**Supplementary Materials:**

**Supplementary Table S1.** Statens Serum Institut strain collection of reference strains harboring the *stx* gene subtypes, their O:H serotype, additional virulence genes, references and identification numbers.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SSI collection D number | Strain  [reference] | Control for toxin subtype | Toxin variant designation | GenBank  accession No. | Results obtained using the subtyping method in [4] | Serotype | Additional virulence genes |
| D2653 1 | EDL933 [30] | Stx1a | Stx1a-O157-EDL933 | M19473 | *stx1a + stx2a* | O157:H7 | *eae, ehxA, astA* |
| D3602 | DG131/3 [31,32] | Stx1c | Stx1c-O174-DG131-3 | Z36901 | *stx1c + stx2b* | O174:K101:H8 |  |
| D3522 | MHI813 [33] | Stx1d | Stx1d-O8-MHI813 | AY170851 | *stx1d* | O8:K85ab:Hrough |  |
| D26531 | EDL933 [30] | Stx2a | Stx2a-O157-EDL933 | X07865 | *stx1a + stx2a* | O157:H7 | *eae, ehxA, astA* |
| D3428 | EH250 [34] | Stx2b | Stx2b-O118-EH250 | AF043627 | *stx2b* | O118:K?:H12 | *astA* |
| D2587 2 | 031 [35] | Stx2c | Stx2c-O174-031 | L11079 | *stx2c* + *stx2b* | O174:K101:H21 |  |
| D3435 3 | C165-02 [36] | Stx2d | Stx2d-O73-C165-02 | DQ059012 | *stx2d* | O73:H18 | *astA* |
| D3648 | S1191 [37] | Stx2e | Stx2e-O139-S1191 | M21534 | *stx2e* | O139:K12:H1 |  |
| D3546 | T4/97 [38] | Stx2f | Stx2f-O128-T4-97 | AJ010730 | *stx2f* | O128ac:[H2] | *eae, bfpA, astA* |
| D3509 | 7v [39] | Stx2g | Stx2g-O2-7v | AY286000 | *stx2g* | O2:K-:H25 | *ehxA, astA, estap* |
| D6061 | STEC299 [5] | Stx2h | Stx2h-O102-STEC299 | CP022279 | *stx2* | O102:K+:H18 | *paa*, *kpsM*II\_K5 |
| D6205A | CB10366 [6] | Stx2i | Stx2i-O9-CB10366 | FN252457 | *stx2* | O9:K39:[H4] | *estb* |
| D6283 | OLC-1685 [1] | Stx2j | Stx2j-O158-OLC-1685 | MZ229608 | *stx2* | O158:H23 |  |
| D6068 | 12GZSW01 [40] | Stx2k | Stx2k-O159-12GZSW01 | KC339670 | *stx2* | O159:K?:H16 | *astA*, *estap*, *estb* |
| D7138 | 1610T27873 [6] | Stx2l | Stx2l-O65-1610T27873 | AM904726 4 | *stx2a* + *stx2d* | O65:H16 | *hlyE*, *lpfA*, *shiB*, *tia* |
| D6276 | 2001F31428 [2] | Stx2m | Stx2m-O96-2001F31428 | OQ054797 | *stx2* | O96:H19 |  |
| D6924 | 2017C-43175 | Stx2n | Stx2n-O23-2017C-4317 | CP113092 | *stx2* | O23:K-:H15 | *chuA*, *kpsE*, *eilA, traT*, *kpsMII\_K5*, *sitA*, |
| D6282 | 03-3638 [1] | Stx2o | Stx2o-O85-03-3638 | MZ229604 | *stx2* | O85:H1 |  |

1 This strain is used for both Stx1a and Stx2a

2 This strain has been replaced by D3431, serotype O157:H7, *stx2c,* *eae, ehxA, astA*

3 May result in both fragments at 179 bp and 280 bp; This strain has been replaced by D4134, serotype O166:H15, *stx2d*; ESBL producing.

4 This is the original Acc. No. for strain FHI 1106-1092 of serotype O8:H2. The presence of the *stx2l* gene is however very unstable and a similar Danish isolate is used as the reference strain for Stx2l.

5Stx2n strain is from this study.

**Supplementary Figure S1**. Location of pro-phages identified by Phaster (<https://phaster.ca>) in the chromosome of the strains carrying novel stx2 types (stx2n and stx2o) reported in this study. The stx2 pro-phages regions are highlighted for each strain.

Diagram

Description automatically generated