Outbreak and genomic Analysis of Shiga-Toxin–Producing EA-*E. coli* O104:H4, 2011

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Hydrid pathotypes

Several types of hybrid pathotypes have been reported:

- STEC/ETEC Hybrids - strains containing Shiga toxin genes (*stx*) together with enterotoxin genes from ETEC (e.g., ST/LT)
  - Taiwan porcine hybrid STEC/ETEC, carrying stx2e + ST + LT genes.
  - Human clinical isolates in Sweden, with rare stx2 subtypes and multiple ST toxins
- STEC/EAEC Hybrids - EAEC strain that acquired Shiga toxin 2 (stx2a)
  - German O104:H4 outbreak strain 2011
- EAEC/UPEC Hybrids
  - Strains that combine EAEC virulence factors (e.g., *aatA, aggR*) with uropathogenic *E. coli* (UPEC) markers (e.g., *fyuA, chuA, pap*, iron acquisition genes)
- Triple-Hybrid DEC Combinations (EPEC + ETEC + DAEC)
  - bfpA (EPEC), staI (ETEC), daaE (DAEC)

Outbreak – short story

From May to July 2011, in Northern Germany

- large outbreak of illnesses characterized by haemolytic-uremic syndrome (HUS) and bloody diarrhea
- associated with infections by enterohemorrhagic *Escherichia coli* (EHEC) of the serotype O104:H4

- Outbreak began at the beginning of May 2011 and reached its peak on 22 May 2011
- Since mid-June, only sporadic cases of HUS occurred
- a total of 855 cases of HUS and almost 3000 cases of acute gastroenteritis were attributed to the outbreak
- more women than men were identified with HUS (68%) and EHEC (58%)
- the majority of cases involved adults

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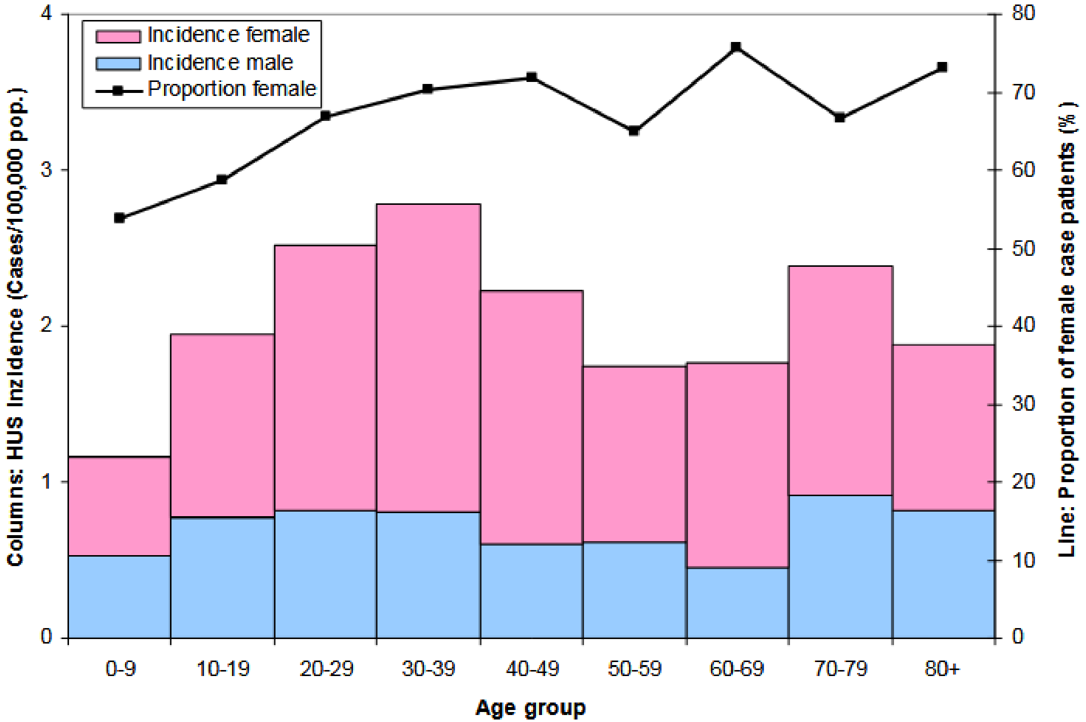
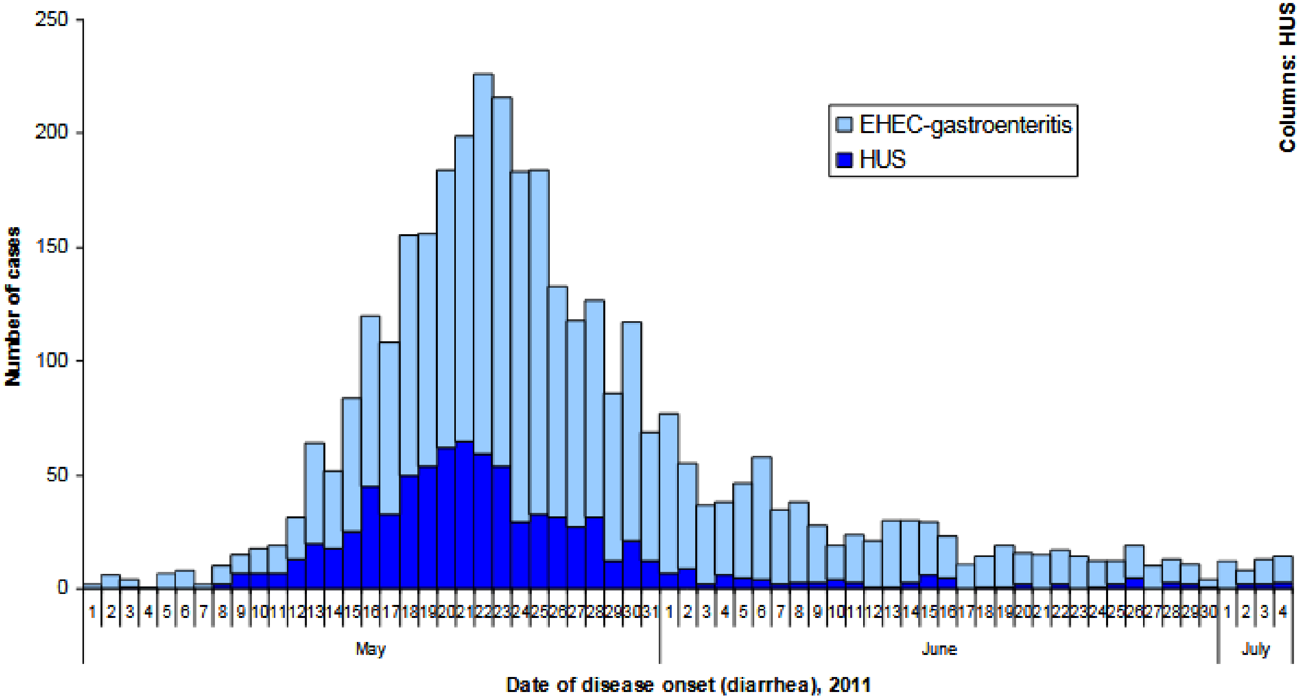
Outbreak – short story

Uncommon:
- size of the outbreak
- most female cases, mostly adults
- food source
- Exceptionally high rate of HUS





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Uncommon:
- STEC serotype (O104:H4)
- AMR profile (ESBL)
- Virulence profile (stx + EAEC pAA plasmid)

Epidemiological investigations

- Case reporting
  - Decentralised public health system – longer reaction time
  - Uncommon serotype – delay in ID
- Food source
  - Interviews were inconlusive
  - Indications of different vegetables
  - Epidemiological investigation consisted of a case-control study involving 26 adults hospitalized with HUS. Univariate analysis linked only the consumption of sprouts with disease
    - But only one quarter of the interviewed had responded eating sprouts
  - Fenugreek seed sprouts
  - Human-to-human transmission

Open-source genomic analysis

An open-source genomic analysis of one outbreak was initiated

This analysis involved
- use of rapid, bench-top DNA sequencing technology
- open-source data release
- prompt crowd-sourced analyses

In less than a week, these studies revealed that the outbreak strain belonged to an enteroaggregative *E. coli* lineage that had acquired genes for Shiga toxin (*stx2*) and for antibiotic resistance (ESBL –CTX-M-15)

Virulence factors

The outbreak strains possessed two different mobile elements, a phage and a plasmid, contributing essential virulence genes

- some EAEC virulence factors on the virulence plasmid pAA
- the epidemic strains acquired a lambdoid prophage carrying the gene for the Shiga toxin
- Additional plasmid carrying ESBL resistance

Genomic overview

- In-depth analysis of where the virulence facters are located
- Indication of how this hybrid pathotype developed

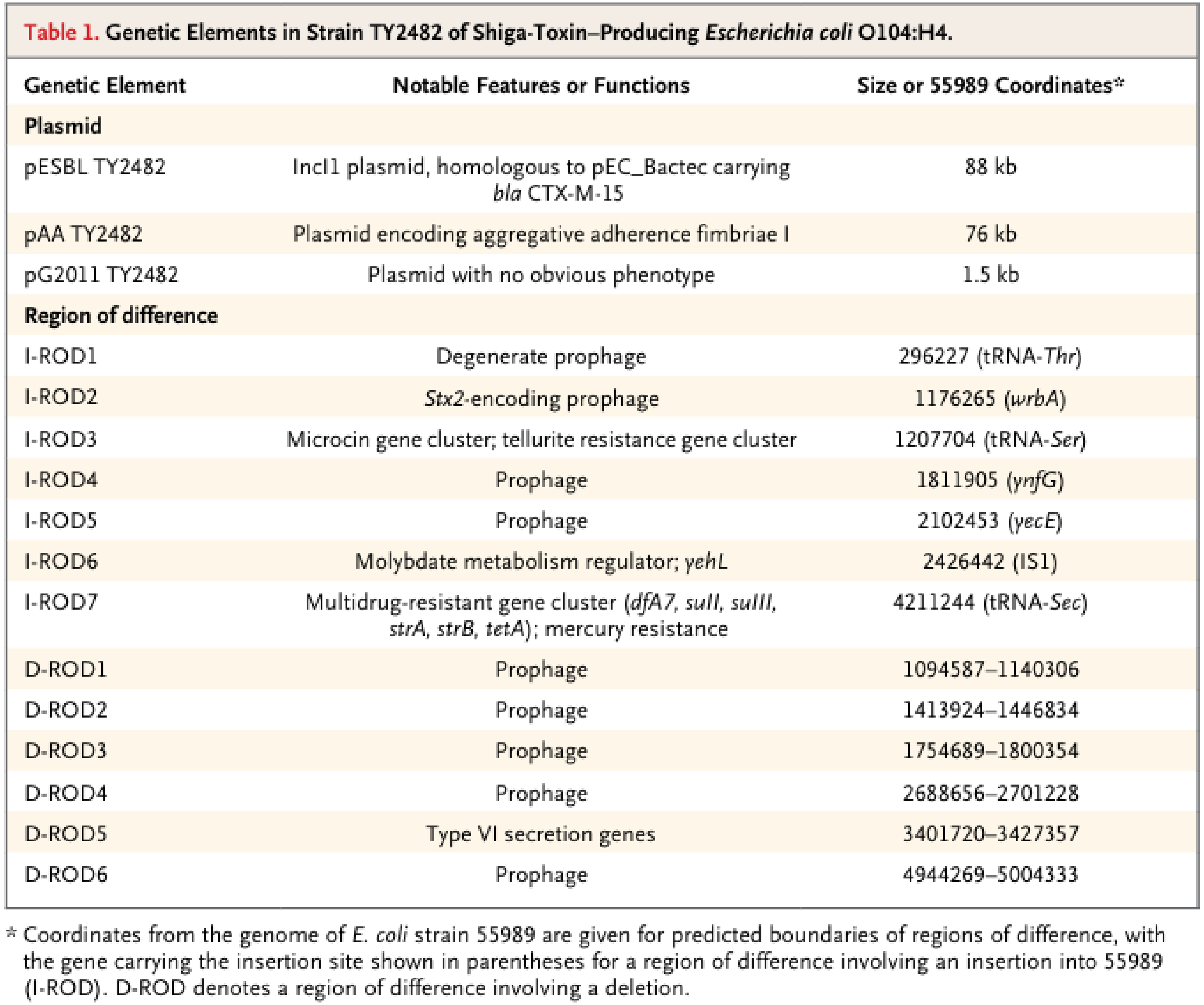

Table 1. Genetic Elements in Strain TY2482 of Shiga-Toxin–Producing *Escherichia coli* O104:H4.

Genetic Element	Notable Features or Functions	Size or 55989 Coordinates*
Plasmid		
pESBL TY2482	IncI1 plasmid, homologous to pEC_Bactec carrying *bla* CTX-M-15	88 kb
pAA TY2482	Plasmid encoding aggregative adherence fimbriae I	76 kb
pG2011 TY2482	Plasmid with no obvious phenotype	1.5 kb
Region of difference		
I-ROD1	Degenerate prophage	296227 (tRNA-*Thr*)
I-ROD2	*Stx2*-encoding prophage	1176265 (*wrbA*)
I-ROD3	Microcin gene cluster; tellurite resistance gene cluster	1207704 (tRNA-*Ser*)
I-ROD4	Prophage	1811905 (*ynfG*)
I-ROD5	Prophage	2102453 (*yecE*)
I-ROD6	Molybdate metabolism regulator; *yehL*	2426442 (IS1)
I-ROD7	Multidrug-resistant gene cluster (*dfA7*, *sulI*, *sulII*, *strA*, *strB*, *tetA*); mercury resistance	4211244 (tRNA-*Sec*)
D-ROD1	Prophage	1094587–1140306
D-ROD2	Prophage	1413924–1446834
D-ROD3	Prophage	1754689–1800354
D-ROD4	Prophage	2688656–2701228
D-ROD5	Type VI secretion genes	3401720–3427357
D-ROD6	Prophage	4944269–5004333

* Coordinates from the genome of *E. coli* strain 55989 are given for predicted boundaries of regions of difference, with the gene carrying the insertion site shown in parentheses for a region of difference involving an insertion into 55989 (I-ROD). D-ROD denotes a region of difference involving a deletion.

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In summary

- Hybrid pathotypes appear
  - Both in human and animals
- Interpretation of these are more complicated
  - Case definition is challenged
  - Problematic for the treatment and prediction on clinical outcome
- Challenging for outbreak management, when the pathogens acts unususal
  - (Food) sources
  - Etiology
  - Initial typing
  - Treatment options
  - Severity

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