Molecular characterisation of Shiga-toxin producing Escherichia coli strains isolated from sheep presented for slaughter in Ireland using whole-genome sequencing

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INTRODUCTION

Shiga toxin-producing Escherichia coli (STEC) are foodborne zoonotic pathogens of serious public health concern that are characterised by the onset of intestinal disorders including diarrhoea and haemolytic uraemic syndrome. Sheep are recognised STEC reservoirs and can shed variable numbers of the pathogen via faecal excretion. Historically, an associated serogroup was considered indicative of strain pathogenicity, but recent scientific opinion has suggested this to be a poor indicator of clinical outcome. A molecular approach to strain evaluation, comprising a thorough characterisation of the virulence determinants, is now considered to provide a more favourable prediction of a strain's pathogenic potential. The primary determinants of bacterial virulence among STEC are the bacteriophage-encoded Shiga toxins (stx) and the intimin adhesion protein-encoding gene (eaeA). A range of additional virulence factors is also encoded on mobile genetic elements. The zoonotic potential posed by STEC circulating in sheep to the food production chain may be evaluated by in-depth characterisation of the hitherto undescribed virulence determinants circulating within strains isolated from this source.

METHODOLOGY

One hundred and seventy-eight (N=178) STEC isolated from Irish sheep were subjected to paired-end whole-genome sequencing using the Illumina MiSeq platform. Raw reads were quality assessed using FastQC (v0.11.9) and trimmed with Fastp (v0.20.1). Trimmed, paired reads were de novo assembled using SPAdes (v3.14.1) and assemblies were quality assessed by QUAST (v5.0.2). Assemblies were filtered to include contigs > 500 bp in size with an average N50 of 163 kbp.

Genome annotation was carried out using a combination of the ABRIcate tool (v1.0.1) and Prokka (v1.24.6). Assembled genomes were serotyped using the EcOH database. Intimin and enterohaemolysin subtyping was performed using the ecoli_vf database. A custom ABRIcate database was created to characterise Shiga toxin, OI-islands and nle gene distribution among strains. Multi-locus sequence typing was performed in silico using the mlst tool (v2.19.0) and the Achtmann sequence typing scheme. Strains were assigned to

one of seven phylogroups using the in silico ClermonTyping tool (v1.4.0). For all analyses, a minimum sequence identity parameter of 95 % and a minimum coverage of 80 % was specified.

RESULTS

Eight stx gene variants were reported, two stx1 and six stx2, while four variant subtypes were reported for stx1 and twelve for stx2, respectively. The most prevalent subtypes were stx1c and stx2b, which were harboured by 66 % and 62 % of strains, respectively. Further, among the observed stx gene profiles, strains harbouring both variants occurred most frequently (30 %), with a slightly smaller percentage (29 %) carrying just stx1c. Three novel Shiga toxin subunit combinations have been reported for the first time.

Thirty-five different STEC serotypes were identified. The most prevalent serotypes were O91:H14 and O128:H2, which occurred at a frequency of 19 % and 18 %, respectively. To the best of our knowledge, fifteen other serotypes were isolated for the first time in sheep.

In total, four STEC strains harboured eaeA and 123 carried hlyA. The ecp operon and fepC were ubiquitously distributed among strains. Other important virulence factors, including cah, hcp, ompT, agn43, tia, senB and espL2, were also prevalent among eaeA-negative strains. Strains harbouring eaeA additionally carried a T3SS, a selection of genes from OI-122, OI-71, OI-57 and OI-36, as well as a number of nle genes.

DISCUSSION

This study observed a wide variety of STEC serotypes and Shiga toxin variants circulating within Irish sheep. The isolates were composed of a variety of non-O157 STEC, whose prevalence and contribution to human disease have been hitherto underestimated for many years. Genome sequencing highlighted the range of Shiga toxin variants and variant subtypes harboured among the strain collection, some of which are considered of high clinical importance. Moreover, many strains additionally carried a range of important adherence and agglutinating factors, as well as genes involved in cell metabolism and survival, indicative of their ability to effectively colonise the host gastrointestinal tract. Importantly, strains harbouring eaeA carried several auxiliary virulence determinants, many of which have been previously isolated from clinical strains associated with serious illness, emphasising their clinical potential. Given the limited information on the diversity of STEC shed by sheep in Ireland, this study highlights the zoonotic potential posed to the food production chain by this ruminant host.