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Review Article

ESCHERICHIA COLI OUTBREAK IN GERMANY: EPIDEMIOLOGY AND IMPACT

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ABSTRACT

Shiga toxin producing *Escherichia coli* (STEC) are serious public health problem and cause millions of illnesses annually in the world. Outbreak due a Shiga toxin producing strain of *E. coli* (STEC O104:H4) has been reported in Germany. This new strain of *E. coli* had many new virulence characteristics and also had a different epidemiology than STEC O157:H7. This outbreak infected a lot of adults and claimed 36 lives. After thorough investigation and with the help of modern molecular biology and genetic tools, the source of the outbreak was traced to imported bean sprouts. This outbreak also resulted in huge economic losses. In this review, the epidemiology and impact of this outbreak has been discussed.

Key words: *Escherichia coli*, Shiga toxin, outbreak, epidemiology

The intestines of humans and other mammals are reservoir of abundant enteric microflora comprising several hundred bacterial species. The family *Enterobacteriaceae* constitutes a major portion of the enteric bacteria as most of the genera and species in this family are able to survive the bile salts and other conditions in the intestinal lumen (Moore and Holdeman, 1974; Zoric *et al.*, 2002). Human colon contains about 320 billion cells per gram of dry weight of colon (Whitman *et al.*, 1998). Among the *Enterobacteriaceae*, the main bacterial species that lives as a commensal in intestines is *Escherichia coli* (*E. coli*). Microscopically *E. coli* appears as a Gram negative coccobacillary organism normally occurring as straight cylindrical rods measuring 1.1-1.5 X 2.0-6.0 µ, present singly or in pairs and conforms to the general definition of the family *Enterobacteriaceae* (Garrity, 2005). The genus *Escherichia* is comprised of several species among which *E. coli* is the most important and commonly occurring bacteria in animals and man. *E. coli* species comprise a large and diverse group of bacteria with most strains as harmless. Several disease syndromes like gastroenteritis resulting in diarrhea, urinary tract infections, respiratory illness and pneumonia have been associated with some strains of *E. coli* (Garrity, 2005; CDC, 2011).

E. coli has been classified into various strains on the basis of somatic and flagellar antigens. The cell wall is a typical Gram negative cell wall having outer membrane, lipid A and O polysaccharide chain. Somatic antigens are the parts of outer membrane of cell wall and are designated by O, while flagellar antigens are designated by H. There are 55 H-antigens and 172 O-antigens. The big six non-O157 serotypes that are commonly associated with the infections in animals and humans are O26, O45, O103, O111, O121 and O145. The shiga toxin producing *E. coli* (STEC) are also called as Verotoxigenic *E. coli* (VTEC) as the shiga toxin has been found to be lethal to Vero cells. The toxin does not show lethality in other types of cell culture systems. Shiga toxin has two types - *Stx1* and *Stx2* and a particular STEC strain may produce one or both of the toxins. *Stx1* and *Stx2* are also called as *Vt1* and *Vt2* due to their cytotoxic effect on Vero cells. The shiga toxin produced by STEC is homologous to the shiga toxins of *Shigella dysenteriae* (Tarr *et al.*, 2005).

Pathogenic *E. coli* has several pathotypes including enterohaemorrhagic *E. coli* (EHEC) which is responsible for causing gastroenteritis and diarrhea or severe inflammatory disease of colon known as hemorrhagic colitis in humans. There are several diagnostic tests and techniques employed to detect an EHEC infection and serotyping is one of these tests in addition to the characterization of other virulence factors. Serotyping and characterization of other virulence factors help to identify an organism as an EHEC. A disease condition of kidneys characterized by small-vessel renal thrombi, thrombocytopenia and non-immune (*i.e.*, Coombs-negative) haemolytic anemia is called as haemolytic uremic syndrome (Gasser *et al.*, 1955; Tarr *et al.*, 2005). The sequel of haemorrhagic colitis occasionally is

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hemolytic uremic syndrome (HUS). HUS results in acute renal failure and even death in children. In elderly, the case fatality rate for HUS can be as high as 50% (Barnard and Kibel, 1965). Many of the strains are not routinely screened and diagnosed in laboratories, so even if these strains might have been as significant in human disease as EHEC O157:H7, they are not reported as labs are unable to grow these strains on media. Several serogroups of *E.coli* including the members of O26, O91, O103, O104, O111, O113, O117, O118, O121, O128 and O145 serogroups are being increasingly recognized as causes of HUS and haemorrhagic colitis (CDC, 2010).

ETIOLOGY OF GERMANY OUTBREAK 2011

After extensive labor input and continuous efforts by all agencies involved, the Fenugreek seeds, which were procured/imported from Egypt, were implicated as the most likely cause of the outbreak. Although it cannot be ruled out that other lots may also be implicated but lot # 48088 of fenugreek seeds was the most likely cause. The cause of this outbreak was ascertained by comprehending the back tracing information from the French and German outbreaks (EFSA, 2011; Wu *et al.*, 2011). The outbreak was caused by a novel strain of shiga toxin producing *E. coli* called as STEC O104:H4. The outbreak started in May 2011 and infected more than 3368 people in Germany. Of the EHEC cases, 852 developed the rare, life-threatening complication, HUS and claimed 36 deaths (Brzuszkiewicz *et al.*, 2011). The contamination of seeds with the STEC O104:H4 strain reflected a flaw or fault in the production or distribution process which allowed contamination of the raw produce with human or animal faeces. The contamination typically could occur during production at the farm level or at subsequent steps in transportation and importing. Rarely, sealed containers got contaminated. Bielaszewska *et al.* (2011) isolated the first outbreak strain of this bacterium in the German National Consulting Laboratory for HUS in Munster on May 24, 2011. The strain was given accession number as 'LB226692'. They found the strain grows well on cefixime tellurite sorbitol MacConkey agar (CTSMAC) and possessed gene encoding tellurite resistance. CTSMAC is used as a selective and differential media for *E. coli* O157:H7 which cannot ferment the sugar sorbitol but tolerates well the cefixime and potassium tellurite.

Bielaszewska *et al*. (2011) carried out the molecular characterization of the isolate and developed a multiplex PCR targeting the genes *rfbO104*, *fliCH4*, *stx2*, and *terD*, which were then used for identification of the subsequent outbreak isolates. They studied 80 outbreak isolates and found that all of them possessed *rfbO104* belonging to the O104 biosynthetic cluster and fliCH4 belonging to the H4 antigen biosynthetic cluster, as determined by the multiplex PCR. Shiga toxin producing *E. coli* (STEC) that belongs to serotype O104:H4 has unanimously been implicated and defined as the German outbreak strain (Bielaszewska *et al.*, 2011; EFSA, 2011).

Microbiological characteristics like presence of shiga toxin *(stx2)*, absence of intimin *(eae)*, hemolysin *(hlyA)* and presence of multi-resistance pattern to antimicrobials were common with the German outbreak strain as they were in the French outbreak strains. When molecular and genetic techniques were used to fully characterize and compare the strains from two countries, the outbreak strains in France and Germany showed genetic relatedness using two molecular techniques such as repetitive sequence based polymerase chain reaction (Rep-PCR) and pulsed-field gel electrophoresis (PFGE) (Gault *et al.*, 2011).

Several virulence factors harbored on horizontally transmitted mobile genetic elements (MGEs), a kind of bacteriophage, confer pathogenicity to STEC (Lothar and Annett, 2012). The locus of enterocyte effacement (LEE), which encodes proteins necessary for the formation of attaching and effacing lesions, is included in the virulence-associated MGE (Oliver *et al.*, 2011). LEE also encodes a type III secretion apparatus, an outer membrane protein, intimin (*eae*), and its translocated intimin receptor (*tir*) and effector proteins translocated by the secretion system.

The German outbreak strain has some of the differential characteristics from the commonly implicated serotype in food borne illnesses (*E. coli* O157) which are summarized in Table 1.

CLINICAL SIGNS AND DISEASE DIAGNOSIS

An important epidemiological factor in this new strain/outbreak was the secondary transmission (human to human and human to food). Haemorrhagic colitis characterized by a bloody diarrhoea is common in EHEC infections. This symptom appeared in most of the cases (Lothar and Annett, 2012). Life threatening complications such as HUS may develop in very young individuals.

Table 1
Characteristics of commonly implicated serotypes of *E. coli* in food borne illnesses

Parameter	Serotype STEC O157:H7	Serotype EAEC STEC O104:H4
Reservoir	Animal reservoir	Human reservoir
Host	Young children	Severe disease-adults
Toxin charac -teristics	*Stx1*, *Stx2* positive Contains intimin and forms 'Attaching and Effacing' lesions on intestinal epithelial cells	*Stx2* positive Lacks intimin and large number of HUS cases. Broad antimicrobial resistance

(Adapted from Soon *et al.*, 2012)

Renal function is lost in HUS which develops in about 15% of the haemorrhagic colitis cases. In the elderly, the absence of 'A Disintegrin and Metalloproteinase with Thrombospondin Motifs' (ADAMTS) proteases causes a neurologic dysfunction called as thrombotic thrombocytopenic purpura (TTP) which has the same symptoms as HUS but can be differentiated in the laboratory (Abumuhor and Kearns, 2002; Tarr *et al.*, 2005). In young animals, some non-O157 EHEC sero-groups including O26, O111, O118 and O103 may cause diarrhoea and other gastrointestinal disorders but no HUS and TTP.

Chronic carriers of the pathogen which shed the bacteria in to the environment can be detected by demonstrating EHEC in freshly voided faecal samples or samples taken directly per-rectal from the animal. Recto-anal swabs may also be used in some cases. It is not easy to identify EHEC because they do not constitute a major population in the faecal flora of animals and they closely resemble commensal *E. coli* except in shiga toxin production. CDC (2010) reported that while many diagnostic laboratories can detect STEC and identify EHEC O157:H7, samples must be sent to a reference laboratory for identification of non-O157 EHEC strains. There is no single technique that can be used to isolate all EHEC serotypes.

The bacteriophage encodes the shiga toxin which inhibits protein synthesis by ribosomes in susceptible eukaryotic cells. The LEE encodes a specific protein secretion system (called a type III secretion system) in strains EHEC that is responsible for attachment to the intestine. HUS is common with infections by EHEC and is characterized by the triad of acute renal failure, haemolytic anemia, and thrombocytopenia in children. In adults *E. coli* O104:H4 is the main etiological agent responsible for kidney damage and eventually HUS.

EPIDEMIOLOGY

The German outbreak was unusual as in that 86% of the cases were aged 18 years or more and 67% of them were females (WHO, 2011). The epidemiological information from a cohort study in Germany suggested that STEC O104-contaminated sprouts were the vehicle of infection. Robert Koch Institute (Frank *et al*., 2011) carried out cohort study involving guests of that point

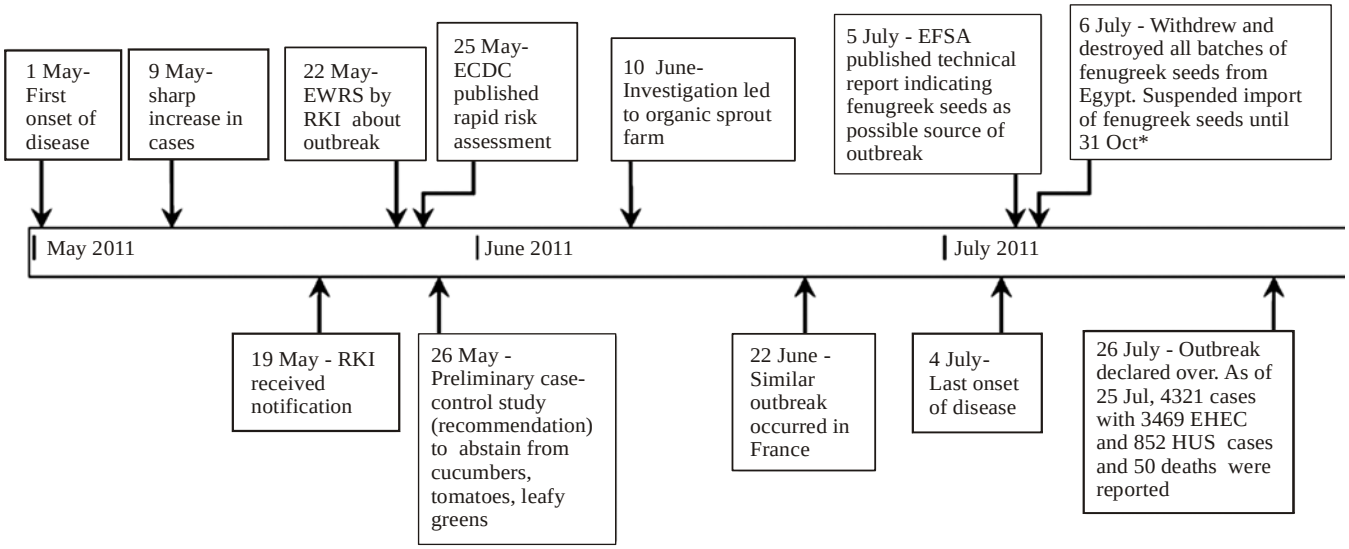

Fig 1. Time line investigation of the German outbreak (Adapted from Soon *et al.*, 2012)

exposure for several restaurants. The food consumption during the period of concern was ascertained by interviews, food delivery lists and receipts. It established a high relative risk to develop bloody diarrhoea related to consumption of sprouts. Scharlach *et al*. (2013) investigated a cluster of STEC and HUS cases within this German outbreak by means of a retrospective cohort study on 72 subjects which had attended a birthday party in the district of Cuxhaven, Lower Saxony, Germany which was hit badly by the outbreak with a large number of STEC and HUS cases. At this party the salad served to guests was a mix of assorted vegetables and garnished with sprouts. However, of all 72 persons, only 35 persons (48.6%) could definitely answer the question of having eaten the sprouts from the salad or not. The sprouts were implicated as the cause of outbreak in these persons based on the interviews, history of food intake and trace back of the sprout delivery from the catering company to the specific sprout producer to whom all other investigations in the whole outbreak converged. Moreover, the catering company also reported that sprouts were used for garnishing the salad (Scharlach *et al.,* 2013).

In France, infection with *E. coli* O104:H4 has been confirmed in four (with HUS) of eleven (seven women and four men) patients. These people had attended an event in Bordeaux, France, where they had eaten sprouts (ECDC, 2011). Later on four more cases of HUS and eight cases of bloody diarrhoea were seen. In the United States, there were six confirmed cases of STEC O104:H4 infections out of which five had recently travelled to Germany.

Exner *et al.* (2011) reported a consensus report on the potential threat of contamination of European water supplies by *E. coli* O104: H4. The report indicated that the strain may become established in human population or perhaps somewhere else in a still unknown animal reservoir and continue to endanger the public health for many years. This report by Exner *et al.* (2011) confirmed that they found no indication that this re-emergent strain has been transmitted by drinking water as all the samples tested negative or unrelated to the outbreak strain.

Halabi *et al.* (2008) tested 2633 water samples between November 2000 and December 2003 and, only 280 were found positive for *E. coli.* Of the positive water samples, 101, 96, 61 and 22 water samples were from drilled wells, dug wells, springs and water supplies, respectively. Of the 280 *E. coli* isolates, eleven isolates were found to be positive for one of the investigated virulence genes (*stx2, eae* or *hlyA*). None of the isolates contained *stx1*. Eight of these were from private and three from public water supplies, respectively. This report suggests that if the water resources are not well maintained and particular attention is not given to such microbial reservoir, an outbreak is likely to occur.

INSIGHT INTO THE GENETICS OF THE PATHOGEN AND MOLECULAR EVOLUTION

There is a limited knowledge about the evolutionary aspects of pathogens and the information on phylogenetic relatedness is also scanty. The shortcomings of our knowledge on the basic principles of evolutionary trends of new pathogens came into lime light and received due recognition due to *E. coli* outbreak in Europe. The knowledge about pathogenesis or spread of this strain was not available although the same strain had caused two sporadic cases in 2005 and 2006 in Germany and Korea, respectively. Large number of isolates from this outbreak were sequenced and annotated and based on the unique combination of genomic features, these strains were suggested to represent a new pathotype called as 'Entero-Aggregative-Haemorrhagic *E. coli* (EAHEC)'. It was suggested that the horizontal gene transfer (HGT) via plasmids which are the mobile genetic elements carrying the extra-chromosomal DNA has played a role in evolution of this strain which carried several new virulence determinants that were absent in the closest relative strain of *E. coli* which had caused an outbreak in central Africa in 2002 (Paramo *et al.,* 2004). The strain that had caused the outbreak in central Africa in 2002 was *E. coli* 55989. However, the origin of this new deadly pathogen from this central African strain remains obscure until now. Mobile genetic elements possess genes that contribute to bacterial speciation and adaptation to different niches, genes for secondary metabolism, antibiotic resistance and symbiotic interactions (Morabito *et al.,* 2003; Holger *et al.,* 2011). Accessory pathogenicity islands (PAIs) encode virulence determinants such as toxins, adhesins, polysaccharide capsule synthesis proteins and iron uptake (Paramo *et al.,* 2004).

OTHER RECENT *E. COLI* OUTBREAKS

The outbreaks due to *E. coli* infection dates back to 1800s and before that it was not reported, although,

there might have been cases of the diseases. The Centers for Disease Control and Prevention (CDC) began keeping records in 1970. In 1993, an outbreak of *E. coli* O157:H7 occurred due to consumption of undercooked hamburgers from a company called Jack in the Box resulting in death of four children and around 700 sicknesses in the Seattle region of the United States and other parts of the Pacific Northwest. There was an outbreak of salmonellosis in 1994 in Minnesota, USA, linked to ice cream. The contamination occurred when raw, unpasteurized eggs were hauled in a tanker truck that later carried pasteurized ice cream to the Schwan's plant. In 1996, there were two outbreaks due to *E. coli* O157:H7, one due to unpasteurized apple juice due to a company in Odwalla, California, USA. The company specialized in selling unpasteurized juices for their supposed health benefits was using blemished fruit and had ignored warnings from in-house safety experts. The other outbreak was due to lettuce in Illinois, New York and Connecticut in 1996. In 1997, there was an outbreak due to *E. coli* O157: H7 in ground beef from Hudson Foods Company. The company had to recall 25 million pounds of ground beef. It is the second largest recall in world history (CDC, 2011).

PREVALENCE AND GROWTH OF PATHOGENS ON SALAD, VEGETABLES, FRUITS AND SPROUTS

Farm produce (vegetables, fruits) get contaminated with pathogenic microorganisms while at farm or during post-harvest handling and processing, transportation and distribution. Outbreaks of infection associated with ingestion of the raw vegetables, fruits and seed sprouts are not rare and have claimed several lives earlier also. Table 2 shows few of the notable outbreaks linked to the seed sprouts.

Consumption of contaminated fresh vegetables, fruits and sprouts has been implicated as the cause of several outbreaks of human gastro-enteritis and diarrhoea (Brackett and Splittstoesser, 1992; Larry, 1995). There are so many reports about the presence of microbial pathogens in vegetables in developing countries like India. In one such study, Poorna and Randhir (2001) collected a total of 120 samples of salad vegetables, fruits and sprouts from street vendors to study the microbial flora of salad vegetables, fruits and sprouts sold by street vendors in Mumbai, India. Samples were collected in sterile containers and analyzed within 1 hour of collection. The organisms recovered from vegetable samples were mainly *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while, fruit samples showed presences of mainly *S. aureus*, *P. aeruginosa* and *Salmonella* spp. The pathogens recovered from sprouts were *P. aeruginosa*, *E. coli* and *S. aureus*. After analyzing the data and other risk factors involved in the manifestation of disease symptoms, Poorna and Randhir (2001) suggested that the high coliform counts ~102-1011 cfu/g and prevalence of *E. coli* (~43%) in salad samples posed imminent threat of food borne illnesses resulting from the consumption of such salad.

Table 2

Notable outbreaks linked to seed sprouts

Year	Bacterial strain	Cases	Country	Food/vector
1996	*E. coli* O157:H7	2764	Japan	Radish
1997	*E. coli* O157:H7	126	Japan	Radish
1997	*E. coli* O157:H7	85	USA	Alfa Alfa
1998	*E. coli* O157:H7	9	USA	Alfa Alfa, clover

(Adapted from Pennington, 2011)

TREATMENT OF ILLNESS AND PREVENTION OF OUTBREAKS

The antibiotic therapy is contraindicated in case of *E. coli* infections particularly those caused by EHEC because of the risk of release of shiga toxin containing bacteriophages which can worsen the infection. Conventional fluid therapy and rehydration are mostly followed by the modern therapeutic agents like the anti-C_5-antibody 'Eculizumab' (Schmidtko *et al.*, 2013). This antibody prevents the self-damage caused by activation of complement proteins in the inflammatory process of infection.

For the reduction in the incidence of outbreaks and prevention of such casualties, various methods for obtaining fresh produce in a hygienic manner have been proposed. One among them can be a combination of physical and chemical methods such as application of high pressure treatment, ultra sound and by using slightly acidic electrolyzed water for raw produce to reduce contaminants. Risk assessment of farms may allow growers to identify causes of contamination and to come up with mitigation strategies. Training of farm workers and raising awareness among public will also help to reduce potential outbreaks (Soon *et al.*, 2012).

CONCLUSION

Escherichia coli is a widespread commensal bacterium and the epidemiology of this commensal is

rapidly changing and new serotypes and strains are emerging with new characteristics distinguishable from the ancestor strains. The fresh produce handling system in food industry is prone to attacks by these newly evolving strains and constant efforts and education in this direction would help in reducing the microbial burden and reducing the chances of an outbreak. The study of epidemiology is of prime concern and it helps in understanding disease pattern and prediction of the etiological agent. Food borne illnesses resulting from fresh produce are on the rise partly due to changing dietary habit and partly due to alteration in the genome of the microbes. To win this battle we must exert every effort to prevent unnecessary contamination of the produce and develop good agricultural practices (GAPs) along with awareness at the grass root level workers and the farmers.

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