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Enterohemorrhagic *Escherichia coli*: A versatile pathogen that should be under surveillance

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ABSTRACT

Out of bacteria which cause food –borne infections, enterohemorrhagic *Escherichia coli* (EHEC) is well known to be pathogen causing serious outbreaks. The first outbreak of EHEC infection occurred in 1982 was due to ingestion of hamburger at restaurant. A rare *Escherichia coli* serotype, 0157:H7 was isolated at that time and the following outbreaks were mostly due to this serotype. However, O26, O111 and O104 were also responsible for EHEC outbreaks. Enterohemorrhagic *E. coli* is an important food and water-borne pathogen. Verotoxins (VTs) produced by this pathogen causes painful hemorrhagic colitis along with major complications of hemolytic uremic syndrome (HUS). The morbidity and significantly high mortality and enormous economic loss are problematic to the health care administrators and EHEC infection is a serious public health issue. Another factor which makes it high transmissibility is the low infectious dose. The German O104:H4 epidemic was caused by the pathogen carrying a combination of virulence genes derived from two well-known pathogens, EHEC and enteroaggregative *E. coli* (EAEC). There is a possibility that two mobile DNA elements can occur again in this versatile pathogen. In this article, some aspects of EHEC infections which were established but not well known to the medical personals were explained to get understanding of why this infection should not be overlooked and should be under surveillance.

Keywords: Enterohemorrhagic *Escherichia coli*, verotoxins, hemolytic uremic syndrome, 0157:H7 serotype.

INTRODUCTION

From February to June, 1982, in Oregon and Michigan states, 47 people were affected by an unusual gastrointestinal illness after they had eatenhamburger, at McDonald's Restaurant. In these two outbreaks, the affected persons had suffered severe abdominal cramp, watery diarrhea followed by bloody diarrhea, and low - grade fever. Bloody diarrhea was described as "all blood and no stool."

A rare *Escherichia coli* serotype, 0157:H7 was isolated from 9 of 12 stools collected within four days of onset of illness. The disease was named hemorrhagic colitis and the bacterium was named enterohemorrhagic *E. coli* by the scientists who studied these outbreaks[1].

While a heat-labile enterotoxin (LT) of enterotoxigenic *E. coli* was studied by Konowalchuk et al, 1977, certain serotypes including O26 and O111 secreted a toxin which killed Vero cells was observed. This toxin was designated Verocytotoxin to be distinct from LT. This was the first report on Vero toxin (VT) produced by *E. coli* strains[2]. This finding was not well received attention by the researchers until occurrence of two outbreaks of hemorrhagic colitis in United States. Because EHEC produced toxin which is cytotoxic to Vero cells, this pathogen was called Verocytotoxin-producing *E. coli* (VTEC) [3].

Verotoxin variants

Two types of Vero toxins, VT1and VT2 were produced by VTEC. VT2 have many variants in contrast to VT1. Takao et al have shown that Shiga toxin and VT 1 are identical in terms of nucleotide and amino acid sequences As a consequence the biological activity of VT1 is neutralized by antiserum to Shiga toxin[4]. In contrary, VT2 and its variants are heterologous to VT1 as well as Shiga toxin. The *vt2*-family genes and *vt1* gene have nucleotide sequence homologies of approximately 55-60%[5,6]. The biologic toxicity of VT2 and its variants are not inhibited by polyclonal antiserum against Shiga toxin and VT1 [7-9].

Structure of the Vero Toxins

Several researchers purified VT1, VT2 and its variants for the study of structures and their biological activities. The Vero toxins are observed to be consisted of two subunits: A subunit which is enzymatically active with N-glycosidase activity and B subunit binds to the Gb3 (globotriaosylceramide) receptor molecule. Although slight variations are found in each type and variants, A and B subunits' molecular weights were approximately 33,000 and 7,500, respectively. One biologically active holotoxin is consisting of one molecule of A subunit and 5 molecules of B subunit[10].

Clinical manifestation of EHEC infection

Most infected persons have watery diarrhea at first. However, some suffers haemorrhagic colitis later after 1-2 days with crampy abdominal pain. HUS occurs in a proportion of cases. HUS includes a triad of conditions acute renal failure, microangiopathic hemolyticanemia and thrombocytopenia. Some patients with HUS develop neurological manifestations which are severe headache, ataxia, convulsions and encephalopathy. Its incidence is higher in extremes of age[11].

Reservoir of EHEC

Although complications are common in humans, EHEC colonization in cattle is asymptomatic. There is no expression of Gb 3 in vascular endothelium and consequently VT was not combine to the blood vessels in the cattle gastrointestinal (GI) tract. Therefore, VT is not carried to other organs to induce vascular damage in cattle[12]. Furthermore EHEC colonizes the recto-anal junction (RAJ) of cattle in contrast to humans where EHEC colonizes in the colon. Because of difference in colonization sites, cattle are more tolerant host for EHEC[13]. Before it reaches RAJ, EHEC must first pass through the acidic barrier of the stomach. EHEC has an intrinsic ability to resistant acid. It has acid resistance system to survive through the acidic environment of the stomach. The infectious dose of it is 10–100 colony-forming units which are very low for the intestinal pathogen[14]. For its colonization at the RAJ, attaching and effacing (A/E) lesions on the mucosal epithelium at the RAJ are formed by EHEC. Destruction of microvilli, intimate attachment of the bacteria to the cell, and formation of a pedestal-like structure are the characteristics feature due to A/E lesions[15]. The locus for enterocyte effacement (LEE) consists of genes within the chromosomal pathogenicity island[16, 17]. As a conclusion, cattle are major reservoir of EHEC without giving rise to symptoms and its site of colonization is RAJ. Factors important for EHEC survival and colonization in cattle are acid resistance systems and LEE-mediated adherence of EHEC to intestinal epithelia.

Pathogenesis of HUS

The sequence of events for HUS was not clear. This is may be due to only the toxin, not the bacterium is transported to the target tissues the glomerular endothelium and brain. The toxin liberated by dying bacteria in the intestinal tract, transport through the gut barrier, reaches the blood, combines with platelets, and gains access to the specific organs. Subunit B of VT reacts with their cellular receptor Gb 3. Receptor-mediated endocytosis occurs and intracellular activity of subunit A starts. It cleaves a specific N-glycosidic bond in the 28S rRNA. This 28S rRNA is important for peptide bond elongation in cellular protein synthesis. Because of its cleavage, elongation factor 1-dependent binding of the aminoacyl-tRNA to the 60S ribosomal subunit is halted and consequently there is inhibition of the peptide chain elongation step of protein synthesis. Besides inhibition of protein synthesis, it causes cytokine release and tissue factor expression. Organs mainly kidney, brain, and others are affected by the toxin. Renal glomerular capillary thrombotic microangiopathy is the pathologic lesion in kidney. In the patient, the VT causes cellular apoptosis, necrosis and thrombotic microangiopathy leading to the acute renal failure. The main action is on Gb3-rich tubular epithelium and glomerular endothelial cells[18]. VT induces inflammatory reaction that causes the thrombi formation in the micro vasculature

during development of HUS. VT2 increases production of chemokines monocyte chemotactic protein-1 and IL-8. This group of chemokines contribute to increased tissue damage[19,20].

As usual, progression to HUS depends on virulence of EHEC strains and host factors. Regarding the characteristics of pathogen, those persons who have been infected by an O157 serotypes had higher chance of HUS as a complication of EHEC infection. However, other non-O157 serotypes also can lead to HUS. Non-O157 strains which gave rise to HUS complication produced only VT2. Lesser incidence of HUS was observed in strains that produce only VT1 or both VT1 and VT2[21].

Transmission and importance of diagnosis

Contaminated foods like undercooked ground beef, unpasteurized milk, juice and raw vegetables can transmit EHEC. Contaminated water ingestion, animals contact, and direct contact from person (e.g., in child-care units) were the other modes of transmission. Immediate and early diagnosis of EHEC infection is important because early appropriate treatment decreases chance of HUS, other complications and patient outcome is favourable. Moreover, prompt diagnosis is essential because antibiotic therapy might aggravate severe complications. From the public health point of view, for implementation of control measures for effective and timely responses, early laboratory identification of EHEC strains is important Although O157:H7 caused most outbreaks, other serotypes such as O104, O26 and O111 were involved. Detection of new serotypes is also critical to give information on epidemiology of disease especially tracing the source of infection and consequently enhance the preventive measures as soon as possible[21].

Role of antibiotics in the treatment of EHEC infections

In vitro studies have shown quinolones increased the level of transcription of the *vt2* gene. These antibiotics induced VT prophage, which is followed by bacterial lysis leading to release of VT from O157 strains[22]. Quinolones also promote transfer of toxin-encoding prophages to uninfected intestinal *E. coli* commensals with subsequent increased VT production[23]. Antibiotics' use are controversial for the treatment of EHEC infections. In one study in hospitalized children, significantly increased the risk of developing HUS is due to antibiotic treatment[24]. Therefore the use of antibiotics should be avoided except the secondary complications which need antibiotic treatment occur.

Germany Outbreak, 2011

The outbreak of EHEC in Northern Germany in 2011 caused 53 deaths out of 855 cases of HUS and 2,987 cases of acute gastroenteritis[25]. Most likely vehicle of infection was Fenugreek sprouts[26]. Because of its severity and its unusual presentation, it became a major challenge to clinicians as well as microbiologists and epidemiologists. HUS incidence rate (22%) was relatively higher than that reported in previous outbreaks (1% - 15%)[27]. More number of cases with severe neurological symptoms (aphasia, seizures, and delirium) were found there[28]. The average incubation period was 8 days and it was longer when compared to other outbreaks[29]. The 2011 outbreak strain O104:H4 had virulence factors of both enteroaggregative *E. coli* (EAEC) and EHEC. The strain carried pAA plasmid of EAEC, as well as a VT2 producing phage and also an extended spectrum beta-lactamase encoding plasmid[30]. The maximum shedding time for outbreak strain was more than one year while it was 124 days for O157 strain. According to the data, the outbreak strain might be carried for longer periods and this information can be explained by the enteroaggregative adherence to human cells[31].

Genetic analysis of EHEC causing Germany Outbreak

In O104:H4, a phage and a plasmid (different mobile elements) were observed in exploration of the genome sequence. There was an unusual combination of virulence genes from EHEC strains and EAEC strains. The former were transferred on the lamboid phage and the latter were mostly carried on the virulence plasmid pAA. Adherence and cytological damage of the intestinal epithelia facilitated systemic adsorption of Shiga toxin that could be explain the high prevalence of HUS in the outbreak[32].

EHEC Outbreaks in Japan and EHEC cases in Thailand

Infectious Disease Surveillance Center (IDSC) of the National Institute of Infectious Diseases (NIID), Japan reported that there are approximately 4000 cases of EHEC annually. Among 60 to 70% of the reported EHEC infections, O157 is the most common EHEC serotype causing infections. Again, O26 is the second most common, followed by serogroups O111, O121, and O103 among the non-O157 EHEC serotypes[33].

The O111 and O157 serotypes were responsible for the serious outbreak in April and May, 2011. These EHEC strains O111:H8 and O157:H7 were originated from raw beef dishes served at barbecue restaurant.. Nineteen % (34 patients) of cases developed HUS out of 181 cases in this outbreak**.** Acute encephalopathy occurred in 21 cases and 5 died. Isolation of *E. coli* strains from the patients stool samples showed O111 and O157 strains. EHEC O111 *vt2* and *vt*-negative *E. coli* O111 strains were detected in a stock of meat block from the restaurant. It was assumed that strain conversion from an EHEC O111 *vt2* to *vt*-negative strain may be occurred during infection because deletion of *vt2*-converting prophage from the EHEC O111 *stx*2 isolates had taken place in the

previous studies. O157 strains had diverse *vt* gene profiles (*vt1* and *vt2*). However, molecular epidemiological methods indicated that these isolates originated from a single clone[33].

Koitabashi et al. observed that some *E. coli* O157:H7 strains isolated in various Asian countries including Thailand possessed a unique q gene and flanking nucleotide sequences which caused them incapable of producing toxin[34]. This information indicates that *E. coli* O157 obtained in Thailand produce VT in a low amount to detect or cannot produce it. These strains may cause antibody production to O157 antigen and protect the hosts to virulent strains. This causes absence of O157 outbreak in Thailand. Although serotypes other than O157 may play in EHEC infections in Thailand, researchers focus on sorbitol fermenter and techniques used for O157 detection with consequent failure in detection of EHEC cases in this country[35].

CONCLUSIONS

The morbidity and significantly high mortality and enormous economic loss are problematic to the health care administrators and EHEC infection is a serious public health issue. Another factor which makes it high transmissibility is the low infectious dose.

The German O104:H4 epidemic was caused by the pathogen carrying a combination of well-investigated virulence genes derived from two well–known pathogens. This pathogen caused new disease manifestations and 855 cases of HUS that made the medical community surprise. The event in Germany outbreak that fusion of two mobile DNA elements could be achieved in *E. coli* suggests that this phenomenon can occur again in this versatile pathogen. The research community on food borne infection and public health specialists were still continuing to study this pathogen, even after the outbreak has been finished[32].

Finally, it can be concluded that regular detection of EHEC in common source of infection like raw beef, unpasteurized milk, etc. should be performed by health care authority so that early preventive measures can be undertaken before the occurrence of serious outbreak.

CONFLICT OF INTEREST: None

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