Review Article

Enteroaggregative E. coli O104 from an outbreak of HUS in Germany 2011, could it happen again?

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Abstract

Enterohaemorrhagic *E. coli* (EHEC) particularly O157:H7 (Sequence type 11 complex), is the best documented and most well-known of *E. coli* that cause diarrhoea. The importance of EHEC lies in the severity of disease. Outbreaks can infect thousands of people causing bloody diarrhoea and haemolytic uremic syndrome (HUS) that in turn can result in protracted illness or even death. The ability of EHEC to colonise the human gut is normally associated with the presence of genes from another group of diarrhoeagenic *E. coli*, the enteropathogenic *E. coli* (EPEC), via the locus of enterocyte effacement. However, the massive outbreak in Germany was caused by an EHEC which had acquired virulence genes from yet another group of diarrhoeagenic *E. coli,* the enteroaggregative *E. coli* (EAEC). In reality EAEC is probably the most common bacterial cause of diarrhoea but is not identified in most diagnostic laboratories. This outbreak emphasises the importance of being able to detect all diarrhoeagenic *E. coli* and not to focus on *E. coli* O157:H7 alone. Routine surveillance systems for EAEC, a once ignored global pathogen, would go a long way to reaching this goal. This review describes methods for identifying non-O157 EHEC and describes the key genetic features of EHEC and EAEC. Our aim is to provide information for laboratories and policy makers which enables them to make informed decisions about the best methods available for detecting newly emergent strains of diarrhoeagenic *E. coli*.

**Key words:** EAEC; EHEC; HUS; Outbreak O104:H4; ST678

Introduction

Beginning in early May 2011, an unusually high number of haemolytic uraemic syndrome (HUS) cases were reported in Germany. The outbreak was caused by an enterohaemorrhagic *E. coli* (EHEC) which had characteristics of both a verotoxigenic *E. coli* (VTEC) (for a guide to nomenclature see Table 1) and of the less well-known diarrhoeagenic *E. coli*, enteroaggregative *E. coli* (EAEC). There is considerable expertise in diarrhoeagenic *E. coli* in Germany but even with support from the reference laboratory in Rome, the pathogen responsible for the outbreak proved challenging to characterize. Within most diagnostic laboratories the current methodology for VTEC detection is aimed at detecting sorbitol negative VTEC O157:H7 and for most European countries, the sorbitol positive outbreak strain O104:H4 could not be detected. It is therefore important that we examine the methods used by diagnostic and public health microbiology laboratories to characterise VTEC isolates and begin the process of global standardisation. A universal approach based on genomic features would be more generally applicable and transportable than current methods.

The medical care provision required to manage thousands of patients with haemolytic uremic syndrome (HUS) was a major challenge. Even the well-funded hospitals in Northern Germany were forced to loan dialysis and other medical equipment to manage the unexpected case load. Boosting diagnostic and epidemiological apparatus to improve source attribution during outbreaks is imperative to reduce the burden on already overstretched health care facilities. It is now clear that the ability to isolate and identify novel, *e.g.* non-O157:H7 VTECs, as well as known diarrhoeagenic *E. coli* (DEC) must be considered.

Strains with combinations of virulence factors from different *E. coli* pathotypes have been described before but it is the size and severity of the outbreak in Germany which has highlighted the importance and unpredictability of the consequences of genetic exchange amongst gut bacteria. This review will present what is currently known about the outbreak strain and discuss the preliminary genomic analysis

Figure 1. Spanning Tree of MLST data for EHEC and EAEC
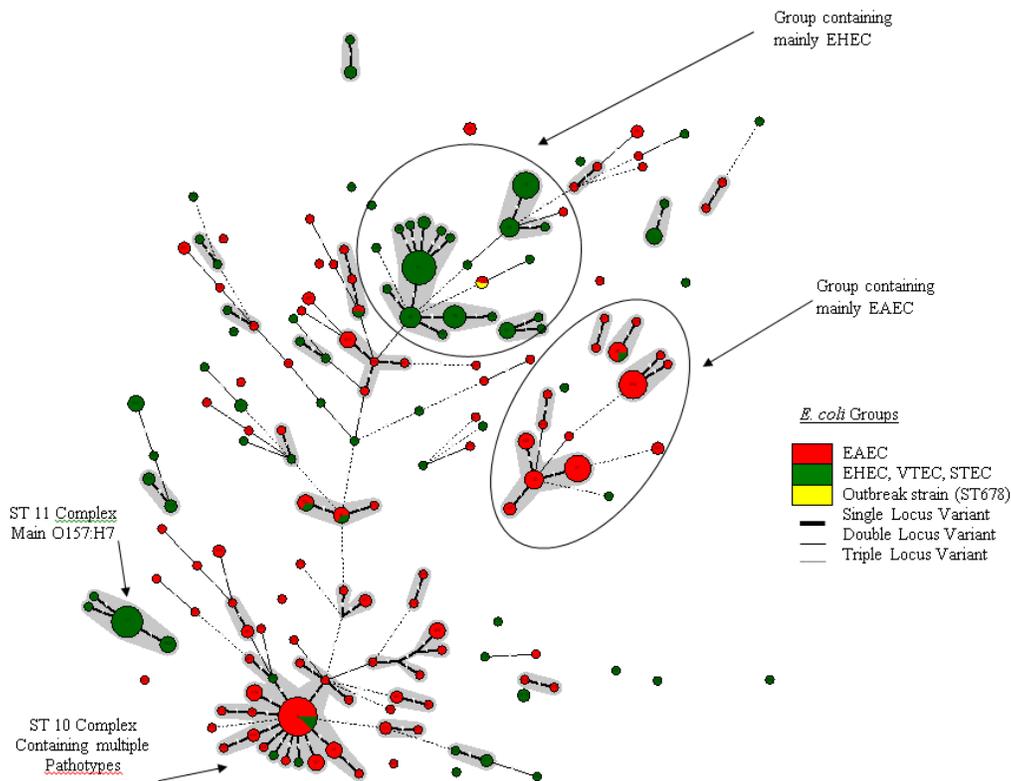
Groupings that are highlighted represent the best known group of EHEC ST11, O157:H7, and two regions of the tree which contain almost exclusively EAEC or EHEC. Other regions of the tree contain both EHECs and EAECs which are distributed widely and evenly. The outbreak strain, *E. coli* ST678, which is positive for both VTEC and EAEC genes, is located in the region of the tree which is mainly non-ST11 EHECs. This suggests that the genes associated with VTEC or EAEC can be acquired by many different lineages of *E. coli* but that some lineages are more successful than others, probably after virulence genes are acquired, and so expand into clusters of one pathotype or the other. The combination of VTEC and EAEC genes in the ST678 *E. coli* led to a particularly virulent strain.

in the context of what is known about other isolates from the EAEC and VTEC pathogroups. It will also highlight an important lesson learned from this outbreak – the importance of a global epidemiological capacity, encompassing the developing world, to detect novel and emerging pathogens in addition to well-known ones.

The outbreak strain

Initial testing by German laboratories showed that the strain associated with the outbreak was of sequence type (ST) 678, serotype O104:H4, and contained genetic elements found in both EHEC (*vtx*) and EAEC (*aggR*). ST678 also contains the EAEC (55989) sequenced strain and the HUS causing O104:H4 VTEC (deposited on the public MLST database by Karch in 2001). The most closely related sequence type (the ST25 group) (Figure 1) is a VTEC O128:H2. The O128:H2 serotype (although no sequence type data is available) has been previously seen in sheep [1] and also isolated from infantile EPEC infections [2]. The group of *E. coli* most

closely related to the outbreak strain are therefore a mixture of pathotypes: EAECs, EHECs and EPECs.

Although serotyping data in the MLST database is incomplete, it is clear that *E. coli* within the O104 serogroup occurs in several different unrelated STs. Therefore, this serogroup does not represent a related group of organisms and comparison of the outbreak strain with other O104 isolates might be redundant. However, including the flagella type, O104:H4 seems to describe a very closely related group of isolates, all within ST678. Members of this sequence type can belong to either the VTEC or the EAEC pathotypes (Figure 1) which can be associated with HUS in humans [3,4] but have not been commonly isolated in Europe. Possibly because detection techniques are optimised for VTEC O157:H7 in diagnostic laboratories. Mthods traditionally used to detect the commonly known VTEC O157 were not successful (O157 agglutination negative, sorbitol positive) and a combination of phenotypic and genotypic methods were necessary. The following sections describe the methods used at the reference laboratories

Figure 2. Flow chart of identification and typing procedures for diarrhoeagenic *e. coli*
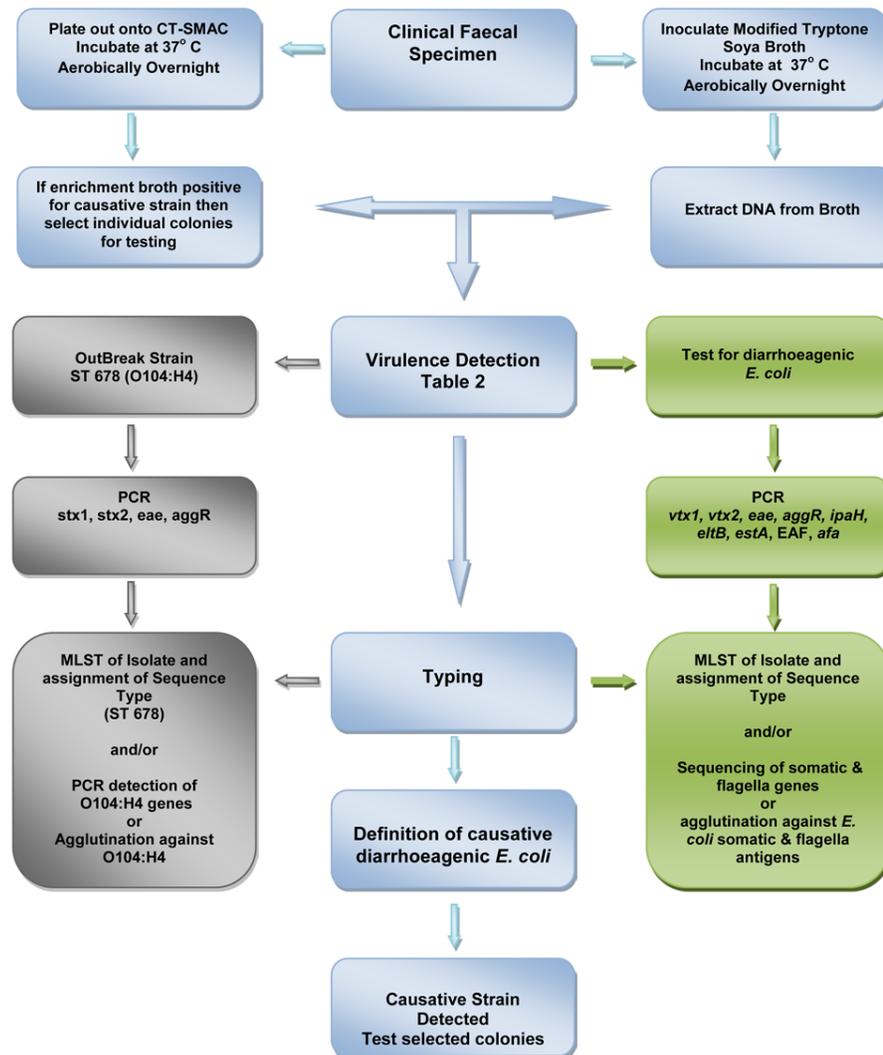
Preliminary report may be generated after initial detection of the strain either from individual colonies or after enrichment. A final report of positive should only be sent after confirmation from individual colonies. Specimens should only be reported as negative after the enrichment broth has tested negative.

supplemented by published methods, and describe the potential for using genomic data.

Enrichment and isolation

Isolation directly from faecal specimens, stored at 4-8°C, was performed as soon as possible as viability of the organism decreases each day and plasmid loss may occur. An enrichment broth of Modified Tryptone Soya was inoculated with mixed faecal matter. The faeces were directly plated onto selective cefixime tellurite sorbitol MacConkey agar (CT-SMAC) and the EHEC ST678 (O104:H4) colonies grew very well producing a creamy pink

morphology. The broth and agar plates were incubated aerobically at 37°C overnight. If CT-SMAC selective plates are unobtainable, other methods can be used such as exploiting the antibiotic resistant properties of this strain and using MacConkey agar supplemented with streptomycin (20 mg/ml) and/or tetracycline (10 mg/ml) [5]; commercial media is now available for this outbreak strain. Although useful for the O104:H4 outbreak strain, it is possible that other outbreaks will occur in which this supplemented media may not be appropriate.

Typing methods

Microbial typing relied on the isolation of the outbreak strain from faeces and this was straightforward because positive cases grew as an almost pure culture on CT-SMAC plates. Single colonies were picked and tested against O104:H7 antigens. Somatic antibodies (including O104) are available commercially. Molecular serotyping was performed using the O104 antigen-associated gene (*wzx*O104) and the gene encoding the H4 flagellar antigen (*fliC*H4) [6,7]. Antibodies raised against the O104 antigen are also positive with the K9 capsular antigen; therefore, O8:K9 and O9:K9 antigens can also be positive. Separate O8, O9 and O9a specific PCR has been carried out in other studies to rule out these other serotypes [8].

For serological typing of unknown isolates, screening against the whole panel of at least 185 somatic and 56 flagella *E. coli* antigens might be necessary. This is likely to be conducted by regional or international reference laboratories; the local testing laboratories can then purchase the antibodies for testing.

Alternatively, the genes that encode the specific O antigens in *E. coli* are clustered in the genome [9] and DNA sequencing can be used to predict the serotype [10].

Virulence detection

For detection of virulence factors by PCR, extraction of DNA was performed from the enrichment broth. Targets recommended for detecting the outbreak strain (Table 2 and Figure 2) include EHEC targets such as the rarely found *vtx1*, the commonly found *vtx2* and intimin (*eae*), an adhesion factor responsible for the attaching and effacing (A/E) lesions found in EHEC and EPEC [11]. The outbreak strain was *vtx2* positive and *vtx1*/*eae* negative.

Although a sub-typing scheme is available for *vtx1* and *vtx2* [12] and described for detecting the outbreak sub-type *vtx2a*, the variation at the nucleotide level is difficult to detect by PCR and needs careful optimisation. Detection using the generic *vtx2* primers and the presence of the EAEC plasmid with the absence of intimin was considered to be sufficient for the screening of the outbreak strain.

The PCR targets described for EAEC are not as stable as EHEC possibly because most are plasmid encoded; plasmids are variable and sometimes they may be lost completely during culture in the laboratory. Targets used for detecting the outbreak strain included a regulator (*agg*R) [13] of multiple EAEC virulence factors including an anti-aggregator transporter gene (*aat*) [14] and a dispersing protein (*aap*) that coats the bacterial surface [15], although this marker has also been found in other *E. coli* [16]. These gene products are linked in that they all play a role in the EAEC colonisation of the gut by aiding the translocation of dispersin across the membrane [17] and are usually found together.

Multilocus sequence typing

Multilocus sequence typing (MLST (http://www.mlst.net/) of seven gene loci (*adk*, *fum*C, *gyr*B, *mdh*, *pur*A & *rec*A) [18], define the outbreak strain as a member of ST 678. The combination of MLST and virulence marker targets (*vtx*1, *vtx*2, *eae*, *agg*R *etc*) is a robust and accessible test that can accurately identify strains of all *E. coli*, including unusual EHECs. Sequence type profiles should ideally be submitted to the public database so that we can start to gain an understanding of the *E. coli* pathotypes causing disease globally and enabling the assignment of new alleles and STs.

Detection of other EHEC strains

Focusing on the serotype of EHEC outbreak strains has led to a bias in laboratory testing for the detection of O157:H7. Recent research studies in both developing and developed countries have shown that non-O157 EHEC strains are prevalent and can be more dominant then O157:H7 in some geographical areas [19-22]. However, front-line laboratories still test only for VTEC O157 and so the true burden of non-O157:H7 EHEC is not known. For example, many O157 EHEC are sorbitol-negative, but the strain in this outbreak was not, nor did it react with the common antisera for EHEC, such as those recognizing O157, O26, O111 and H7 antigens.

A multiplex PCR has recently been described to specifically detect the most common toxin producing VTEC serogroups (O157, O103, O91, O113, O145, O111, and O26) [10] yet O104 had not been recognised as a potential pathogen. A microarray has also been designed to detect the most clinically relevant EHEC with additional targets for O104, O121, O118, O45 and O55 included [6]; however, microarray technology is not feasible to implement in most laboratories. A comprehensive selection of EHEC flagella antigens was included in these studies but they were selected from a historical prospective

Table 1. Nomenclature of Diarrhoeagenic *E.coli* and Enterohaemorrhagic *E. coli* toxin sub-types

Nomenclature

Abbreviation	Meaning	Description
DEC	Diarrhoeagenic *Escherichia coli*	Any defined group of *E. coli* which has been associated with the ability to cause diarrhoea
DEAC	Diffusely-adherent *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea defined by a specific pattern of adherence using the HEp-2 cell assay
EAEC	Enteroaggregative *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea defined by a specific pattern of aggregation using the HEp-2 cell assay
EIEC	Enteroinvasive *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea defined by the presence of invasion genes also found in *Shigella*.
EPEC	Enteropathogenic *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea originally defined as specific serotypes and by a specific pattern of adherence using the HEp-2 cell assay but now by the presence of certain virulence factors including the locus of enterocyte effacement and associated effectors
ETEC	Enterotoxigenic *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea defined by the presence of heat stable or heat labile toxins
VTEC	Verocytotoxic *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea defined by the presence of a toxin gene, *vtx*, which has activity against cultured vero cells
STEC	Shiga Toxin-Producing *Escherichia coli*	A group of *E. coli* which been associated with the ability to cause diarrhoea defined by the presence of a toxin gene, *stx*, because of genetic similarity with the toxin of *Shigella dysenteriae*.
stx/vtx	Toxin genes	For *E. coli* these two gene names are synonymous – only in *Shigella dysenteriae* type 1 is *stx* used exclusively. The discussion about which name should be used revolves around the scientifically agreed use of the same gene name for genes which show homology (shared ancestry); *vtx1* and *stx* are homologues but for *vtx2/stx* this may not be true.
EHEC	Enterohemorrhagic *Escherichia coli*	VTEC/STEC patients that have the symptoms of bloody diarrhoea/ haemorrhagic colitis. This infection can lead to haemolytic uraemic syndrome (HUS) characterised by acute renal failure, haemolytic anaemia (anaemia due to haemolysis) and thrombocytopenia (low number of platelets).
*stx*1/*vtx*1	Toxin gene type 1	Several genetic variants including *vtx*1a, *vtx*1c, *vtx*1d
*stx*2/*vtx*2	Toxin gene type 2	Several genetic variants including *vtx*2a, *vtx*2b, *vtx*2c, *vtx*2d, *vtx*2e, *vtx*2f and *vtx*2g.

The terms *stx*/*vtx* or STEC/VTEC are entirely interchangeable and here we follow the European reference laboratories guidance and use *vtx* and VTEC.

Table 2. List of PCR targets with primers for Diarrhoeagenic *E.coli*

Target	Function	Pathotype	Primer Sequence	Reference
aat	Anti-aggregator transporter gene	EAEC	Forward: CTG GCG AAA GAC TGT ATC AT Reverse: CAA TGT ATA GAA ATC CGC TGT T	[58]
*agg*R	Regulator multiple EAEC virulence factors	EAEC	Forward CTA ATT GTA CAA TCG ATG TA Reverse: AGA GTC CAT CTC TTT GAT AAG	[59]
aap	Anti-aggregation protein (dispersin)	EAEC	Forward: CTT GGG TAT CAG CCT GAA TG Reverse: AAC CCA TTC GGT TAG AGC AC	[59]
*elt*B	Heal Liable Toxin	ETEC	Forward: TCT CTA TGT GCA TAC GGA GC Reverse: CCA TAC TGA TTG CCG CAA T	[60]
*est*A	Heat Stable Toxin	ETEC	Forward: AAT TTT MTT TCT GTA TTR TCT T Reverse: CAC CCG GTA CAR GCA GGA TT	[61]
Ipa-H	Invasion plasmid	EIEC	Forward: GTT CCT TGA CCG CCT TTC CGA TAC CGT C Reverse: GCC GGT CAG CCA CCC TCT GAG AGT AC	[62]
EAF	EPEC adherence factor	EPEC	Forward: CAG GGT AAA AGA AAG ATG ATA A Reverse: TAT GGG GAC CAT GTA TTA TCA	[63]
*eae*A	ettaching and effacing gene	EPEC/ EHEC	Forward: CTG AAC GGC GAT TAC GCG AA Reverse: CCA GAC GAT ACG ATC CAG	[64]
afa	Afrimbrial adhesion	DAEC	Forward: GCT GGG CAG CAA ACT GAT AAC TCT Reverse: CAT CAA GCT GTT TGT TCG TCC GCC G	[65]
Stx1	Shiga toxin 1	EHEC	Forward: TTTGTYACTGTSACAGCWGAAGCYTTACG Reverse: CCCCAGTTCARWGTRAGRTCMACRTC Probe : Cy5-CTGGATGATCTCAGTGGGCGTTCTTATGTAA-BHQ	[66]
eae	intimin	EHEC	Forward: CAT TGA TCA GGA TTT TTC TGG TGA TA Reverse: CTC ATG CGG AAA TAG CCG TTA Probe : Yak-ATAGTCTCGCCAGTATTCGCCACCAATACC-BHQ	[67]
Stx2	Shiga toxin 2	EHEC	Forward: TTTGTYACTGTSACAGCWGAAGCYTTACG Reverse: CCCCAGTTCARWGTRAGRTCMACRTC Probe : Yak-TCGTCAGGCACTGTCTGAAACTGCTCC-BHQ	[66]
rfbE(O157)	O157 antigen associated gene	VTEC O157	Forward: TTTCACACTTATTGGATGGTCTCAA Reverse: CGATGAGTTTATCTGCAAGGTGAT Probe : FAM-AGGACCGCAGAGGAAAGAGAGGAATTAAGG-BHQ	[66]
Additional Specific ST 678 (O104:H4) Outbreak Targets				
wzxO104	O104 antigen-associated gene	*E. coli*	Forward: TGTCGCGCAAAGAATTTCAAC Reverse: AAAATCCTTTAAACTATACGCCC Probe : FAM-TTGGTTTTTTTGTATTAGCAATAAGTGGTGTC-BHQ	[6]
fliCH4	Gene encoding H4 flagella antigen	*E. coli*	Forward: GCTGGGGGTAAACAAGTCAA Reverse: CCAGTGCTTTTAACGGATCG Probe : FAM-TCTTACACTGACACCGCGTC-HEX	[7]
Stx2a	Sub-type of toxin 2a	EHEC	Forward: GCGATACTGRGBACTGTGGCC Reverse 3: CCGKCAACCTTCACTGTAAATGTG Reverse 2 : GGCCACCTTCACTGTGAATGTG	[12]

These are examples of published primers used, ideally primers should be self designed and optimised to keep up with sequence variation.
EPEC: enteropathogenic *E. coli*; EHEC: enterohaemorrhagic *E. coli*; ETEC: enterotoxigenic *E. coli*; EIEC: enteroinvasive *E. coli*; DAEC: diffusely-adherent *E. coli*; EAEC: enteroaggregative *E. coli*.

and so emergence of unforeseen serological profiles such as H4 was unexpected. VTEC isolates can be of 60 O/H types [22] and to include all O/H types for detection on the front line is impractical. Relying on serological typing of common EHEC antigens has led to insufficient systems within the front-line laboratories to detect emerging EHEC outbreaks. The switch to molecular serotyping will overcome this problem but will always be problematic for new serotypes.

The importance of designing globally relevant tests to detect the virulence and background of circulating strains is clear and this outbreak has shown the adaptability and ability of *E. coli* to accumulate virulence genes. Therefore, several pathotypes of *E. coli* should now be considered when screening for EHEC strains to help identify emerging hybrid strains. To do this for verocytotoxin producing *E. coli* strains belonging to different serotype requires toxin assays or molecular identification of the toxin genes.

The best option for the majority of laboratories is DNA-based diagnostics for multiple DEC genes (Figure 2). Multiple genes must be sought since different strains can harbour different combinations of known virulence loci, especially in heterogeneous groups such as EAEC. Thus isolates such as the recent ST678 (O104:H4) outbreak strain can only be reliably identified using molecular methods. Molecular methods can also be used to track virulence genes in specimens or suspected sources that may no longer contain live organisms, an important feature for outbreak analyses. Moreover, the versatility of these methods means that they can be adapted when new strains appear, which is important because we cannot predict when or where a new hypervirulent *E. coli* strain will appear.

For detecting future EHEC strains, the same methodology used for the detection of the outbreak strain could be employed but with the addition of other DEC virulent targets: the invasive gene (*ipa*H) for enteroinvasive *E. coli* (EIEC); heat labile (*elt*B) and heat stable toxin (*est*A) for enterotoxigenic *E. coli* (ETEC); the enteropathogenic *E. coli* (EPEC) adherence factor (EAF) and the afrimbrial adhesion gene (*afa*) for diffusely adherent *E. coli* (DAEC) (Table 2 and Figure 2).

O104:H4 outbreak strain – genetic content

The O104:H4 outbreak was a verocytotoxin producing *E. coli* strain containing *vtx*2; however, the strain is different from many VTEC strains because it lacks both the locus for the enterocyte effacement (LEE) pathogenicity island and the EHEC virulence plasmid. The strain tested positive in initial screens for *agg*R, which encodes a transcriptional regulator of aggregative adherence genes and is located on the virulence plasmid of many EAEC strains. The strain has since been shown to possess an aggregative adherence plasmid and to demonstrate aggregative adherence; it carries an aggregative adherence plasmid as well as the verocytotoxin gene, the two elements that were of most interest to clinical microbiologists. However, there are also other multiple prophages, transposons and a number of horizontally-acquired antimicrobial resistance genes.

Enteroaggregative *E. coli (*EAEC*)*

Enteroaggregative *E. coli* (EAEC) is a large, diverse pathogroup of diarrhoeagenic *E. coli* (DEC) which was defined in 1987 when it was observed that some non-toxigenic strains of *E. coli* from cases of diarrhoea were not adhering to HEp-2 cells in the localised pattern typical of classical enteropathogenic *E. coli* (EPEC) but aggregated in a stacked brick formation [23,24]. Early research on EAEC linked these strains to persistent diarrhoea in children in developing countries but EAEC have since been shown to be an important cause of acute diarrhoea as well, and to be important in the etiology of intestinal infections in industrialized countries [25].

EAEC are known for their heterogeneity and although there are serotypes associated with this group, such as O44:H18, O111:H12, O125, and O126:H7 [25-29], they are not unique to EAEC. Studies have shown a wide selection of EAEC serotypes and many are untypeable [30-32]; therefore, serotyping is not a useful tool in distinguishing this problematic group.

The group contains organisms of multiple lineages [24] which harbour a virulence plasmid; because the HEp-2 assay is difficult to perform and interpret, it is detection of the virulence plasmid which forms the mainstay for identification and so diagnosis of the disease. The following are problems associated with the use of plasmid markers: plasmids have variable gene content; plasmids may be lost on sub-culture; and the plasmid may transfer and be detected in entirely unrelated bacteria which are not actually able to cause diarrhoea.

This group is a main cause of health costs in the developing world but its variable pathogenicity means that funding has not been a priority and comparative pangenome analysis of EAEC in relation

Table 3. Table of sequenced strains for Enteroaggregative *E. coli* and Enterohaemorrhagic *E. coli*

Strain Name	Accession Number	Genome size (MB)	Serotype	ST	AAF	Phylotype	VT
042	FN554766	5.35	O44:H18	414	II	D	N/A
101-1	AAMK00000000	4.98	ONT:H10	493	II	B2	N/A
55989	CU928145.2	5.15	Unknown	678	III	B1	N/A
H112180280	AFPN00000000	5.5	O104:H4	678	I	B1	*stx2*
O111	AP010960.1	5.80	O111:H-	16	N/A	B1	*stx1/stx2*
O26	AP010953.1	5.86	O26:H11	21	N/A	B1	*stx2*
O103	AP010958.1	5.48	O103:H2	17	N/A	B1	*stx1/stx2*
Sakai	BA000007.2	5.60	O157:H7	11	N/A	E	*stx1/stx2*

to other *E. coli* pathotypes and commensals has not been extensively conducted. It has therefore not been possible to define unique stable chromosomal markers for identification. One chromosomal maker (also known to be plasmid encoded) is the *pic* gene which is present in the sequenced 042 strain [33]. This gene is a multi-functional secreted protease but is not unique in the Enterobacteriaceae. Flanking sequence around this gene in EAEC and *Shigella* is different, suggesting that this gene has been acquired by horizontal transfer [34].

These problems with diagnostics have resulted in a poor understanding of this heterogeneous pathotype, which has in turn led to a lack of knowledge of its true burden and impact on human health. Despite ample evidence that EAEC is the most common DEC [35-39], it remains less well-known compared to EPEC, EIEC AND ETEC (Table 1).

Outbreaks of EAEC

There have been some reports of this organism being associated with outbreaks, the largest of which was in Japan in 1993 when 2,697 schoolchildren became ill after eating food contaminated with EAEC with their lunch [40]. Although evidence pointed to white radish sprouts in the stir-fried vegetables, the bacteria were never isolated from the most likely food source. Multiple outbreaks in association with EAEC have been reported in the United Kingdom in association with public functions such as restaurants, hotels and conference centres [41]. EAEC has also caused outbreaks in hospitals (in Serbia 19 babies were infected in a neonatal ward [42]); from well water (in India 20 cases were reported including multiple age groups [43]); and from food (seen in 24 cases in an Italian holiday resort associated with cheese made with unpasteurized sheep milk). Furthermore, they may be an animal reservoir for

some EAEC strains [44]. There is no common serotype associated with EAEC outbreaks.

Genomics of EAEC

At least four EAEC genomes have been completed, are nearly completed, or are in progress (Table 3). EAEC strain 042 produced diarrhoea in three of five adult volunteers in a challenge study in which other EAEC strains tested did not produce symptoms [45]. Strain 101-1 was responsible for the largest documented EAEC outbreak prior to 2011 [40]. Interestingly, although the 101-1 did harbour some virulence genes seen in strain 042, its presumed hypervirulence as suggested by the outbreak remained enigmatic for several years. Recent data demonstrates that in addition to multiple horizontally acquired virulence genes, strain 101-1 harbours a pathoadaptive mutation [46]. Like *Shigella* and some EHEC lineages, it has lost the lysine decarboxylase or *cad* genes. Inserting these genes onto the chromosome of 101-1 attenuates the strain. [46]. The genome of strain 55989 (source) is also in progress; of the four fully or partially sequenced EAEC genomes begun in June, it is this strain that shares the most genomic sequence with the ST 678 (O104:H4) outbreak isolate whose draft genome sequence was completed in June.

Enterohemorrhagic *E. coli* (EHEC)

Enterohemorrhagi*c E. coli* (EHEC) causes haemorrhage of the intestinal tract of humans. The mechanism for this is complex but, for EHEC infection, always involves a toxin [47] called verotoxin (*vtx*) or shigatoxin (*stx*) (Table 1). Originally described as a rare *E. coli* serotype in 1983 [48] causing hemorrhagic colitis, O157:H7 VTEC, a cow-adapted *E. coli*, has since expanded in the bovine population and spill-over into humans, associated with disease, is such that it is currently the

Table 4. List of Useful Links

Links to other resources

There are many useful links which describe information in relation to the outbreak strain including

- Health Protection Agency (http://www.hpa.org.uk/)
- Robert Koch Institute (http://www.rki.de/EN/Home/homepage__node.html)
- Eurosurveillance (http://www.eurosurveillance.org/)
- European food safety authority (http://www.efsa.europa.eu/)
- Centers for disease control and prevention (http://www.cdc.gov/index.htm)
- World Health Organisation (http://www.who.int/en/)
- NCBI BioProject page (http://www.ncbi.nlm.nih.gov/bioproject/68275)
- github repository for the "crowdsharing" efforts (https://github.com/ehec-outbreak-crowdsourced/)

most commonly isolated EHEC (ST11 complex). Another commonly isolated sub-type is the ST21 complex EHECs which are predominantly serotype O26:H11 but may also be O111:H- or O111:H8. The outbreak EHEC ST678 strain clusters away from these "common" EHECs but clusters, as a double locus variant, with EHECs of serotype 0128:H2. It is clear that EHEC, as with EAEC, represent a diverse group of *E. coli* which have acquired virulence genes on several different occasions (Figure 1). It is not just the virulence genes, but also the background into which the virulence genes are acquired, which results in the ability of a strain to cause disease and spread; adherence and toxin production have both been implicated for EHEC. Cases of EHEC infection normally present to health facilities as bloody diarrhoea although more severe complications can occur. The frequency of these complications is dependent on the toxin encoded; the presence of *vtx2a* has been shown to be associated with a more virulent infection [49] partly due to increased expression [50]. The ability to adhere to intestinal cells has also been shown to be associated with virulence and although EHEC, as with EPEC, normally adhere using the LEE [47], there are other mechanisms of attachment within *E. coli* and outbreaks have been caused by several different lineages of EHEC using non-LEE mediated attachment. For the outbreak strain, adherence is presumably mediated via the acquired EAEC virulence factors. It is possible that this adherence is more effective than LEE mediated adherence and so may explain why the outbreak strain caused such a virulent infection.

Outbreaks of EHEC

EHEC outbreaks are more often reported from industrialised countries than from developing countries because surveillance and reporting systems are in place. The most common type from outbreaks is O157:H7 which was responsible for one of the largest outbreaks which included 106 HUS cases from 2,764 confirmed infections in Japan in 1996 [51]. Outbreaks caused by non-O157 EHEC have for several years been highlighted as a potential risk [52] and are well documented again in some industrialised countries [53]. One of the common non-O157 VTECs in the USA is O111:H8 and one of the largest outbreaks was caused by an EHEC O111 (ST and H group not given) in the USA in 2008 causing 341 illnesses [54]. Another strain of EHEC O111:H2 (unknown ST) caused an outbreak in 1998 [55] and had features very similar to those of the German outbreak strain: *eae* negative, EAEC aggregative adherence, and associated with HUS. In 2007 there was an outbreak of EHEC in which five children were infected by two serotypes (O145 and O26) from consumption of ice-cream produced from a Belgium farm [56], perhaps showing the widespread nature of non-O157 EHECs and emphasising their potential to contaminate food handled by people. Thus highly virulent non-O157 *E. coli* has been circulating for some time but the potential impact may not be fully appreciated.

Genomics of EHEC

It is clear that EHEC, as with EAEC, is a heterogeneous group of DECs defined by a virulence factor (*vtx*). The best studied, single locus variants of ST11 share the serotype O157:H7 and show a

conserved genome containing around 1.5 Mb of horizontally acquired DNA which includes a type III secretion system and effectors, the LEE. The LEE contains around 30 coding sequences in 5 operons and encodes the ability of both EPEC and EHEC to attach to the gut and cause disease. Several (currently 24) non-LEE effectors have been described [47] for which the cellular function is being investigated. There is some variability within the EHEC ST11 (O157:H7) group in toxins (Table 1) and in the other accessory genes. This suggests that acquisition has occurred on several occasions and that the genomes of these closely related bacteria are dynamically exchanging DNA with other gut flora. It is believed that O157:H7 as a group evolved from the O55 ancestor, after the horizontal acquisition of genes encoding the O157 antigen, and then branched into two lineages O157:H7 and O157:H-. There has been little radiation in human isolates since this occurred and comparison with the cattle strains (the normal host for ST11 VTEC) suggests that it is a limited subset of cattle-adapted strains which cause infection in humans. This may be due to the source-sink nature of the population dynamics. The source is cattle which support the majority of the bacterial population whilst spill-over into the human population occurs with a restricted set of strains that have the ability to shed in high numbers from cattle and/or to amplify in the environment as well as the ability to colonise and cause disease in humans.

There are several non-O157 EHECs now described and there is sequence data available for ST/serotype: ST21/O26:H11, ST16/O111:H- and ST17/O103:H2 (Table 3). These non-O157 EHECs are from different lineages and yet contain a set of relatively conserved accessory genes [57]. Although analysis of the accessory genome suggests that selective forces within the same environment have led to the acquisition and maintenance of a similar accessory gene content (parallel evolution), there is high level clustering of several of the non-O157 EHECs suggesting a common ancestry. It seems likely that some *E. coli* lineages acquire genetic material via horizontal exchange more often than others; however, whether this is driven by a pathogenic lifestyle [18] or whether pathogens have emerged from strains with a commensal lifestyle within such lineages is not clear. What is clear is that there are many diverse *E. coli* in which *vtx* genes have been found but it is only those that can also adhere to the intestine which will remain in the *E. coli* population and come to our notice as a cause of

infectious disease in humans or animals. The latest of these emergent *E. coli* caused the massive outbreak of HUS in Germany (see the useful links in Table 4).

The emergence of new pathogenic *E. coli*

The distribution of EAEC and EHEC across the tree drawn from MLST (Figure 1) suggests that both pathotypes have arisen on several occasions from several ancestral strains. However, there are clear patterns in the ancestry; the majority of VTECs are clustered around two STs: ST11 (O157:H7) and ST21 (O26:H11). The ST678 (O104:H4) strain from the outbreak clusters with other non-O157 VTECs, possibly around ST25, but most closely with the strain 55989 (also ST678 and also an EAEC). However, this is away from most EAEC isolates suggesting that the acquisition of the plasmid encoding the EAEC phenotype has occurred independently into the ST678 lineage and is not a previously widespread EAEC strain. It seems likely that the emergence of "new" VTECs will be from lineages of *E. coli* which have the ability to adhere to the gut of an animal host, which may be human, either by the mechanisms classically shown by EPEC (the LEE) or by the virulence plasmid of EAEC.

Conclusion

Although EHEC is the best documented and most severe of the DEC it is not the most common cause of *E. coli* diarrhoea. The importance of EAEC as the most common causative agent is now being realised. This may be due to improved testing rather than a recent increase in identified cases, in which case as testing improves further so will estimates of the burden of EAEC disease. The recent combination of EAEC and EHEC virulence factors in a single outbreak strain causing such severe disease emphasises the importance of being able to detect all DEC using appropriate genetic methods and not to just focus on *E. coli* O157:H7. Routine surveillance systems for EAEC, a once ignored global pathogen, would go a long way to reaching this goal.

References

1. Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F (1993) Prevalence and some properties of verotoxin (Shiga-like toxin)-producing Escherichia coli in seven different species of healthy domestic animals. J Clin Microbiol 31: 2483-2488.

2. Knutton S, Lloyd DR, McNeish AS (1987) Adhesion of enteropathogenic Escherichia coli to human intestinal enterocytes and cultured human intestinal mucosa. Infect Immun 55: 69-77.

3. Bae WK, Lee YK, Cho MS, Ma SK, Kim SW, Kim NH, Choi KC (2006) A case of hemolytic uremic syndrome caused by Escherichia coli O104:H4. Yonsei Med J 47: 437-439.

4. Mellmann A, Bielaszewska M, Kock R, Friedrich AW, Fruth A, Middendorf B, Harmsen D, Schmidt MA, Karch H (2008) Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic Escherichia coli. Emerg Infect Dis 14: 1287-1290.

5. Scheutz F, Nielsen EM, Frobe I, Frimodt-Moller J, Boisen N, Morabito S, Tozzoli R, Nataro J, Caprioli A (2011) Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill 16: 19889.

6. Bugarel M, Beutin L, Martin A, Gill A, Fach P (2010) Micro-array for the identification of Shiga toxin-producing Escherichia coli (STEC) seropathotypes associated with Hemorrhagic Colitis and Hemolytic Uremic Syndrome in humans. Int J Food Microbiol 142: 318-329.

7. EU Reference Laboratory for E.coli Department of Veterinary Public Health and Food Safety (2011) Detection and identification of Verocytotoxin-producing Escherichia coli (VTEC) O104:H4 in food by Real Time PCR. Available from: [Accessed 11 Jun 2011] http://www.iss.it/binary/vtec/cont/ Lab_Proc_VTEC_O104.pdf

8. Wang L, Briggs CE, Rothemund D, Fratamico P, Luchansky JB, Reeves PR (2001) Sequence of the E. coli O104 antigen gene cluster and identification of O104 specific genes. Gene 270: 231-236.

9. Reeves PR, Hobbs M, Valvano MA, Skurnik M, Whitfield C, Coplin D, Kido N, Klena J, Maskell D, Raetz CR, Rick PD (1996) Bacterial polysaccharide synthesis and gene nomenclature. Trends Microbiol 4: 495-503.

10. Debroy C, Roberts E, Valadez AM, Dudley EG, Cutter CN (2011) Detection of Shiga Toxin-Producing Escherichia coli O26, O45, O103, O111, O113, O121, O145, and O157 Serogroups by Multiplex Polymerase Chain Reaction of the wzx Gene of the O-Antigen Gene Cluster. Foodborne Pathog Dis 8: 651-652.

11. Law D(2000) Virulence factors of Escherichia coli O157 and other Shiga toxin-producing E. coli. J Appl Microbiol 88: 729-745.

12. WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella(2011) Identification of three vtx1 and seven vtx2 subtypes of Verocytotoxin encoding genes of Escherichia coli by conventional PCR amplification. Copenhagen: Statens Serum Institut..Available from: [Accessed 11 Jun 2011] http://www.ssi.dk/English/PublicHealth/

13. Morin N, Tirling C, Ivison SM, Kaur AP, Nataro JP, Steiner TS (2010) Autoactivation of the AggR regulator of enteroaggregative Escherichia coli in vitro and in vivo. FEMS Immunol Med Microbiol 58: 344-355.

14. Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM (1990) A sensitive and specific DNA probe to identify enteroaggregative Escherichia coli, a recently discovered diarrheal pathogen. J Infect Dis 161: 1249-1251.

15. Sheikh J, Czeczulin JR, Harrington S, Hicks S, Henderson IR, Le BC, Gounon P, Phillips A, Nataro JP (2002) A novel dispersin protein in enteroaggregative Escherichia coli. J Clin Invest 110: 1329-1337.

16. Monteiro BT, Campos LC, Sircili MP, Franzolin MR, Bevilacqua LF, Nataro JP, Elias WP (2009) The dispersin-encoding gene (aap) is not restricted to enteroaggregative Escherichia coli. Diagn Microbiol Infect Dis 65: 81-84.

17. Nishi J, Sheikh J, Mizuguchi K, Luisi B, Burland V, Boutin A, Rose DJ, Blattner FR, Nataro JP (2003) The export of coat protein from enteroaggregative Escherichia coli by a specific ATP-binding cassette transporter system. J Biol Chem 278: 45680-45689.

18. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M (2006) Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol 60: 1136-1151.

19. Bonyadian M, Momtaz H, Rahimi E, Habibian R, Yazdani A, Zamani M (2010) Identification & characterization of Shiga toxin-producing Escherichia coli isolates from patients with diarrhoea in Iran. Indian J Med Res 132: 328-331.

20. Burnens AP, Boss P, Orskov F, Orskov I, Schaad UB, Muller F, Heinzle R, Nicolet J (1992) Occurrence and phenotypic properties of verotoxin producing Escherichia coli in sporadic cases of gastroenteritis. Eur J Clin Microbiol Infect Dis 11: 631-634.

21. Kehl SC(2002) Role of the laboratory in the diagnosis of enterohemorrhagic Escherichia coli infections. J Clin Microbiol 40: 2711-2715.

22. Blanco JE, Blanco M, Alonso MP, Mora A, Dahbi G, Coira MA, Blanco J (2004) Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing Escherichia coli isolates from human patients: prevalence in Lugo, Spain, from 1992 through 1999. J Clin Microbiol 42: 311-319.

23. Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial P, Levine MM (1987) Patterns of adherence of diarrheagenic Escherichia coli to HEp-2 cells. Pediatr Infect Dis J 6: 829-831.

24. Okeke IN, Wallace-Gadsden F, Simons HR, Matthews N, Labar AS, Hwang J, Wain J (2010) Multi-locus sequence typing of enteroaggregative Escherichia coli isolates from Nigerian children uncovers multiple lineages. PLoS One 5: e14093.

25. Okeke IN, Nataro JP (2001) Enteroaggregative Escherichia coli. Lancet Infect Dis 1: 304-313.

26. Shazberg G, Wolk M, Schmidt H, Sechter I, Gottesman G, Miron D (2003) Enteroaggregative Escherichia coli serotype O126:H27, Israel. Emerg Infect Dis 9: 1170-1173.

27. Abe CM, Knutton S, Pedroso MZ, Freymuller E, Gomes TA (2001) An enteroaggregative Escherichia coli strain of serotype O111:H12 damages and invades cultured T84 cells and human colonic mucosa. FEMS Microbiol Lett 203: 199-205.

28. Chart H, Spencer J, Smith HR, Rowe B (1997) Magnesium ions are required for HEp-2 cell adhesion by

enteroaggregative strains of Escherichia coli O126:H27 and O44:H18. FEMS Microbiol Lett 148: 49-52.

29. Smith HR, Scotland SM, Willshaw GA, Rowe B, Cravioto A, Eslava C (1994) Isolates of Escherichia coli O44:H18 of diverse origin are enteroaggregative. J Infect Dis 170: 1610-1613.

30. Jenkins C, Tembo M, Chart H, Cheasty T, Willshaw GA, Phillips AD, Tompkins D, Smith H (2006) Detection of enteroaggregative Escherichia coli in faecal samples from patients in the community with diarrhoea. J Med Microbiol 55: 1493-1497.

31. Jenkins C, Chart H, Willshaw GA, Cheasty T, Smith HR (2006) Genotyping of enteroaggregative Escherichia coli and identification of target genes for the detection of both typical and atypical strains. Diagn Microbiol Infect Dis 55: 13-19.

32. Kaur P, Chakraborti A, Asea A (2010) Enteroaggregative Escherichia coli: An Emerging Enteric Food Borne Pathogen. Interdiscip Perspect Infect Dis 2010: 254159.

33. Chaudhuri RR, Sebaihia M, Hobman JL, Webber MA, Leyton DL, Goldberg MD, Cunningham AF, Scott-Tucker A, Ferguson PR, Thomas CM, Frankel G, Tang CM, Dudley EG, Roberts IS, Rasko DA, Pallen MJ, Parkhill J, Nataro JP, Thomson NR, Henderson IR (2010) Complete genome sequence and comparative metabolic profiling of the prototypical enteroaggregative Escherichia coli strain 042. PLoS One 5: e8801.

34. Henderson IR, Czeczulin J, Eslava C, Noriega F, Nataro JP (1999) Characterization of pic, a secreted protease of Shigella flexneri and enteroaggregative Escherichia coli. Infect Immun 67: 5587-5596.

35. Weintraub A(2007) Enteroaggregative Escherichia coli: epidemiology, virulence and detection. J Med Microbiol 56: 4-8.

36. Tompkins DS, Hudson MJ, Smith HR, Eglin RP, Wheeler JG, Brett MM, Owen RJ, Brazier JS, Cumberland P, King V, Cook PE (1999) A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun Dis Public Health 2: 108-113.

37. Presterl E, Zwick RH, Reichmann S, Aichelburg A, Winkler S, Kremsner PG, Graninger W (2003) Frequency and virulence properties of diarrheagenic Escherichia coli in children with diarrhea in Gabon. Am J Trop Med Hyg 69: 406-410.

38. Knutton S, Shaw R, Phillips AD, Smith HR, Willshaw GA, Watson P, Price E (2001) Phenotypic and genetic analysis of diarrhea-associated Escherichia coli isolated from children in the United Kingdom. J Pediatr Gastroenterol Nutr 33: 32-40.

39. Araujo JM, Tabarelli GF, Aranda KR, Fabbricotti SH, Fagundes-Neto U, Mendes CM, Scaletsky IC (2007) Typical enteroaggregative and atypical enteropathogenic types of Escherichia coli are the most prevalent diarrhea-associated pathotypes among Brazilian children. J Clin Microbiol 45: 3396-3399.

40. Itoh Y, Nagano I, Kunishima M, Ezaki T (1997) Laboratory investigation of enteroaggregative Escherichia coli O:untypeable:H10 associated with a massive outbreak of gastrointestinal illness. J Clin Microbiol 35: 2546-2550.

41. Smith HR, Cheasty T, Rowe B (1997) Enteroaggregative Escherichia coli and outbreaks of gastroenteritis in UK. Lancet 350: 814-815.

42. Cobeljic M, Miljkovic-Selimovic B, Paunovic-Todosijevic D, Velickovic Z, Lepsanovic Z, Zec N, Savic D, Ilic R, Konstantinovic S, Jovanovic B, Kostic V (1996) Enteroaggregative Escherichia coli associated with an outbreak of diarrhoea in a neonatal nursery ward. Epidemiol Infect 117: 11-16.

43. Pai M, Kang G, Ramakrishna BS, Venkataraman A, Muliyil J (1997) An epidemic of diarrhoea in south India caused by enteroaggregative Escherichia coli. Indian J Med Res 106: 7-12.

44. Scavia G, Staffolani M, Fisichella S, Striano G, Colletta S, Ferri G, Escher M, Minelli F, Caprioli A (2008) Enteroaggregative Escherichia coli associated with a foodborne outbreak of gastroenteritis. J Med Microbiol 57: 1141-1146.

45. Nataro JP, Deng Y, Cookson S, Cravioto A, Savarino SJ, Guers LD, Levine MM, Tacket CO (1995) Heterogeneity of enteroaggregative Escherichia coli virulence demonstrated in volunteers. J Infect Dis 171: 465-468.

46. Hwang J, Mattei LM, VanArendonk LG, Meneely PM, Okeke IN (2010) A pathoadaptive deletion in an enteroaggregative Escherichia coli outbreak strain enhances virulence in a Caenorhabditis elegans model. Infect Immun 78: 4068-4076.

47. Wong AR, Pearson JS, Bright MD, Munera D, Robinson KS, Lee SF, Frankel G, Hartland EL (2011) Enteropathogenic and enterohaemorrhagic Escherichia coli: even more subversive elements. Mol Microbiol 80: 1420-1438.

48. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML (1983) Hemorrhagic colitis associated with a rare Escherichia coli serotype. N Engl J Med 308: 681-685.

49. Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, Mladonicky JM, Somsel P, Rudrik JT, Dietrich SE, Zhang W, Swaminathan B, Alland D, Whittam TS (2008) Variation in virulence among clades of Escherichia coli O157:H7 associated with disease outbreaks. Proc Natl Acad Sci U S A 105: 4868-4873.

50. Abu-Ali GS, Ouellette LM, Henderson ST, Lacher DW, Riordan JT, Whittam TS, Manning SD (2010) Increased adherence and expression of virulence genes in a lineage of Escherichia coli O157:H7 commonly associated with human infections. PLoS One 5: e10167.

51. Pennington H(2010) Escherichia coli O157. Lancet 376: 1428-1435.

52. Johnson KE, Thorpe CM, Sears CL (2006) The emerging clinical importance of non-O157 Shiga toxin-producing Escherichia coli. Clin Infect Dis 43: 1587-1595.

53. Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA (2005) Non-O157 Shiga toxin-producing Escherichia coli infections in the United States, 1983-2002. J Infect Dis 192: 1422-1429.

54. Piercefield EW, Bradley KK, Coffman RL, Mallonee SM (2010) Hemolytic Uremic Syndrome After an Escherichia coli O111 Outbreak. Arch Intern Med 170: 1656-1663.

55. Morabito S, Karch H, Mariani-Kurkdjian P, Schmidt H, Minelli F, Bingen E, Caprioli A (1998) Enteroaggregative, Shiga toxin-producing Escherichia coli O111:H2 associated with an outbreak of hemolytic-uremic syndrome. J Clin Microbiol 36: 840-842.

56. De SK, Buvens G, Posse B, Van den Branden D, Oosterlynck O, De ZL, Eilers K, Pierard D, Dierick K, Van Damme-Lombaerts R, Lauwers C, Jacobs R (2008) Outbreak of verocytotoxin-producing E. coli O145 and O26 infections associated with the consumption of ice cream produced at a farm, Belgium, 2007. Euro Surveill 13: 8041.

57. Ogura Y, Ooka T, Iguchi A, Toh H, Asadulghani M, Oshima K, Kodama T, Abe H, Nakayama K, Kurokawa K, Tobe T, Hattori M, Hayashi T (2009) Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic Escherichia coli. Proc Natl Acad Sci U S A 20;106: 17939-17944.

58. Schmidt H, Knop C, Franke S, Aleksic S, Heesemann J, Karch H (1995) Development of PCR for screening of enteroaggregative Escherichia coli. J Clin Microbiol 33: 701-705.

59. Cerna JF, Nataro JP, Estrada-Garcia T (2003) Multiplex PCR for detection of three plasmid-borne genes of enteroaggregative Escherichia coli strains. J Clin Microbiol 41: 2138-2140.

60. Blomen I, Lofdahl S, Stenstrom TA, Norberg R (1993) Identification of enterotoxigenic Escherichia coli isolates, a comparison of PCR, DNA hybridisation, ELISAs and bioassays. J Microbiol Methods 17: 181-191.

61. Stacy-Phipps S, Mecca JJ, Weiss JB (1995) Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic Escherichia coli DNA during course of infection. J Clin Microbiol 33: 1054-1059.

62. Sethabutr O, Venkatesan M, Murphy GS, Eampokalap B, Hoge CW, Echeverria P (1993) Detection of Shigellae and enteroinvasive Escherichia coli by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. J Infect Dis 167: 458-461.

63. Franke J, Franke S, Schmidt H, Schwarzkopf A, Wieler LH, Baljer G, Beutin L, Karch H (1994) Nucleotide sequence analysis of enteropathogenic Escherichia coli (EPEC) adherence factor probe and development of PCR for rapid detection of EPEC harboring virulence plasmids. J Clin Microbiol 32: 2460-2463.

64. Reid SD, Betting DJ, Whittam TS (1999) Molecular detection and identification of intimin alleles in pathogenic Escherichia coli by multiplex PCR. J Clin Microbiol 37: 2719-2722.

65. Le BC, Archambaud M, Labigne A (1992) Rapid and specific detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic Escherichia coli strains by polymerase chain reaction. J Clin Microbiol 30: 1189-1193.

66. Perelle S, Dilasser F, Grout J, Fach P (2004) Detection by 5'-nuclease PCR of Shiga-toxin producing Escherichia coli O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. Mol Cell Probes 18: 185-192.

67. Nielsen EM, Andersen MT (2003) Detection and characterization of verocytotoxin-producing Escherichia coli by automated 5' nuclease PCR assay. J Clin Microbiol 41: 2884-2893.

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