



## Emergence and genetic heterogeneity of STEC O113:H4: insights from whole-genome sequences of isolates across human and non-human sources

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### ARTICLE INFO

#### Keywords:

Shiga toxin-producing *Escherichia coli*  
STEC  
O113  
Virulence  
Reservoir

### ABSTRACT

The increased detection of Shiga toxin-producing *Escherichia coli* (STEC) O113:H4 among human cases in Belgium questions the importance of this serotype as an emerging pathogen. However, detailed information focusing on serotype O113:H4 from human and non-human sources remains limited. We analysed a collection of 140 STEC O113:H4 isolates and their whole genomes, originating from animal hosts (cattle, deer, goats, and sheep), food, and humans, to determine their genetic relationship and assess key virulence genes. All STEC O113:H4 genomes lacked the locus of enterocyte effacement (LEE) and belonged to Pasteur Sequence Type (pST) 367 complex, dominated by pST367 (*ehxA*<sup>-</sup>, *stx*<sub>2d</sub>) and pST1729 (*ehxA*<sup>+</sup>, *stx*<sub>2b</sub>). Compared to *stx*<sub>2d</sub> isolates, *stx*<sub>2b</sub> isolates carried on median more virulence factors, which might thus contribute to enhanced pathogenicity. Besides, humans appear to be infected with distinct subgroups of STEC O113:H4 carrying distinct *stx* subtypes and originating from potentially different sources: deer, goats, and sheep for STEC carrying *stx*<sub>2b</sub> (alone or in combination with *stx*<sub>1c</sub>) and mainly cattle for STEC carrying *stx*<sub>2d</sub>. Our results call for improved understanding and continuous surveillance of emerging STEC O113:H4.

### 1. Introduction

Globally, Shiga toxin-producing *Escherichia coli* (STEC) of serogroup O113 is considered as one of the relevant non-O157 serogroups related to foodborne illnesses (ECDC, 2025; Monaghan et al., 2012). Within this serogroup, particular attention has been given to serotype O113:H21 as this serotype has been associated with severe human disease including the haemolytic uraemic syndrome (HUS) (Allué-Guardia et al., 2022). Nevertheless, STEC O113:H4 infections with a high virulence potential

have also been reported (Jenkins et al., 2003; Vanesse et al., 2023; present study). Also, in 2023, serotype O113:H4 was among the ten most common STEC serotypes reported in the European Union/European Economic Area (EU/EEA) (data from 18 EU/EEA countries) (ECDC, 2025; Monaghan et al., 2012).

In the Netherlands, O113:H4 (n = 79) appears to be the second most common serotype of all STEC isolates (n = 1527) in the Wageningen Food Safety Research BioBank, based on samples collected by the Netherlands Food and Consumer Product Safety Authority (NVWA)

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<https://doi.org/10.1016/j.ijmm.2025.151688>

Received 17 July 2025; Received in revised form 25 November 2025; Accepted 25 November 2025

Available online 26 November 2025

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through Dutch Food Monitoring and Animal Surveillance Programs between 2017 and 2023 (Litjens and In 't Veld, 2024). Besides food, STEC O113:H4 has been isolated from domestic animals such as cattle (Feng et al., 2017; Monaghan et al., 2012), goat (Schilling et al., 2012; van Hoek et al., 2023) and sheep (Schilling et al., 2012), and from wild animals such as deer (Askari Badouei et al., 2023; Lauzi et al., 2022) and wild birds (Carter et al., 2023).

Phylogenetic studies of Irish O113:H4 isolates (n = 39) based on the commonly used Achtman Multilocus Sequence Type (MLST) scheme showed that all belonged to Sequence Type (ST) 10 and, thus, were within the same ST complex (Cplx), ST10 Cplx (Monaghan et al., 2012), which corresponds to Pasteur ST (pST) 367 Cplx in the MLST Pasteur scheme. In line with this, STEC O113:H4 of ST10 has been reported in various other European countries (Nouws et al., 2023; Werber et al., 2008). Yet, detailed information focusing on serotype O113:H4 from human and non-human sources is limited in the literature.

STEC are characterized by the presence of Shiga toxin genes (*stx*<sub>1</sub> and/or *stx*<sub>2</sub> subtypes, i.e. 1a, 1c-1e, 2a-2o) (Lindsey et al., 2023). The toxin encoded by the *stx*<sub>2</sub> gene, and mainly *stx*<sub>2a</sub> and *stx*<sub>2d</sub>, is a major virulence factor associated with the development of severe symptoms including HUS (De Rauw et al., 2018). Outside of humans, various *stx* subtypes have been found in STEC from ruminants and many different animal hosts (Persad and LeJeune, 2014; Wang et al., 2024). However, previous reports have shown that certain *stx* subtypes could be associated to specific animal reservoirs (Den Ouden et al., 2023). Briefly, STEC carrying *stx*<sub>1a</sub>, *stx*<sub>1c</sub> and *stx*<sub>2a-2e</sub> and *stx*<sub>2g</sub> have been isolated from cattle, with *stx*<sub>1a</sub> and *stx*<sub>2a</sub> predominating, *stx*<sub>1a</sub>, *stx*<sub>1c</sub> and *stx*<sub>2a-2b</sub> from sheep, with *stx*<sub>1c</sub> and *stx*<sub>2b</sub> mostly found, *stx*<sub>1a</sub>, *stx*<sub>1c</sub>, *stx*<sub>2a-2d</sub> and *stx*<sub>2k</sub> from goats, with *stx*<sub>1c</sub> mostly found and *stx*<sub>1a</sub>, *stx*<sub>1c</sub>, *stx*<sub>2a-2d</sub> and *stx*<sub>2g</sub> from deer (Wang et al., 2024). The *stx*<sub>2b</sub>, alone or in combination with *stx*<sub>1a</sub> or *stx*<sub>1a+1c</sub>, is common in wildlife, including wild ruminants, wild boars, and red deer (Alonso et al., 2017). While *stx*<sub>2e</sub>-positive STEC have a porcine reservoir (Marques et al., 1987; Wang et al., 2024), STEC harbouring *stx*<sub>2f</sub> have been detected in many different bird species (Schmidt et al., 2000).

In addition to a *stx* determinant, a subset of STEC isolates linked to human disease possesses the locus of enterocyte effacement (LEE) pathogenicity island (PAI), which contains genes encoding type III secretion system (T3SS) proteins, the adherence factor intimin (*eae*) and its translocated receptor (*tir*), as well as chaperones, regulators, and secreted effector proteins (*esp*). LEE-negative STEC are thought to possess alternative mechanisms for adherence that favour pathogenicity, such as STEC autoagglutination adhesin (encoded by *saa*) or the IrgA homologue adhesin encoded by the *iha* gene (Nuesch-Inderbinen et al., 2021). They also frequently carry subtilase cytotoxin encoding gene *subAB* (Montero et al., 2019). Remarkably, all STEC of serogroup O113 lack the LEE PAI while carriage of non-LEE genes such as *saa* (Jenkins et al., 2003), *subAB* (Allué-Guardia et al., 2022) and enterohemolysin gene *ehxA* (Feng et al., 2017; Feng et al., 2014) may possibly be serotype-specific. Genes such as *saa*, *subAB* and *ehxA* seem to be very common in O113:H21 and are often absent in O113:H4 (Allué-Guardia et al., 2022; Feng et al., 2017; Jenkins et al., 2003). The *iha* gene seems to present among all STEC of serogroup O113 (Allué-Guardia et al., 2022; Feng et al., 2017; Monaghan et al., 2012). It is to be noted that all examined O113:H4 isolates are *stx*<sub>2d</sub>-positive (*stx*<sub>2a</sub>) (with or without additional *stx*<sub>1</sub> or *stx*<sub>2</sub> subtypes) (Feng et al., 2017; Monaghan et al., 2012).

The growing number of human cases linked to STEC O113:H4 in Belgium challenges the notion of this serotype as an emerging pathogen. To characterize O113:H4 isolates circulating in Belgium, we firstly assembled a collection of 62 Belgian O113 isolates and genomes - 30 O113:H4 and 32 O113:non-H4 - from cattle, contaminated food, and humans. Secondly, to gain more insight into its clinical significance and its potential reservoir, we assembled a collection containing 110 additional O113:H4 genomes of European isolates originating from animals, contaminated food, and humans.

## 2. Materials and methods

### 2.1. Collection of STEC isolates

A collection of 172 STEC O113 isolates and genomes - 140 O113:H4 and 32 O113:non-H4 - were investigated in this study. The STEC O113:H4 isolates and genomes originated from the animal reservoir (cattle [n = 15], deer [n = 4], goats [n = 5] and sheep [n = 1]), food (n = 85), and humans (n = 30) (Supplementary Table S1). They included 30 Belgian STEC O113:H4 isolates as well as 110 publicly available O113:H4 genomes from various European countries (Supplementary Table S1).

The 30 Belgian STEC O113:H4 were isolated between 1991 and 2023, from cattle, food and humans by the Faculty of Veterinary Medicine at the University of Liège, the National Reference Laboratory for STEC (NRL STEC) at Sciensano, and the National Reference Centre for STEC (NRC STEC) at the UZ Brussel, respectively. The isolate genomes provided by the Belgian NRL STEC were already publicly available (Nouws et al., 2023).

The 110 publicly available O113:H4 genomes correspond to 90 Dutch isolates including seven from human stools, 14 from cattle faeces, five from goat faeces and 64 isolates from cattle and sheep food products, in addition to nine French human isolates, one Irish isolate from the sheep recto-anal junction, four Norwegian isolates from deer manure and one from dairy cheese, one Scottish venison isolate and four isolates from England recovered from cattle, goat and pork food products. It is to be noted that, for food products, only the country of sampling is mentioned which might differ from the country of origin of the product investigated.

The 32 available Belgian STEC O113:non-H4 (O113:H6 [n = 2] and O113:H21 [n = 30]) isolates were collected between 2001 and 2023, from cattle, food and humans (Supplementary Table S1).

### 2.2. Whole-genome sequencing (WGS) and de novo assembly

Bacterial DNA of the STEC isolates was sequenced using distinct Illumina® instruments (San Diego, CA, USA), according to the country of sampling: Belgium (MiSeq and NovaSeq6000); England (HiSeq 2500 and NextSeq 1000); France (NovaSeq 6000); Ireland (MiSeq); Norway (MiSeq and NextSeq 550); Scotland (HiSeq 2500); the Netherlands (HiSeq 2500 and NovaSeq 6000).

The raw reads of all STEC genomes were uploaded and *de novo* assembled, using SPAdes v.3.7.1, in BioNumerics v.8.1.1 (Applied Maths, BioMérieux, Sint-Martens-Latem, Belgium). Sequence quality was assessed using the quality metrics incorporated in BioNumerics. The major quality parameters are summarized in Supplementary Table S2.

### 2.3. In silico identification of genes linked to serotype and virulence

The *E. coli* genotyping tool, available in BioNumerics v.8.1.1, was used to predict *E. coli* serotypes and virulence gene profiles starting from the genome assemblies. Key virulence genes were examined (Supplementary Table S3). The presence of virulence genes was determined with a minimum % identity (ID) threshold of 85 % and a minimum length for coverage of 80 %.

An *in-silico* PCR-based knowledgebase was created for the STEC autoagglutinating adhesin (*saa*) gene using the primers of Paton and Paton (2002) in BioNumerics.

The odds ratio was estimated to investigate the association of the presence or absence of virulence-related gene with the profiles for *stx*<sub>2b</sub> and *stx*<sub>2d</sub>. The statistical significance of the odds ratio was calculated by Fisher's exact test, where the p-value was set to 0.05 to reject the hypothesis that the true odds ratio is equal to 1 (van Hoek et al., 2023).

## 2.4. Core genome MLST (cgMLST) analysis

The assembled sequencing data were analysed using the *Escherichia/Shigella* cgMLST typing scheme in BioNumerics v.8.1.1 (core Enterobase) (Applied Maths, BioMérieux, Sint-Martens-Latem, Belgium), which consists of 2513 loci as defined by Enterobase (<https://enterobase.warwick.ac.uk/>). The assembly-based BLAST approach, with default settings, was used for allele calling. The quality of the assembly-based allele calls was verified using the quality statistics window in BioNumerics. The MLST profile of each isolate was determined using both the PubMLST (Achtman) and the MLST Pasteur schemes (Wirth et al., 2006; Jauregui et al., 2008) - targeting, respectively, seven and eight different housekeeping genes - incorporated in BioNumerics. Only the latter scheme will be described in the present study. Phylogenetic dendrograms were generated from the cgMLST allelic profiles of the isolates using the categorical similarity coefficient and the Unweighted-Pair Group Method with Arithmetic Mean (UPGMA) algorithm in BioNumerics. The dendrograms were circularized and annotated using iTOL v 6.9.1 (Letunic and Bork, 2024). Minimal spanning trees (MSTs) were generated from both the MLST Pasteur and cgMLST allelic profiles of the isolates using the predefined template "MST for categorical data" in BioNumerics. Branch lengths reflect the number of allele differences (AD) between the allelic profiles of the isolates in the connected nodes.

## 2.5. Data availability

All O113 genomes are available online in the NCBI GenBank

database. The NCBI accession numbers are provided in [Supplementary Table S1](#).

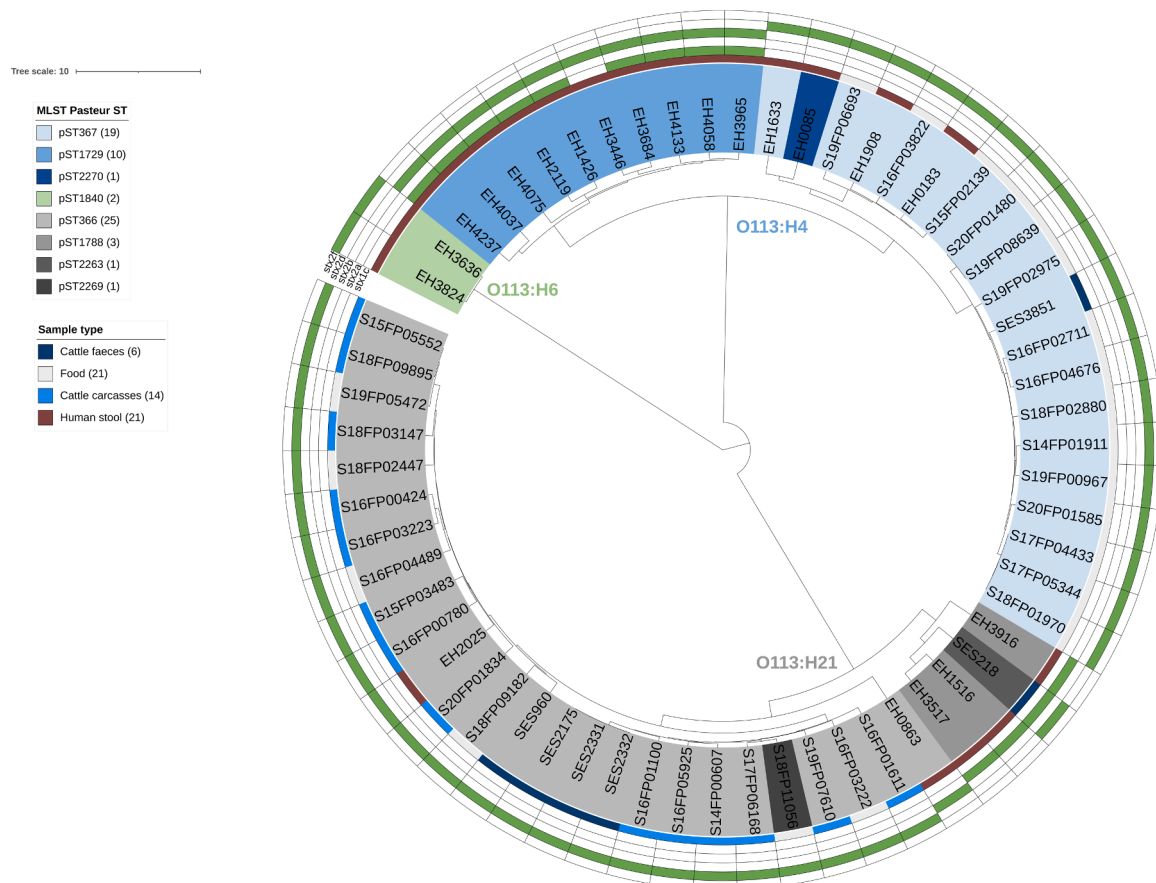
## 3. Results

### 3.1. CgMLST analysis of 62 Belgian O113 isolates and Shiga toxin genes

Based on cgMLST analysis, the 62 Belgian O113 genomes were grouped according to their H-antigen: H4 (n = 30), H6 (n = 2) and H21 (n = 30) (Fig. 1 and [Supplementary Table S4](#) and [Table S5](#)). Also, the genomes could be subdivided according to their MLST pST Cplx (Fig. 1). Indeed, the O113:H4 genomes belonged to pST367 Cplx encompassing mostly pST367 (n = 19) as well as pST1729 (n = 10) and pST2270 (n = 1), which differed by one allele - *dinB* and *trpB*, respectively - from pST367 (Fig. 1 and [Supplementary Table S6](#)). The two O113:H6 genomes belonged to pST1840 while the 30 O113:H21 belonged to pST366 Cplx: pST366 (n = 25), pST1788 (n = 3), pST2263 (n = 1) and pST2269 (n = 1) (Fig. 1 and [Supplementary Table S5](#)).

Focusing on O113:H4, the isolate genomes appear to be subdivided based on the *stx*<sub>2</sub> subtype; 2b (alone or in combination with *stx*<sub>1c</sub>) on the one hand and 2d on the other hand (similarity coefficient of 85.8 %) (Fig. 1). On top of this, isolates harbouring *stx*<sub>2b</sub> alone (n = 1) or in combination with *stx*<sub>1c</sub> (n = 9) were from human origin only while the *stx*<sub>2d</sub> isolates were obtained from all three sources (animal [n = 1], food [n = 15]) and human [n = 4]) (Fig. 1).

The majority of the O113:H21 isolates carried *stx*<sub>2d</sub> alone (n = 25) and were mainly derived from cattle faeces and carcasses. Four O113:H21 isolates harboured *stx*<sub>2a</sub> - either alone (n = 3; human origin) or in



**Fig. 1.** UPGMA phylogenetic dendrogram based on cgMLST allelic profiles of 62 Belgian STEC O113 genomes - 30 O113:H4, 2 O113:H6 and 30 O113:H21 - built from cgMLST analysis (core Enterobase, BioNumerics v.8.1.1). The dendrogram was circularized and annotated using iTOL. The five outer rings, coloured in green, relate to *stx* subtypes (from the inside to the outside: 1c, 2a, 2b, 2d and 2f). The inner ring refers to the isolate source as labelled (Sample type). Tip labels are coloured according to the MLST Pasteur Sequence Type (pST).

combination with *stx*<sub>2d</sub> (n = 1; cattle faeces) -, and one human isolate carried *stx*<sub>2b</sub>. Both O113:H6 human isolates carried *stx*<sub>2f</sub> (Fig. 1 and Supplementary Table S5).

### 3.2. Comparison of multi-country European STEC O113:H4 genomes and Shiga toxin genes

Based on the above-mentioned observations, 110 publicly available STEC O113:H4 genomes originating from multiple sources (cattle [n = 77], human [n = 16], goat [n = 6], deer [n = 5], sheep [n = 5], and pork [n = 1]) were added to the study collection. Accordingly, a total of 140 STEC O113:H4 genomes were analysed, including genomes from the Netherlands (90), Belgium (30), France (9), Norway (5), England (4), Ireland (1), and Scotland (1) (Supplementary Table S1).

As expected from the above-mentioned results, all O113:H4 genomes belonged to pST367 Cplx. Pasteur MLST clustered most of the O113:H4 genomes into two major subgroups, pST367 (*stx*<sub>1a+2d</sub>; n = 1 and *stx*<sub>2d</sub>; n = 83) and pST1729 (*stx*<sub>2b</sub>, n = 6 and *stx*<sub>1c+2b</sub>, n = 26), differing by one allele (*dinB*) (Supplementary Fig. S1 and Table S6). The 24 other O113:H4 isolates belonged to eight pSTs with pST1738 (*stx*<sub>2a</sub>; n = 1 and *stx*<sub>2d</sub>; n = 15), pST2265 (*stx*<sub>1c+2b</sub>; n = 1) and pST2267 (*stx*<sub>1c</sub>; n = 1 and *stx*<sub>1c+2b</sub>; n = 1) differing by one allele from pST1729; pST2266 (*stx*<sub>2c</sub>; n = 1) and pST2270 (*stx*<sub>2d</sub>; n = 1) differing by one allele from pST367; and pST2264 (*stx*<sub>1c+2b</sub>; n = 1), pST2268 (*stx*<sub>2d</sub>; n = 1) and pST2271 (*stx*<sub>2b</sub>; n = 1) differing by one allele from both pST367 and pST1729 (Supplementary Table S6).

The cgMLST phylogenetic tree, decorated with the defined MLST pSTs, reflected the partitioning observed in the MLST Pasteur based

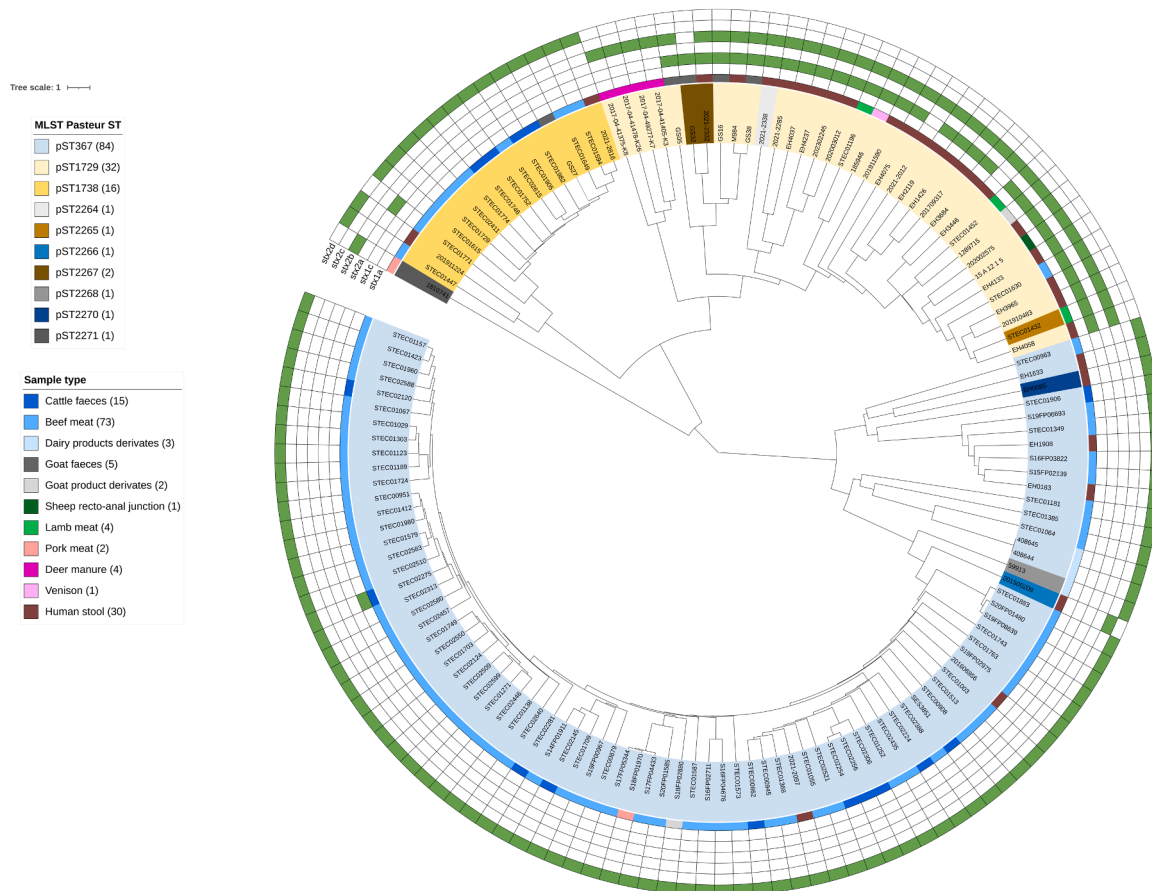
phylogenetic tree (Supplementary Fig. S1) but provided a higher resolution (Supplementary Fig. S2). Indeed, two major pST367 and pST1729 subgroups were observed, corresponding to a similarity threshold of 86.0 %. pST1738 isolates appeared to be positioned in the phylogenetic boundary between both pST367 and pST1729 subgroups (Supplementary Fig. S2).

Interestingly, subgroup pST367 included a majority of bovine (11/84) and cattle-derived food isolates (66/84) and a limited number of human isolates (5/84) (Fig. 2). The same was observed for subgroup pST1738 (Fig. 2). Subgroup pST1729, on the other hand, included mainly human isolates (19/32) and a few animal (8/32) and food isolates (5/32), which were originating from deer, caprine or ovine hosts (Fig. 2). Both pST367 and pST1738 subgroups were dominated by *stx*<sub>2d</sub> while pST1729 was dominated by *stx*<sub>2b</sub> (Fig. 2 and Supplementary Table S4).

### 3.3. Comparative analysis of virulence genes

Consistent with the literature, the virulence genes *ee*, *tir*, *espA* and *espB* carried by the LEE PAI required for intimate attachment and the formation of attaching and effacing lesions were absent in the examined O113:H4 isolates (Supplementary Table S4). Other clinically significant virulence genes encoding adherence factors (*lpfA*, *saa*), toxins (*hlyA*, *toxB*), proteases (*espP*, *ompT*) and other pathogenicity-related factors (*hlyF*, *katP*, *usp*) were also absent (Supplementary Table S4).

In contrast, multiple virulence genes were identified among the isolates including genes encoding adhesion factors (*csgA*, *yehABCD*), toxin (*hlyE*; also named cytotoxin A [*clyA*]), regulation (*terC*) and other



**Fig. 2.** UPGMA phylogenetic dendrogram based on cgMLST allelic profiles of 140 multi-country European STEC O113:H4 genomes built from cgMLST analysis (core Enterobase, BioNumerics v.8.1.1). The dendrogram was circularized and annotated using iTOL. The five outer rings, coloured in green, relate to *stx* subtypes (from the inside to the outside: 1a, 1c, 2a, 2b, 2c and 2d). The inner ring refers to the isolate source as labelled (Sample type). Tip labels are coloured according to the MLST Pasteur Sequence Type (pST).

pathogenicity-related factors (*aslA*, *gad*) (Supplementary Fig. S3 and Table S4). All but one cattle isolate harboured the adherence-mediating *fdeC* gene while all but two goat isolates harboured the adherence-mediating *iha* gene (Supplementary Fig. S3 and Table S4).

Interestingly, certain virulence genes were identified in relation to  $stx_{2b}$  – mainly in pST1729 subgroup - or  $stx_{2d}$  – mainly in pST367 or pST1738 subgroups - (Supplementary Fig. S3 and Table S4). A strict association with  $stx_{2b}$  was observed for at least thirteen genes (Supplementary Table S7). These included nine genes that were mostly present in  $stx_{2b}$ -positive ( $stx_{2b}^+$ ) isolates ( $n = 36$ ) but absent in  $stx_{2d}^+$  isolates: *cba* ( $n = 31$ ), *cma* ( $n = 31$ ), *ehxA* ( $n = 35$ ), *ireA* ( $n = 35$ ), *kpsE* ( $n = 34$ ), *kpsMII\_K5*, *senB* ( $n = 30$ ), *stx<sub>1c</sub>* ( $n = 29$ ) and *subA* ( $n = 27$ ). Remarkably, the subtilase cytotoxin gene *subA* and the plasmid-encoded enterotoxin gene *senB* were absent in the  $stx_{2b}^+$  isolates originating from deer manure as for the  $stx_{2d}^+$  isolates. Only four genes encoding heat-resistant agglutinin (*hra*), the bacteriocin microcin B17 (*mcbA*), serine protease autotransporter of *Enterobacteriaceae* (SPATE) (*espI*) and the homolog of *Shigella flexneri* SHI-2 PAI (*shIB*) were associated with the presence of  $stx_{2d}$ . Indeed, 64 out of 101  $stx_{2d}^+$  isolates contained *hra*, whereas only four out of 36  $stx_{2b}^+$  isolates – originating from deer manure - contained this gene. Similarly, *espI* was common among the  $stx_{2d}^+$  isolates ( $n = 85/101$ ) in contrast to the  $stx_{2b}^+$  isolates ( $n = 6/36$ ).

The microcin genes (*mcmA*, *mchB*, *mchC* and *mchF*) were exclusively detected in the four  $stx_{2b}$  isolates originating from deer manure, which formed a separate subcluster within pST1729 (Supplementary Fig. S3 and Table S4). The same was observed for the afimbrial adhesin genes *afaA* and *afaB* (without *afaC*) (Supplementary Fig. S3 and Table S4).

In summary,  $stx_{2b}$  isolates carried a median of 28 virulence genes compared to a median of 19 among the  $stx_{2d}^+$  isolates (Supplementary Table S8). Translating this observation to pSTs, less virulence genes were observed within pST367 (19;  $n = 84$ ), pST1738 (19;  $n = 16$ ), pST2268 (18;  $n = 1$ ) and pST2270 (17;  $n = 1$ ) as compared to pST1729 (28;  $n = 32$ ), pST2264 (28;  $n = 1$ ), pST2265 (29;  $n = 1$ ), pST2267 (27;  $n = 2$ ) and pST2271 (23;  $n = 1$ ).

#### 4. Discussion

Here, we provided genomic insights into STEC O113:H4, an emerging serotype of human clinical significance (ECDC, 2025; Monaghan et al., 2012).

In line with previous reports (Feng et al., 2017; Monaghan et al., 2012; Nouws et al., 2023), all O113:H4 isolates analysed in this study were within the same pST367 Cplx. Two major subgroups, i.e. pST367 and pST1729, - differing by one allele from each other - were identified. Eight additional pSTs were identified that correspond to single locus variants of pST367 and/or pST1729. Of note, pST1738 isolates occupied an in-between position between these two major subgroups.

Consistent with the literature, all O113:H4 isolates analysed in this study were LEE-negative (lacking *eae* and *tir*) and lacked clinically significant virulence genes such as *saa*. However, multiple virulence genes were identified among all O113:H4 isolates, independent of the associated pSTs: *aslA*, *csgA*, *gad*, *hlyE*, *terC* and *yehABCD*. Yet, this study revealed substantial variability in the composition of STEC O113:H4 virulence factors. Thirteen out of the 87 virulence genes studied were strictly associated with the presence of  $stx_{2b}$ , while only four genes were strictly associated with  $stx_{2d}$ . Consequently,  $stx_{2b}^+$  isolates carried on median more virulence factors as compared to  $stx_{2d}^+$  isolates. This might account for enhanced pathogenicity of these  $stx_{2b}^+$  isolates, compensating for the absence of the high-risk virulence genes  $stx_{2a}$  or  $stx_{2d}$ . Moreover, we identified a distinct virulence gene profile associated with either of the two major pSTs:  $ehxA^-$ ,  $stx_{2d}^+$  for pST367 and  $ehxA^+$ ,  $stx_{2b}^+$  for pST1729 isolates. This is in line with a previous study describing the absence of the enterohemolysin gene *ehxA* in the examined O113:H4 isolates carrying  $stx_{2d}$  (alone or in combination with other *stx*-subtypes such as 1a, 2a or 2c) (Feng et al., 2017).

Of note is the presence of the microcin genes (*mcmA*, *mchB*, *mchC* and

*mchF*) as well as the afimbrial adhesin genes *afaA* and *afaB* (without *afaC*) exclusively in the  $stx_{2b}$  pST1729 isolates originating from deer manure. Microcin genes (*mchB*, *mchC* and *mchF*) have already been observed in STEC O27:H30, O146:H21 and O146:H28 isolates obtained from wildlife (Dias et al., 2022). Direct contact with wildlife and their environment could therefore pose a risk to humans.

Interestingly, an association between the pST and the *stx* subtype was observed; pST367 isolates carried high risk  $stx_{2d}$  (with or without  $stx_{1a}$ ) while pST1729 isolates carried  $stx_{2b}$  (with or without  $stx_{1c}$ ), which is generally associated with mild disease although HUS cases associated to  $stx_{2b}$  STEC have been described in Europe (ECDC, 2025; Fravallo et al., 2025). Like pST367, pST1738 isolates carried mainly  $stx_{2d}$ . Based on these observations, isolates of pST367 and pST1738 belonged to the high risk group for HUS development as defined in a previous report (De Rauw et al., 2018), which means these isolates are more prone to cause HUS, while pST1729 isolates belonged to the intermediate risk group for HUS development. However, this contrasts sharply with the fact that isolates of human origin carried more frequently  $stx_{2b}$  (70.0 %; 21/30) than  $stx_{2d}$  (26.7 %; 8/30). Also, all three HUS cases, out of the 23 cases with known clinical manifestations, were associated with  $stx_{2b}$  (alone or in combination with  $stx_{1c}$ ). These observations could be explained by the higher number of virulence genes found in  $stx_{2b}^+$  isolates, possibly contributing to enhanced pathogenicity. Notably, bacteriocins (*cba*, *cia* and *cma*) could enhance intestinal implantation by successfully out-competing other *E. coli* species; toxins *ehxA*, *senB* and *subA* could influence the pathogenic potential as these genes have been implicated in the development of severe disease (Brown et al., 2023; Nuesch-Inderbinen et al., 2021); *ireA* could contribute to enhanced iron acquisition (Li et al., 2016), which is essential for bacterial survival in their infected host; and the extraintestinal pathogenic *E. coli*-associated *kps* genes (*kpsE* and *kpsMIII*), encoding capsule transport proteins, could facilitate resistance against host immune defences (Zong et al., 2016).

Remarkably, this study also revealed that humans appeared to be infected with distinct subgroups of STEC O113:H4, and that these could originate from different potential sources: deer, goats and sheep for strains carrying  $stx_{2b}$  (alone or in combination with  $stx_{1c}$ ) and mainly cattle for strains carrying  $stx_{2d}$ .

Taken together, our findings call for improved understanding and continuous surveillance of emerging STEC of serotype O113:H4. Subgroups of this serotype exhibit the capacity for severe disease. A broad range of ruminants serve as potential reservoirs of human infection.

#### Funding

Part of this work was performed in the frame of the Belgian National Reference Centre for Shiga toxin-producing *Escherichia coli* supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System. The isolation and sequencing of the deer isolates from Norway were part of DiSCoVeR, a project in the One Health European Joint Programme (EJP) receiving funding from EU's Horizon 2020 Research and innovation Programme (Grant Agreement No 773820).

#### Ethical statement

The isolates of human origin that were used in this study were all collected in the frame of routine public health surveillance from faecal samples. No additional testing was performed on any of these faecal samples. As no additional sampling or information was asked from patients, no formal approval from an ethical committee or informed consents were needed.

#### CRedit authorship contribution statement

**Joost Stassen:** Writing – review & editing, Resources. **Crombé Florence:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Formal analysis. **Ralph Litjens:**

Writing – review & editing, Resources. **van Hoek Angela H. A. M.:** Writing – review & editing, Writing – original draft, Resources, Formal analysis. **Denis Piérard:** Writing – review & editing, Writing – original draft, Supervision. **Frederic Auvray:** Writing – review & editing, Writing – original draft, Formal analysis. **De Keersmaecker Sigrîd C. J.:** Writing – review & editing, Resources. **Bavo Verhaegen:** Writing – review & editing, Resources. **Carolina Silva Nodari:** Writing – review & editing, Resources. **Jacques Mainil:** Writing – review & editing, Resources. **Aurélie Cointe:** Writing – review & editing, Resources. **Gro S. Johannessen:** Writing – review & editing, Resources. **Caroline Willis:** Writing – review & editing, Resources.

## Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose. The authors declare no conflict of interest.

## Acknowledgements

We would like to thank the scientists and technicians of the Brussels Interuniversity Genomics High Throughput core (BRIGHTcore; [www.brightcore.be](http://www.brightcore.be)). We would like to thank former and current technicians for their contribution to the work on STEC O113:H4 and O113:H21 (in alphabetical order): Johan Breynaert, Bart De Cock, Jean-Noël Duprez, Linda Godau, Iris Ottoy and Anita Wyns. We thank the Institut Pasteur teams for the curation and maintenance of BIGSdb-Pasteur databases at <http://bigsdb.pasteur.fr/>.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ijmm.2025.151688](https://doi.org/10.1016/j.ijmm.2025.151688).

## Data availability

Sequence data analysed in this study can be found under the BioProject numbers provided in [Supplementary Table S1](#).

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