rare at 20°C but was observed at low levels at 37°C and the strains were also observed to form weak to moderate levels of biofilm and showed greater adhesion to biotic surfaces. The biofilm-forming abilities of *E. coli* O157:H7 isolated from super-shedding and low-shedding cattle were strain dependent and not linked to shedding status.

- Genomic differences were observed between strains but were not sufficient to differentiate isolates from low-shedding or super-shedding cattle (Figure 1).

- Clustering of isolates from cattle from the same farm was observed. However, clusters of isolates from disparate geographical locations were also observed.

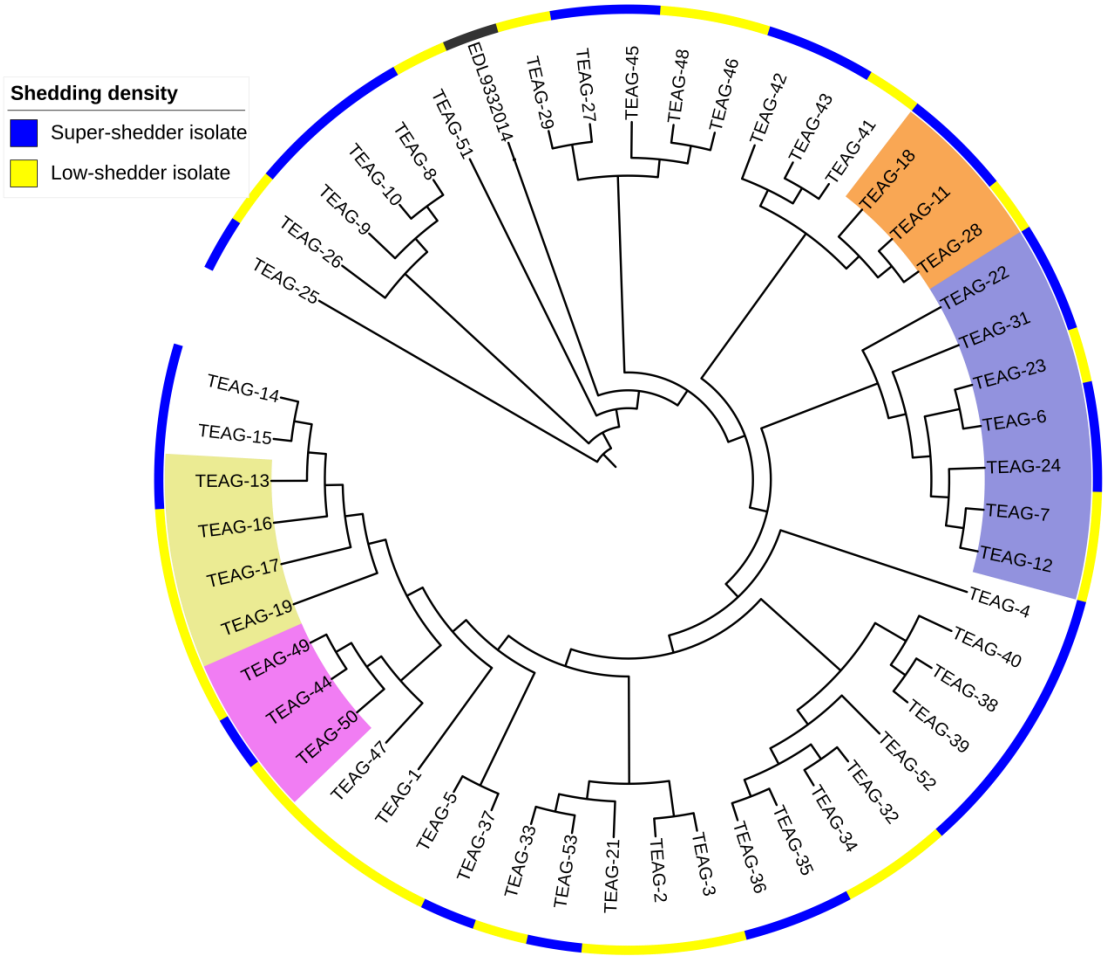

Figure 1: Maximum-likelihood phylogenetic tree of STEC O157 strains. Super-shedding isolates are indicated in blue and low-shedding isolates are indicated in yellow. Strains which clustered in groups of 3 or more via cgMLST analysis are represented in shaded regions.

4. Opportunity/Benefit:

This study demonstrated that in the bank of *E. coli* isolates examined, phenotypic traits including biofilm formation and adhesion were, in general, strain dependent and not linked to pathogroup or shedding status. Whilst moderate, many strains were able to form biofilm which may provide protection from cleaning regimes/environmental stresses and enhance survival in adverse conditions. Many strains also showed the

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ability to adhere to collagen-I, which may influence adherence to meat surfaces.

Advances in sequencing technology have provided an ability to type strains in much greater detail and show phylogenetic linkages, as well as exploring individual strains' virulence gene profiles in much greater detail. This will support outbreak investigations and provide a greater understanding of the transmission of this pathogen of significant public health concern.

5. Dissemination:

Burgess C, Gray, J., Rani, S., Duffy, G., Fanning, S. Investigating factors influencing *E. coli* strains' persistence in the primary production environment. Microbiology Society Annual Conference, Birmingham April 2018.

Gray, J., Rani, S., Duffy, G., Fanning, S. and Burgess, C. Analysis of the phenotypic traits which may impact long term survival of different *Escherichia coli* pathotypes. Presented at IFSTI, 47th Annual Food Science and Technology Conference, UCC, Cork. 6th-7th December, 2018.

Gray, J., Rani, S., Duffy, G., Fanning, S. and Burgess, C. Analysis of phenotypic traits which may impact long term survival of different *Escherichia coli* pathotypes. Presented at Microbiology Society Annual Conference 2019, ICC Belfast, UK, 8th-11th April, 2019.

Gray, J., Duffy, G., Fanning, S. and Burgess, C. Comparison of the phenotypic traits of *E. coli* O157 isolates from super shedder and low shedder cattle. Presented at One Health EJP ASM 2019 Conference, Teagasc Conference Centre, Ashtown, Dublin.22nd – 24th May, 2019.

6. Compiled by: Kaye Burgess

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