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Review (Invited)

Shiga Toxin (Verotoxin)-producing *Escherichia coli* and Foodborne Disease: A Review

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Shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) is an important cause of foodborne disease. Since outcomes of the infections with STEC have a broad range of manifestation from asymptomatic infection or mild intestinal discomfort, to bloody diarrhea, hemolytic uremic syndrome (HUS), end-stage renal disease (ESRD), and death, the disease is a serious burden in public health and classified as a notifiable infectious disease in many countries. Cattle and other ruminants are considered to be the major reservoirs of STEC though isolation of STEC from other animals have been reported. Hence, the source of contamination extends to a wide range of foods, not only beef products but also fresh produce, water, and environment contaminated by excretes from the animals, mainly cattle. A low- infectious dose of STEC makes the disease relatively contagious, and causes outbreaks with unknown contamination sources and, therefore, as a preventive measure against STEC infection, it is important to obtain characteristics of prevailing STEC isolates in the region through robust surveillance. Analysis of the isolates by pulsed-field gel electrophoresis (PFGE) and multiple-locus variable-number tandem repeat analysis (MLVA) could help finding unrecognized foodborne outbreaks due to consumption of respective contaminated sources. However, though the results of molecular analysis of the isolates could indicate linkage of sporadic cases of STEC infection, it is hardly concluded that the cases are related via contaminated food source if it were not for epidemiological information. Therefore, it is essential to combine the results of strain analysis and epidemiological investigation rapidly to detect rapidly foodborne outbreaks caused by bacteria. This article reviews STEC infection as foodborne disease and further discusses key characteristics of STEC including pathogenesis, clinical manifestation, prevention and control of STEC infection. We also present the recent situation of the disease in Japan based on the surveillance of STEC infection.

Keywords: Shiga toxin-producing *E. coli*, foodborne disease, infection, HUS, Japan

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The contents of this article reflect solely the view of the author(s).

Abbreviations: A/E: attaching and effacing, CDC: Centers for Disease Control and Prevention, CFU: colony forming unit, CNS: central nervous system, DAEC: diffusely adherent *E. coli*, EAEC: enteroaggregative *E. coli*, EC: European Commission EFSA: European Food Safety Authority, EHEC: enterohemorrhagic *E. coli*, EIEC: enteroinvasive *E. coli*, EPEC: enteropathogenic *E. coli*, ER: endoplasmic reticulum, ESRD: end-stage renal disease, ETEC: enterotoxigenic *E. coli*, EU: European Union, FDA: U.S. Food and Drug Administration, FoodNet: Foodborne Diseases Active Surveillance Network, FSIS: Food Safety and Inspection Service, Gb3: glycosphingolipid globotriaosylceramide, HUS: hemolytic uremic syndrome, IDSC: Infectious Disease Surveillance Center, LD_{50}: median lethal dose, LEE: locus of enterocyte effacement, MHLW: Ministry of Health, Labour and Welfare, MLVA: Multiple-locus variable-number tandem repeat analysis, NESID: National Epidemiological Surveillance of Infectious Diseases, NIID: National Institute of Infectious Diseases, PFGE: Pulsedfield gel electrophoresis, STEC: Shiga toxin-producing *E. coli*, Stx: Shiga toxin, Stx1: Shiga toxin type 1, Stx2: Shiga toxin type 2, USDA: U.S. Department of Agriculture

1. General introduction

As an important cause of foodborne disease, it is estimated by searching references published between January 1, 1990 and April 30, 2012 that Shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) causes 2,801,000 acute illnesses annually, and leads to 3,890 cases of hemolytic uremic syndrome (HUS), 270 cases of ESRD, and 230 deaths globally[1]. Similar estimation for STEC global burden, that is 2.5 million illnesses and 1.2 million foodborne illnesses annually, has been given by WHO, although Norovirus was the leading cause of foodborne illness, causing 125 million cases and *Campylobacter* spp. caused 96 million foodborne illnesses[2,3].

According to food poisoning statistics by the Ministry of Health, Labour and Welfare (MHLW) in Japan, the average number of food poisoning incidents and cases for the decade from 1981 to 1990 and from 2005 to 2014 were 967 and 35,618 for the former and 1,207 and 25,852 for the latter, respectively[4]. However, approximately 28% of reduction was observed in the number of the cases as a whole and the number of cases per incidents decreased from 36.8 to 21.4 between the two decades. On the other hand, as the number of incidents in the latter grew 1.25 times of that of the former, large foodborne outbreaks have been decreasing in number and small outbreaks and/or sporadic cases have been increasing. There are two different reporting systems for surveillance of STEC infections in Japan. One is based on the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections (the Infectious Diseases Control Law) and its purpose is to collect and compile reports of nationally notifiable infectious diseases, including STEC infections regardless of the route of infection. Since STEC infection is defined by isolation of STEC from the person except in the case of HUS, when serodiagnosis was possible, asymptomatic patients are also found in this system. The other is based on the Food Sanitation Law that collects reports of foodborne illness from municipal public health agencies and the system focuses on collecting symptomatic patients of food poisoning. Though the major cause of STEC infections is considered to be foodborne, there is a quite big difference between the numbers of STEC infection cases and the numbers of STEC food poisoning reported through each surveillance system (**Fig. 1**). The number of patients in food poisoning due to *Salmonella* spps. or *Vibrio parahaemolyticus* has gradually decreased since 2001 but that of patients due to *Campylobacter jejuni*/*coli* has remained relatively high compared to that of *Staphylococcus* spps. and STEC. The average number of the cases of STEC infection in the 11-year period from 2005 to 2015 was 3,995 but that of food poisoning due to STEC was only 364 for the same period. Although the number of cases of STEC infection includes 34% of asymptomatic patients during the period of 2009 to 2015[5–10], number of patients with STEC food poisoning is by far too small to compare to that of the cases of STEC infection. In this article we focus on STEC and STEC infection as foodborne illness discussing potential

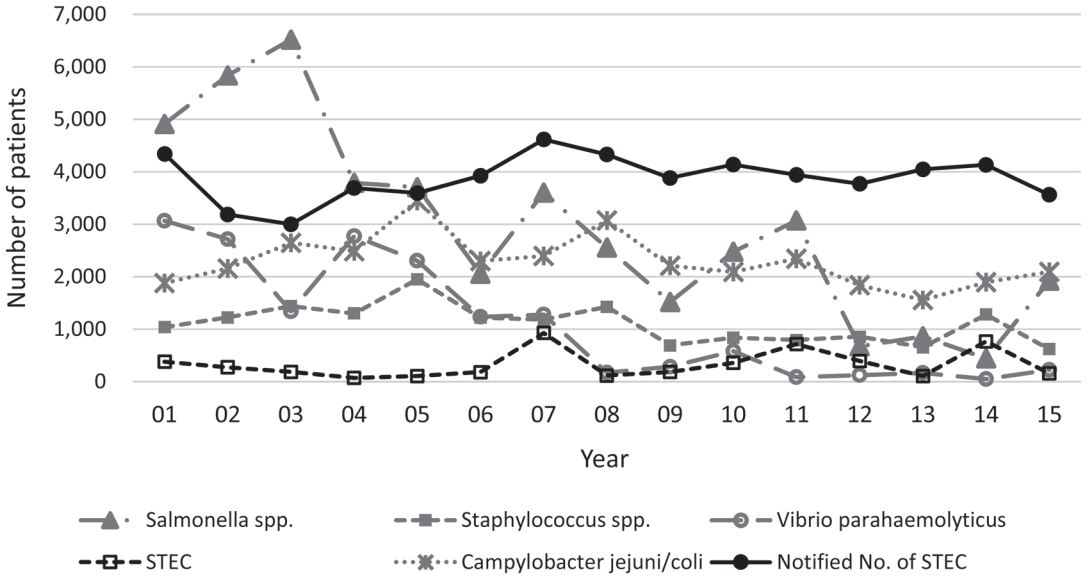

Fig. 1. The number of patients of food poisoning by various causal agents in Japan from 2001 to 2015 and the number of notified STEC infection during the same period. Note that the number of patients of food poisoning by STEC is strikingly smaller than that of notified STEC infection. The number of patients with *Salmonella* species and *Vibrio parahaemolyticus* are in decline.

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preventive measures against STEC infection.

2. Shiga toxin (verotoxin)-producing *E. coli* (STEC) and STEC infection

2-1. Introduction

Diarrheagenic *E. coli* that are capable of causing disease in healthy individuals can be categorized into six well-described categories: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC)[11]. The categories of diarrheagenic *E. coli* are differentiated on the basis of pathogenic features reflecting profile of virulence factors of the isolate. We will use the term STEC to denote strains possessing Shiga toxin independent of accompanying virulence factors.

STEC was first recognized as a human pathogen in 1982, when *E.coli* O157:H7 caused two outbreaks of hemorrhagic colitis associated with consumption of undercooked ground beef[12,13]. Since then, a number of foodborne outbreaks of hemorrhagic colitis and HUS due to not only STEC O157:H7 but also other serotypes of STEC have been reported worldwide. Since STEC resides in the gastrointestinal tract of cattle and other ruminants, contamination of meat with STEC during slaughter is a principal route by which these pathogens enter the food supply. However, a variety of foods have been identified as vehicles of STEC-associated illnesses; these include ground beef[14], roast beef[15], salami[16], raw milk[17,18], cheese[19,20], ice-cream[21], yogurt[22], Romaine lettuce[23], lettuce[24], unpasteurized apple cider or juice[25–28], cantaloupe[29], spinach[30], radish sprouts[31,32], and alfalfa sprouts[33]. STEC O157:H7 have been predominant serotype of STEC associated with human illness and the serotype had been the major target of most detection methods. But as Shiga toxin and/or genes for Shiga toxin have become the main target of most detection methods in STEC surveillance, more cases of non-O157 STEC are reported. STEC infection remains a leading cause of gastroenteritis among notifiable disease in Japan and is sometimes followed by a severe life-threatening complication such as HUS.

2-2. Pathogenesis

2-2-1 Shiga toxins

Shiga toxins (Stxs) are key virulence factors produced by *Shigella dysenteriae* serotype 1 and STEC. Stxs have been shown to be responsible for exacerbating intestinal damage, and cause systemic complications involving the kidneys and central nervous system (CNS). The toxin is named after Dr. Kiyoshi Shiga, who identified the causative agent of dysentery, Shiga's bacillus, in an outbreak of dysentery in Japan in 1897[34]. In 1977, Konowalchuk et al. showed that some strains of *E. coli* produced a cytotoxin capable of killing Vero cell, and the cytotoxin was referred to as Vero cytotoxin or Verotoxin[35]. In addition to the findings with the purification of Shiga toxin from *S. dysenteriae* serotype 1 in 1980[36,37], it was reported that a Shiga-like toxin was produced by *E. coli* O157:H7 strain that had caused an outbreak of hemorrhagic colitis in the United States[38] and that this toxin was the same as the verotoxin shown to be produced by *E. coli* O157:H7[39]. Now, the terms Shiga toxins or Verotoxins are used to describe the same toxin[40].

There are two types of Stxs produced by STEC, Shiga toxin type 1 (Stx1) and Shiga toxin type 2 (Stx2), based on their antigenic characteristics compared to the prototypical Shiga toxin produced by *S. dysenteriae* serotype 1[41–43]. It has been shown that purified Stx2 has 400-fold lower median lethal dose (LD_{50}) in mice[44] and is 1,000 times more toxic to human renal endothelial cells than Stx1[45]. Epidemiological evidence also shows that Stx2-producing strains of STEC O157:H7 are more frequently associated with HUS than are strains producing Stx1[46,47].

These Shiga toxin family members have a monomeric A and pentameric B molecular configuration, as revealed by X-ray crystallography[48,49]. A catalytic A subunit is non-covalently associated with a pentamer of identical B fragments that form the B subunit, which is responsible for binding to cell surface receptors, glycosphingolipid globotriaosylceramide (Gb3; also known as CD77 or the Pk blood group antigen)[50–52]. After binding to Gb3 receptors, the toxins are internalized and undergo retrograde intracellular transport to the endoplasmic reticulum (ER). During transport to the ER, catalytic A subunits dissociate from B subunits by proteolysis and disulfide bond reduction[53–56]. Since catalytic A subunit has a specific RNA N-glycosidase activity that cleaves an adenine base at position 4,324 of 28S ribosomal RNA of eukaryotic ribosomes[57,58], it inhibits elongation factor-dependent amino-acyl tRNA binding and subsequent chain elongation[59]. However, delivery of the toxins to the ER and following retrotranslocation of the catalytic A subunits into the cytoplasm result in not only host cell protein synthesis inhibition, but activation of the ribotoxic stress and ER stress response and, in some cases, the induction of apoptosis, cytokines and chemokines[60].

The *stx* genes in STEC strains are encoded by the genomes of prophages of the lambdoid family and are located downstream of the phage late gene promoter[61]. While *stx1* is under control of the iron-regulated authentic promoter, which will result in induction of Stx1 expression at low concentration of iron[62], the expression of *stx2* depends primarily on the

late promoter[63,64]. DNA-damaging agents, such as mitomycin C have been shown to increase Stx production through prophage induction[65,66]. Stx production in STEC strains, therefore, is intimately correlated with the Stx-encoding phages.

2-2-2 Adhesins

Locus of enterocyte effacement

The ability of STEC to induce attaching and effacing (A/E) lesions of intestinal epithelia is shared by EPEC, *Escherichia albertii* (previously classified as *Hafnia alvei*), and *Citrobacter rodentium*[67]. The A/E lesions were typical histopathological observations in intestinal biopsy specimens from patients and infected animals originally reported with EPEC infection, which are characterized by effacement of microvilli and intimate adherence between the bacterium and the surface of epithelial cells[68,69].

The bacterial genes involved in formation of A/E lesion were shown to be located on a 35-kb locus of the chromosome of EPEC and STEC isolates[67]. This locus, called locus of enterocyte effacement (LEE) is not present in non-pathogenic strains of *E. coli* but is found in EPEC and STEC strains capable of producing the A/E lesion. LEE-positive STEC serotypes have been referred to as enterohemorrhagic *E. coli* (EHEC)[70] and LEE-positive STEC serotypes (such as O157:H7, O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM) are much more commonly associated with HUS and with epidemic diseases than are LEE-negative serotypes[11,70,71].The LEE consists of five major operons, which encode a type III secretion system, multiple secreted proteins, a bacterial adhesin called intimin, and a translocated receptor for intimin, Tir[72–75]. Intimin is a 95-kDa outer membrane protein that is encoded by *eae* gene (*E. coli* attaching and effacing) and necessary for the formation of A/E lesions[69,76]. The delivery of Tir into the host cell through type III secretion system is followed by binding of intimin to Tir that was recruited to the surface of the host cell membrane, which initiates formation of A/E lesions[77]. Although intimin is the primary adhesin in STEC, there are other adhesins contributing to the adhesive capabilities of STEC, including fimbrial adhesin proteins such as long-polar fimbriae[78], autotransporters, flagella, and other adhesin proteins reviewed in reference[79].

2-2-3 Acid tolerance

The infectious dose of STEC is estimated to be as low as or less than 100 organism[80,81], which is attributed to its acid-resistant nature[82–85]. In addition to increasing the possibility of survival of the bacterium under gastric acid environment, the acid tolerance has enabled the pathogen to survive in various acidic food; apple cider (pH 3.7 to 4.0)[86], buttermilk (pH 4.1)[87], yogurt (pH4.17 to 4.39)[87,88], and sour cream (pH 4.3)[87]. Acid resistance mechanism in *E. coli* includes a glucose-repressed system, glutamate- and arginine-dependent systems[89]. While *rpoS* (encoding sigma factor) is essential for expression of glucose-repressed system, glutamate- and arginine decarboxylase are required in amino acid-dependent systems. The two decarboxylase systems are believed to consume protons during the decarboxylation of glutamate or arginine, thereby preventing internal pH of the cell from decreasing to lethal levels[90].

2-3. Animal Reservoir of STEC

Cattle and other ruminants[91,92] are considered to be the major reservoirs of STEC, though STEC has also been isolated from other animals, such as dogs, cats, swine[93], and horses[94]. Animal reservoirs for STEC O157:H7 including amphibians and fish, as well as invertebrates, such as insects and mollusks, were reviewed elsewhere[95,96]. Aquatic species such as finfish and shellfish as dead-end hosts[96] could transmit the organism to other animals, when they are consumed[97–99].

In a survey performed on rectal content samples from 250 beef cattle on 25 beef farms and 250 dairy cows on 25 dairy farms during summer in 2011 in Japan, STEC O157 was isolated from 16 (6.4%) beef cattle on 7 (28%) beef farms, but not obtained from any dairy cows tested[100], and the previous investigation performed by the same authors four years apart showed very similar prevalence of STEC O157 (8.9%)[101]. In another study, prevalence of STEC strains in 932 healthy dairy cows from 123 farms was 12%, and 31 different O-serogroups, including O26 but not O157, were identified[102]. Using stx-PCRs for screening, the same study also found that the prevalence of the *stx* gene positive samples among the dairy cows was 30.4%. Hussein[103] reviewed published reports and summarized that the prevalence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlots and from 0.7 to 27.3% on pasture with regard to beef cattle and that corresponding prevalence rates of non-O157 STEC were 4.6 to 55.9% and 4.7 to 44.8%, respectively.

2-4. Sources of Human Infection

2-4-1. Undercooked Contaminated Beef Products

Since the most common source of STEC infection in human is consumption of contaminated foods, consumption of raw or undercooked foods of bovine origin has been the most common means of transmitting STEC infection. Ground beef is an especially efficient transmission vehicle of STEC and a multistate outbreak was traced to hamburgers distributed by a restaurant chain in 1993[104], and undercooked hamburg-

ers were implicated in a number of other outbreaks[105–108]. Hamburgers prepared at home were also implicated[109,110]. Needless to say, raw or undercooked beef products have a higher risk of transmitting contaminated bacteria to human and, in fact, the STEC O157 infections due to consumption of raw beef liver in 2010[6], a large STEC O111 outbreak due to consumption of Yukhoe, a Korean dish of raw beef and egg yolk[7], and a diffuse outbreak from a restaurant chain due to cubically assembled meat[111] were reported in Japan.

2-4-2. Contaminated Fresh Produce

Because STEC can attach to raw or processed fruits and vegetables, produce has also been a vehicle for transmission of the bacteria. Major outbreaks were linked to lettuce[112], including a multistate outbreak; sprouts[32] and spinach[113] are implicated in numerous HUS cases, and the large outbreak of O104:H4 in 2011 in the European Union (EU) also implicated the consumption of sprouts[114]. Produce-associated outbreak surveillance data from the Centers for Disease Control and Prevention (CDC, U.S.A.) for the period from 2000 to 2009 showed that, among produce commodities, leafy greens were the most frequently linked to outbreaks[115]. Typically, produce-mediated outbreaks were linked to foliage contaminated by irrigation/spray water[116]; STEC O157:H7 were shown to adhere to and penetrate roots[117].

Fermentation of food products can reduce the viability of STEC. STEC O157:H7 declined up to 3.5 logs in *soudjouk* sausage[118,119], but fermented products may provide a vehicle for infection if curing conditions are inadequate; an outbreak in Sweden was traced to improperly processed sausage[120]. Pickled vegetables[7] and lightly salted vegetables[8,121,122], which were eaten fresh, as salad, have also been implicated in the outbreaks. Salmon roe that was lightly salted as a topping of sushi (*ikura*-sushi) was contaminated with STEC O157:H7 and caused an outbreak in Japan[123].

2-4-3. Environment-mediated Transmission

Manure is a good vehicle of STEC and some outbreaks have been associated with public events held on grazing areas, presumably strewn with manure. A scouting event held in Scotland on a muddy field grazed by sheep resulted in an outbreak with Pulsedfield gel electrophoresis (PFGE) indistinguishable isolates from patients, the field, and sheep feces[124]; culturable bacteria were isolated for 15 weeks from the field soil after the outbreak[125]. In a sporadic case of STEC O157 infection in Minnesota, the isolates in garden soil linked to the case could survive on manure-amended soil for more than two months[126].

Agricultural fairs exhibiting livestock are often implicated in human STEC outbreaks[127–129]. An investigation of a fair-associated STEC O157 outbreak suggested that infections can be caused by widespread contamination of a building, since there was no evidence implicating specific food or beverage sources but STEC O157 was recovered from the rafters of the building[130]. In an outbreak of STEC O157:H7 infection associated with attendance at multiple rodeos that had used bulls from the same cattle supplier, isolates from all 14 patients showed indistinguishable PFGE pattern and isolates from nine patients had identical multiple-locus variable-number tandem repeat analysis (MLVA) patterns and five had minor differences, and an isolate of STEC O157 identified from a dirt sample collected from the bullpens of one of the attended rodeos was indistinguishable by PFGE and MLVA form the main outbreak strain[131]. STEC O157:H7 was recovered from 3.5% of leafy green samples of the plot at 60 m away from a cattle feedlot, whereas that was recovered from 1.8% of the samples at 180 m away from the same feedlot, suggesting airborne contamination with STEC O157:H7 from cattle production area[132].

Waterborne STEC infection has been implicated in a number of sporadic cases and outbreaks[133,134]. STEC outbreaks were associated with swimming in lakes[135,136] and pools[137], and consumption of water from a private water supply[138–140].

2-4-4. Direct Contact Transmission

Direct human-animal contact can transmit STEC, and the most important prevention step to reduce transmission resulting from human-animal contact is hand-washing[141]. STEC outbreaks have often been associated with animals in public settings. Children are at most risk, as highlighted by HUS cases in petting zoo[142] or farm[143,144] visit and in participation in lamb feeding event[145].

STEC can be transmitted to humans through person-to-person transmission. Through National Outbreak Reporting system in USA, estimated 40 foodborne outbreaks of STEC were reported in 2009, and five additional STEC outbreaks were reported as transmitted by person-to-person contact[146]. Person-to-person transmission of STEC is a recognized cause of outbreaks in childcare settings[147–150], which may be related to close contact of children with immature immune systems and underdeveloped personal hygiene skills.

2-5. Epidemiology

2-5-1. Clinical course

STEC infection has a broad spectrum of clinical manifestation; asymptomatic infection, nonbloody diarrhea, bloody diarrhea (hemorrhagic colitis), and HUS. Typical clinical course of STEC O157:H7 infection begins with ingestion of the organisms, followed by a 3-to-4-day incubation period before the first loose stool. Illness then begins with nonbloody

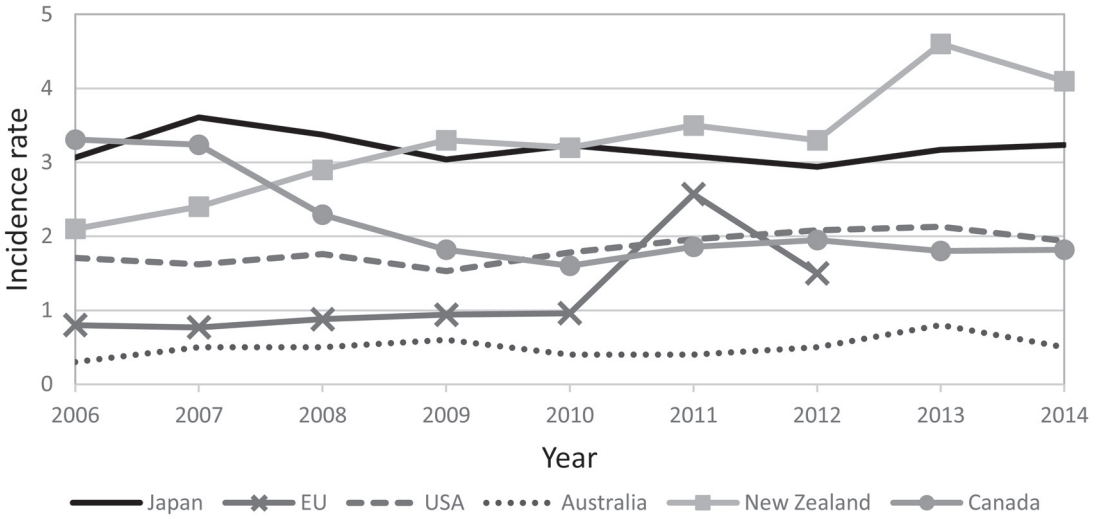

Fig. 2. Annual incidence rate per 100,000 population of STEC infection in Japan, EU, United States, Australia, New Zealand, and Canada, 2006 to 2014, except for the EU, for which data was available up to 2012.

diarrhea and abdominal cramps. Most persons who seek medical attention develop bloody diarrhea, a typical feature of STEC O157:H7 infection, in the second or third day of illness[151]. Symptoms of infection with STEC O157:H7 usually subside in about a week, with no obvious sequelae. However, about 6 percent of patients develop HUS[152] and it is usually diagnosed two to 14 days after the onset of diarrhea[153]. HUS is most likely to occur in young children and elderly[152]. In addition to age, risk factors for development of HUS include bloody diarrhea, fever, an elevated leukocyte count, antibiotic administration, and use of antimotility agents[154–156]. Although an association between non-O157 STEC and milder clinical symptoms have been reported[157–159], further investigation is needed on whether features of the clinical illness vary among serotypes and how difference in virulence factors might result in differences in clinical outcomes. Although limited data are available on dose response, some findings indicate that the infectious doses of STEC are relatively low. For example, from an outbreak of O111 STEC in beef sausage in Australia, investigators extrapolated a dose range of 1 to 10 organisms, given as few as 1 cell per 10 g of sausage[80]. Using the concentrations of STEC O145 in contaminated ice cream in an outbreak in Belgium, the estimated infective dose was 400 colony forming unit (CFU)[21]. This is comparable to illness from STEC O157:H7, which can result from infection with as few as 10 cells[81].

2-5-2. Surveillance

In the United States, all STEC infections that cause human illness are notifiable to the Nationally Notifiable Diseases Surveillance System. Incidence rate for STEC are shown in **Fig. 2**, and the mean incidence rate for STEC in the period from 2010 to 2014 was 1.98 in the United States[160]. On the other hand, in 2014, the Centers for Disease Control and Prevention (CDC) Emerging Infections Program analyzed the data gathered from the Foodborne Diseases Active Surveillance Network (FoodNet). A total of 697 laboratory-confirmed cases of STEC non-O157 and 444 of STEC O157 infections were identified, with incidence rates of 1.43 and 0.91 per 100,000 persons, respectively[161]. An examination of STEC cases from Michigan demonstrated an increase in non-O157 STEC[162] and similar increases in non-O157 STEC were reported in other studies[163–165]. In both the Ontario and British Columbia sentinel sites in Canada, a total of 61 cases of STEC infections were reported between 2011 and 2012, representing an incidence rate of 3.1 cases/100,000 person-years[166]. In comparison, the annual combined incidence rate for STEC infection as notifiable disease in Canada for both years was 1.9 cases/100,000 person-years (**Fig. 2**). While a slight decrease in the incidence rate has been observed in Canada, the apparent increasing trend of the incidence rate was reported from New Zealand[167]. Except for the sudden increase of the incidence rate in the EU in 2011--probably due to a large outbreak of STEC O104 in Germany--it remained less than 1.5 cases/100, 000 in the EU, though the data was available only up to 2012[168], and the incidence rate in Australia has been about 0.5 since 2007[169–173].

In Japan, STEC infection is a category III notifiable infectious disease, along with other bacterial infections caused by *Vibrio cholerae* O1 or O139, *Shigella* species, *Salmonella enterica* serovar Typhi, and *Salmonella enterica* serovar Paratyphi A in the National Epidemiological Surveillance of Infectious Diseases (NESID) under the Infectious Diseases Control Law enacted in April 1999. Despite control measures

instituted since 1996, including designating STEC infection as a notifiable disease, and the disease being monitored effectively through nationwide surveillance, the annual incidence rate remains around 3.0 per 100,000 population (**Fig. 2**). Under a surveillance system for food poisoning based on the Food Sanitation Law, an STEC infection is reported as food poisoning by physicians or judged as such by the director of the health center and reported as such by the local government to the MHLW. During the investigation of the outbreaks, family members and colleagues of the patients were encouraged to have stool examination and it was revealed that approximately 35% of STEC infections were asymptomatic and that the proportion of asymptomatic infection was high among the middle-aged group whereas symptomatic cases were more frequent in young and old age groups[174].

Apart from NESID, results of characterization (serotypes, Stx types, etc.) of STEC isolates at prefectural and municipal PHIs are reported to the Infectious Disease Surveillance Center (IDSC) at the National Institute of Infectious Diseases (NIID). The summary showed that STEC O157 serogroup is the predominant one, followed by O26, O111, O103, O121, O145, and others. However, as seen in the United States[163] and continental Europe[175,176], the percentage of non-STEC O157 serogroups among all STEC isolates from human infection has been increasing slightly; the rate of isolation frequency of STEC O157 has declined from approximately 70% of all STEC isolates in 2000 to about 60% in 2015[177,178]. As a collaborating laboratory surveillance system between prefectural and municipal PHIs and NIID, PulseNet Japan, a national laboratory network that connects foodborne illness cases to detect outbreaks by the use of DNA fingerprinting of the isolates, has been established[179]. It constitutes a part of PulseNet International[180], and has contributed to investigations of domestic[181] and international STEC O157 outbreaks[182].

2-5-3. Outbreaks in Japan

In the outbreaks with more than 10 culture-positive patients reported to the IDSC from 2000 to 2012, the main mode of transmission of the infection was person to person (41%), food borne (29%), and water borne (3%,), and in about one-third of the outbreaks, the mode of transmission of the infection remains unknown[174]. One major setting of these outbreaks was nursery schools, which may account for the high proportion of person-to-person transmissions of the infection in the outbreaks. The most prevalent serogroup of STEC in the outbreaks in nursery schools was O26 (52%), followed by O157 (27%), O111 (9%), O103 (4%), O121 (4%), O145 (3%), and OUT[174], which may account for relatively mild clinical manifestations, including asymptomatic cases and, consequently, frequent person-to-person transmission observed in these outbreaks. Increased STEC outbreaks in childcare facilities due to non-O157 serogroups, particularly O26 and O111 during 2010 to 2013, were also reported by another group in Japan[150]. There were 13 STEC outbreaks that had more than 100 culture positive cases between 2000 and 2015(**Table 1**)[183,184]. All 13 outbreaks appear to have resulted from consumption of contaminated foods, and, in some of these outbreaks, microbiological testing confirmed the implicated foods. These included beef products[121]; lightly salted cucumber[122]; *Koumi-ae* consisting of boiled spinach and steamed chicken meat seasoned with welsh onion, ginger, and soy sauce[122]; boxed meals[185]; lettuce[5]; school lunches[6]; *Yukhoe* (raw beef)[7], and Japanese rice cakes[7].

Low infectious doses of STEC--possibly fewer than 10 organisms[80,81]-- is a critical factor in the transmission of the STEC, when people consume raw or lightly cooked foods such as sushi and vegetables. Because sushi and raw or lightly cooked meat are popular foods in Japan, there have been outbreaks associated with consumption of salmon roe sushi in 1998[123] and "rare" roast beef contaminated with STEC O157 in 2001[121]. In an STEC O111 outbreak associated with consumption of *Yukhoe* at Yakiniku chain restaurants, STEC O111:H8 was isolated from 85 of 181 patients (median age 20 years); in 34 of those patients HUS developed; encephalopathy developed in 21 patients; and 5 patients died[7]. HUS occurred most frequently in individuals aged 5–9 years, and this age group was significantly associated with acute encephalopathy[186]. STEC O111:H8 was also isolated from the conserved part of the original meat preparations distributed to the chain restaurants.

Some of the outbreaks were associated with consumption of vegetables. In addition to two large outbreaks in 2011 (**Table 1**), there were four outbreaks associated with consumption of vegetables[7]; cabbage was identified as a vehicle of STEC O26:H11 in an outbreak and STEC O157:H7 was isolated from pickled eggplant and green perilla, green perilla served with grated radish, and cucumber in three outbreaks, respectively. A large outbreak of STEC O157:H7 infection traced to a brand of lightly salted vegetables occurred in Sapporo, Hokkaido in 2012[8]. STEC O157:H7 was isolated from the implicated product. Since the products were widely distributed, 169 patients were reported from five facilities for the elderly, hotels, restaurants, and families in Hokkaido and included four cases in different prefectures from which STEC O157:H7--with indistinguishable PFGE patterns and identical MLVA type--was isolated. STEC O157:H7 was isolated from 73 of 169 patients, eight of whom, mostly elderly,

Table 1 Foodborne Outbreaks Caused by STEC in Japan

Year	Prefecture/City	Setting (reference)	Serotype	Stx type	Symptomatic cases	Culture positives	Likely mode of transmission
2001	Chiba P.	Patient's home (113)	O157:H7	Stx1&2	195	257	beef products (a)
2002	Fukuoka C.	Nursery school (114)	O157:H-	Stx2	74	112	lightly salted cucumber (a)
2002	Utsunomiya C.	Hospital and home for the elderly (114)	O157:H7	Stx1&2	123	111	Koumi-ae (a)
2003	Yokohama C.	Kindergarten (183)	O26:H11	Stx1	141	449	Foodborne
2004	Ishikawa P.	High school (184)	O111:H-	Stx1&2	110	103	Foodborne
2007	Tokyo M.	School refectory (173)	O157:H7	Stx2	467	204	Foodborne
2007	Miyagi P., Sendai C. & Akita C.	Restaurant (173)	O157:H7	Stx1&2	314	173	boxed meals (a)
2009	Saga P.	Nursery school (5)	O26:H11	Stx1	N.D.	133	lettuce (a)
2010	Mie P.	High school (6)	O157:H7	Stx2	138	164	school lunch (a)
2011	Toyama P.	Chain restaurants (7)	O111:H8	Stx2, Stx-	181	102	Yukhoe (raw beef) (a)
			O157:H7	Stx1, Stx2,Stx1&2		38	
2011	Yamagata P.	Festival (7)	O157:H7	Stx1&2	287	189	Japanese rice cakes (a)
2012	Osaka C.	Nursery school (8)	O26:H-	Stx1	68	115	Foodborne
2014	Shizuoka C.	Street stall (10)	O157:H7	Stx1&2	510	193	Foodborne

(a) Confirmed microbiologically; ND, no data.

died. In August 2014, there was STEC O157 food poisoning of 510 cases, who consumed contaminated lightly pickled cucumbers sold at food stands during a fireworks display in Shizuoka Prefecture[10].

2-6. HUS

HUS is a life-threatening illness characterized by hemolytic anemia, thrombocytopenia, and renal failure, and it is the most common cause of acute renal failure among children in the United States[187]. Foodborne Diseases Active Surveillance Network reported that among 3464 STEC O157 infections in the period of 2000-2006, 218 persons (6.3%) developed HUS and the highest proportion of HUS cases (15.3%) occurred among children aged less than 5 years and that death occurred in 0.6% of all patients with STEC O157 infection and in 4.6% of those with HUS[188]. In Italy, an average of 33 sporadic cases of HUS per year were observed between 1988 and 2012, with a mean annual incidence of 0.4 cases per 100,000 residents aged 0–15 years[176]. In 15 EU countries, A total of 382 (6.6%) confirmed STEC cases (n=5746) developed HUS in 2012 and 59 per cent of HUS cases (n=226) were reported in 0–4 year-old children with O157 and O26 as dominant serogroups followed by 5–14 year old children with O157 as a dominant serogroup (74%)[168]. In Argentina, postdiarrheal HUS is endemic and approximately 400 HUS cases were reported annually between 2002 and 2011. The incidence ranged from 10 to 17 cases per 100,000 children less than 5 years of age, and lethality was between 1 and 4%[189].

In Japan, due to an amendment of the case definition of STEC in the Infectious Diseases Control Law in 2006, HUS patient should be reported as having STEC infection if Stx was detected in feces, or O-antigen agglutinating antibody or anti-Stx antibody was detected in the serum of the patient. From 2006 to 2015, the average annual number of HUS cases (including serodiagnosed cases) was 99 and the incidence rate of HUS (HUS cases/symptomatic cases) was 3.6% (**Fig. 3**)[111,190–197]. As reported in previous studies, increased rates of HUS in children less than 10 years old and the elderly[198,199] are shown in **Fig. 3**.

From 2006 to 2015, 65% of the 985 HUS cases were culture-confirmed by laboratory testing, and the rest of the cases were diagnosed by detecting antilipopolysaccharide antibody of *E. coli* in the serum of the patients or Stx in the stool samples of the patients.

STEC O157 was the predominant serogroup, occupying

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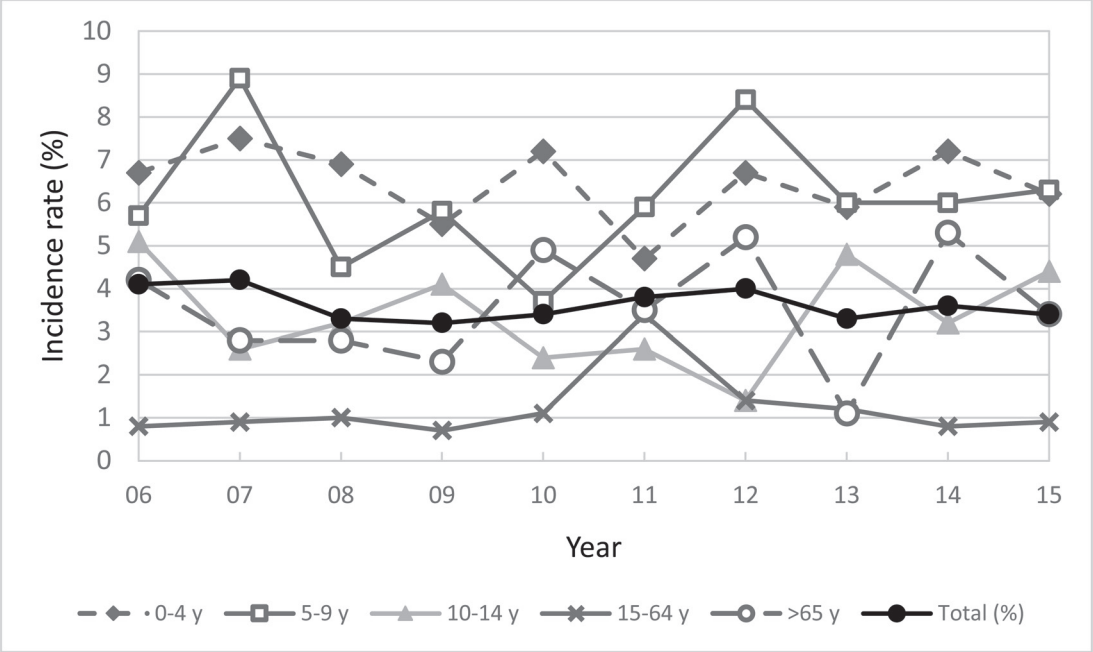

Fig. 3. Incidence rate of HUS in STEC infection by age groups in Japan, 2006 to 2015. Incidence rate (%) was calculated as (number of patients with HUS) ÷ (number of symptomatic cases) × 100%.

85% of all isolates in culture-confirmed HUS cases, followed by O111 (4.4%), O26 (2.7%), O121 (2.3%), O165 (1.2%), O145 (0.6%), and the rest of the O serogroups, including O55, O74, O76, O115, O174, O183, and unknown serogroup samples. Although non-O157 serogroup strains were isolated in the culture-confirmed HUS cases, 94% of all STEC isolates in the culture-confirmed HUS cases were either Stx2 or both Stx1 and Stx2 producers, which is consistent with epidemiological evidence that Stx2-containing STEC O157:H7 strains are more frequently associated with HUS than the strains containing Stx1[46,47,71].

2-7. Infection Control

Although it is rare to be able to find the source of infection in sporadic cases of STEC infection, many outbreaks of STEC infection have been associated with foods that become contaminated through direct or indirect environmental exposure to waste products of cattle. Therefore, implementing effective measures to reduce or eliminate STEC from all stages of the food chain, starting from production to consumption would lead to reducing STEC infection in humans. All steps ranging from reducing carriage of STEC by cattle used in food production to proper food preparation to kill STEC before consumption should be included. Certain farm management practices, especially those related to prevention of contamination and multiplication of STEC in feed and water, may provide practical means to reduce the prevalence of STEC in cattle on farms and in slaughter plants[200]. Good hygienic practices during food production are essential to keep microbiological contamination to a minimum. The most effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating[201,202] or irradiation[203–206]. Since person-to-person contact is an important mode of transmission through the oral-fecal route in STEC infection, good hygiene practice is especially important in settings such as child-care facilities, where persons at high risk for STEC infection spend considerable time together. In addition, as the median duration of shedding reported from previous outbreaks in childcare facilities has been found to be between 20 and 50 days[147,207–209], exclusion of ill persons from the facilities until the diarrhea has resolved should be considered. In general, routine handwashing before eating and after diaper changes and toileting is the best way to prevent the spread of infection in child-care facilities.

3. Control Measures Against Foodborne Disease Due to STEC

Among preharvest food safety interventions to reduce the prevalence and shedding level of STEC O157 by cattle, vaccines have been the most effective interventions documented to date.

Currently, only two commercially available vaccines against *E. coli* O157:H7 in cattle exist: a type III secreted proteins (Bioniche Life Sciences Inc., Belleville, Ontario, Canada), and a siderophore receptor and porin protein (Epi-

topix, LLC, Wilmar, MN, USA). Several systematic reviews and meta-analyses suggested that both vaccines effectively reduce the probability of feedlot cattle to shed STEC O157 in feces[210–213].

Some of the lessons learned from STEC outbreaks have resulted in long-term improvements in food safety. In 1994, the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) declared STEC O157:H7 an adulterant in ground beef in response to the multistate outbreak caused in 1993 by undercooked hamburgers contaminated with STEC O157[214]. Following these outbreaks, the U.S. Food and Drug Administration (FDA) issued more stringent guidelines for the internal temperature of cooked hamburgers[215]. The pronouncement from FSIS was extended in 1999 to all nonintact raw beef products[216]. Since the number of infections and outbreaks due to STEC non-O157 has increased, FSIS documented risk profile for pathogenic non-O157 STEC and concluded that STEC O157 was not the only STEC representing a hazard[217]. Furthermore, FSIS decided on the implementation of sampling and testing manufacturing trim and other raw ground beef product components for STEC non-O157 in 2012[218]. STEC strains of serogroups O26, O111, O103, O145, O121, and O45 were declared as adulterant in these food commodities and included in the sampling plans in addition to STEC O157.

In the European Union (EU), although the limitations exist in categorizing the level of danger associated with STEC from nonhuman sources, the seropathotypes A and B of Karmali's scheme[219] formed a large consensus in the scientific community and were endorsed by the European Food Safety Authority (EFSA). The scheme was based on the evaluation of the virulence and serological features of the strains combined with their association with severe disease and epidemic outbreaks. STEC strains belonging to serogroups O157, O26, O111, O103, and O145 were included in the seropathotypes A and B of the scheme. EFSA recommended focusing food testing for STEC on the seropathotype A and B groups[220,221]. However, the massive outbreak caused by an enteroaggregative STEC O104:H4 strain in 2011 in Germany and other 12 European countries forced the European Commission (EC) to take measures against the possibility of other STEC crises in the EU. EFSA was asked to assess the public health risk caused by STEC and other pathogenic bacteria that may contaminate both seeds and sprouted seeds intended for direct human consumption[222,223]. Finally, the European Commission issued Regulation (EU) 209/2013, containing the microbiological criteria for STEC in sprouts and amending Regulation (EC) 2073/2005, which introduced for the first time in EU legislation a specific criterion for STEC regarding the presence in sprouts of the five STEC serogroups, i.e. O157, O26, O111, O103, and O145 plus STEC O104:H4[224].

In Japan, STEC infections have been routinely notifiable since 1996. They are also reported as food poisoning by physicians or judged as such by the director of the health center under a surveillance system for food poisoning. Furthermore, the Abattoir Law Enforcement Regulation (Ministry of Health and Welfare Ordinance No. 44, September 28, 1953) was amended by the MHLW so that the measures for prevention and reduction of STEC contamination at every step of the processes such as tying the rectum before evisceration at slaughter houses could be fulfilled. The MHLW has also formulated standards of a hygienic control manual for large cooking facilities based on the HACCP (Hazard Analysis and Critical Control Point) to prevent food poisoning due to food provided by these facilities. Although the official detection methods for STEC O26, O111, and O157[225] was in use, a newer detection method for STEC O26, O103, O111, O121, O145, and O157 in food[226] has been established and validated,[227] reflecting a steady increase in non-O157 STEC infections. The method involved a combination of various chromogenic agars, targeting particular STEC serogroups, and molecular approaches such as real-time PCR based on the methods used by the USDA[228] and EFSA[221]. In response to persistent food poisonings caused by raw beef, the MHLW revised the standards of beef product quality for raw-eating and put them into operation in October 2011. Further, after STEC O157 was detected in the inner part of cattle livers, the MHLW banned the marketing of cattle liver intended to be eaten raw. Probably as a consequence of these preventive measures, the incidence of STEC O157 cases related to consumption of raw meat decreased by almost half in one year, from 2011 to 2012[8].

4. Conclusions

STEC infections continue to occur due to a variety of foods contaminated by this bacterium. Although foods of bovine origin contaminated with this pathogen have been a major source for infection, evidence of disease linked to other sources, including contaminated produce, water, and other environmental exposures, indicates a necessity for a comprehensive approach to STEC reduction or elimination at all levels of food production. However, despite various control measures taken today, reported incidence of non-O157 STEC has been gradually increasing, partially due to recent improved detection methods, and massive outbreak due to a rare category of STEC that belonged to enteroaggregative *E. coli* occurred in Europe, indicating the complexity of securing food safety when dealing with STEC.

Surveillance of STEC infections, especially laboratory investigation of the implicated isolates based on molecular analytical methods, have revealed that complex ecology and genetics of this pathogen existed and indicated that combining the results of isolate analysis with epidemiological information were important for accurate determination of the infectious source. Therefore, cooperative interplay of relevant authorities referring to public health, food, and veterinary science are indispensable for establishment of effective control measures and prevention against STEC infection.

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Conflict of interest statement

The authors had no conflicts of interest to declare in this article.

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