OVERVIEW OF O157 IN PAKISTAN: AN IMPORTANT FOOD-BORNE DISEASE OF PUBLIC HEALTH

Sadaf Ismail
Department of Microbiology, University of Balochistan, Quetta-87300, Pakistan.

Kaleem Ullah*
Department of Microbiology, University of Balochistan, Quetta-87300, Pakistan.

Hafsa sunnya
Department of Microbiology, University of Balochistan, Quetta-87300, Pakistan.

School of Life Science and Technology, Beijing Institute of Technology, Beijing, 100081, China

Xie Bingjie
School of Life Science and Technology, Beijing Institute of Technology, Beijing, 100081, China

Corresponding Author: kaleemattal@yahoo.com

Abstract

Food-borne microorganisms are the source of 65% of the human food-originating ailments present globally and with the highest load in under-developing nations including Pakistan. Thus, this review focused on the background of E. coli O157, a significant food-borne bacteria which is pathogenic, and its public health perspective in general and particularly in Pakistan. Dairy animals and their products are the chief means of spreading and challenging public health. Dairy products, meat, and eggs are the main items through which the public is vulnerable to E. coli O157, which leads to food-borne illness and consequently death. Virulence and production of toxins are accountable for the pathogenesis of E. coli O157, which are categorized chiefly by gastrointestinal disorders including moderate and severe nausea, abdominal cramps, vomiting, diarrhea, and other species-specific symptoms. Proper hygiene, good manufacturing practices, sanitation in operating procedures of products, by-products,
and execution of standardized hazard investigation and critical control points (HACCP) plus pasteurization measures are important approaches for the control and safety of Public Health. In recent times, the emergence of \textit{E. coli O157} multidrug-resistant (MDR) related with ingesting of polluted animal originating food products and by-products are a great alarm for public health and should be managed by surveillance and monitoring of \textit{E. coli O157}, particularly in developing countries including Pakistan.

**Keywords:** \textit{E.coli}, Gastrointestinal disorders, Dairy products, Food-borne bacteria

### I. Introduction

The awareness of affiliation between food intake and human illnesses was vigilantly perceived early in the 460 B.C. Hippocrates stated a vigorous acquaintance between food consumption and human diseases (Hutt \textit{et al.}, 1984). Food-borne illness is demonstrated as a batch of disorders, that turn out as a result of foodstuff ingestion either of microbial contamination or chemical contamination (Javed, 2016). The incidence of further cases of identical illness emanating from the digestion of the same food is interpreted as a food-borne outbreak. Biological agents that cause food-borne disease incidences include viruses, bacteria, and parasites (CDC, 2012). Likewise, 200 varied food-borne diseases have been diagnosed, the severity of which is equally present in the older age group people, in the young, in the immune compromised people and healthy individuals (CDC, 2012; Ishaq \textit{et al.}, 2020).

In the 21\textsuperscript{st} century, food-borne illnesses have been declared as one of the major medical problems (Beyi \textit{et al.}, 2017). They exemplify crucial public health predicaments all over the globe. The potency of pathogens causing fatalities and illnesses features the influence of such incidences which subsequently need monitoring and prevention. Every year, many countries report millions of cases of food-borne
disease and chronic complications (Schirone et al., 2019). The dilemma is worse in on-progress states because of lower infrastructure, resources and inadequate public awareness (Zhoa et al., 2001).

**Food-borne Pathogen**

 Universally, food-borne illnesses are produced via abundant sets of bacteria, prions, viruses, parasites, and fungi (Rodríguez-Morales et al., 2016). Among food-borne health matters, it is ascertained that microbial sources are more quotidian than the chemical ones (Javed, 2016). As regards "The WHO food-borne Diseases Burden Epidemiology Reference Group (FERG)" documented in 2015 the estimated prevalence using 2010 as the reference year, death rate and disease burden in term of "Disability Adjust Life Years (DALYs)". It illustrated marked dissimilarity heeding food-borne illness load among Subregion demarcate on the grounds of child and adult fatalities. This document states the estimation of 31 food-borne hazards causing 32 diseases in which 11 are diarrhea associated causative agents in a ratio of (1 virus, 7 bacteria, 3 protozoans) that collectively instigated 600 (95% uncertainty interval [UI] 420 to 960) million food originating diseases and 420,000 (95% UI 310,000 to 600,000) fatalities in 2010. Globally 18 million (95% UI 12 to 25) DALYs were credited to food-borne diarrheal illness causative agents, mainly pathogenic *E. coli* (FAO/WHO, 2018).

**Escherichia coli**

 *E. coli* is a rod-shaped, gram-negative, non-spore-forming facultative anaerobic group of bacteria (Razzaq et al., 2014) that may or may not hold flagella (Bintsis, 2017). It was primarily described by Theodor Escherichia in 1885 (Lim et al., 2010). It is taxonomically a well-demonstrated member of the family *Enterobacteriaceae*. Most strains of *E. coli*, enter the intestinal region of humans and also animals without defacement like usual flora. Although, few strains are equipped with virulence factors via plasmids, transposons, bacteriophages, pathogenic islands, some strains have come
up with the production of toxins. Divergent pathovars or pathotypes of *E. coli* effectuate the greater number of deaths and diseases for a reason of sustaining the low infectious dose and may disseminate by way of all-pervasive mediums, embracing food and water (Croxen *et al.*, 2013).

**ECO: Pathotypes**

The infective *E. coli* are assorted on the foundation of serogroups, virulence issues, clinical symptoms, and pathogenicity mechanisms (Kaper *et al.*, 2004). It can be categorized into six groups (Bokhari *et al.*, 2013).

- Enteroaggregative *E. coli* (EAEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enterohemorrhagic *E. coli* (EHEC)
- Shiga-toxin producing *E. coli* (STEC) or Verotoxigenic *E. coli* (VTEC)
- Enteroinvasive *E. coli* (EIEC)
- Diffusely Adhesive *E. coli* (DAEC)

Certain EHEC serotypes intermittently cause human-linked diseases including O26:H11, O91:H21, O111:H8, O157: NM, O157:H7 (Paton *et al.*, 1999). STEC, apart from O157 are generally termed non-O157 STEC. Non-O157 STEC followed as O26, O45, O103, O111, O121 and O145 have come out as serious food originating pathogens in diverse areas of the globe (Valilis *et al.*, 2018).

**II. STEC O157:H7: Infections**

The STEC ancestry is multitudinous. An extensive array of O: H serotypes have been deemed to be kindred with human illnesses. Nevertheless, empirical evidence denotes precise STEC serogroups, in particular O157:H7 constitutes an uneven massive number of crucial infections (Karmali, 1998).
*E. coli* strain O157:H7 is the domineer serotype rendering a Shiga-toxin producing *E. coli* breed cognate with human illnesses (Beauvais *et al.*, 2017). It was initially spotted as a pathogen in 1982 (Lupindu *et al.*, 2018) in a course of an outbreak investigation of hemorrhagic colitis (Riley *et al.*, 1983). Meanwhile, numerous epidemics have been described from various parts of the world. STEC O157: H7 are contemplated influential food-borne and waterborne pathogens of the zoonotic implication related to distinct clinical indicators fluctuating from asymptomatic to disease severity. A few sole can thrive potentially lethal and devastating convolutions such as diarrhea, thrombotic thrombocytopenic purpura (TTP) (Elmali *et al.*, 2005), hemorrhagic colitis (HC) an acute foodborne disease (Carney *et al.*, 2006), and deadly sequela hemolytic uremic syndrome (HUS)-a serious problem intuited by prolific bleeding headed up to a failure of kidney and death (Lim *et al.*, 2010; Valilis *et al.*, 2018), a chronic renal sequel may abide in survivals (Beauvais *et al.*, 2017).

The ability of prevalence and biological characterizations following low infectious dose, expression of distinct virulence factors, prolonged survival in environment, this rare serotype STEC has widely prevailed in food products and environment, causing infections in humans. The arduousness in treatment of STEC O157:H7 is making it an enteric pathogen of problematic concern all over the world (Lupindu, 2018).

**Virulence Factors**

STEC holds multiple pathogenic factors comprising Shiga toxin (*stx*), *E. coli* attachment, and the enterohemolysin (*ehxA*) and effacing (*eae*) genes. They are distinguished with one or more Shiga toxins (*Stxs*) that are the chief pathogenic marker linked with predominant HC and HUS cases of humans. *Stxs* impedes the synthesis of protein inside the cells, eminently renal endothelial cells (Mccarthy *et al.*, 2021). Furthermore, they target the globotriaosylceramide (Gb3) receptor observed beside human renal endothelial cells and monocytes. The absence of this receptor in ruminants
that acquire Gb4 receptor variant instead, makes them shelter the pathogen symptoms-free for an extended period. The two utmost groups of Stx toxins (Stx1 and Stx2) share 55% sequence of amino acid homology (Caprioli et al., 2005; Gyles, 2007). The E. coli effacing (eae) and attaching gene is sited on the locus of the enterocyte effacement (LEE) encoding protein of outermost membrane intimin which aids in effacement and attachment of E. coli to the epithelial coating of the intestine (Xu et al., 2016). Attachment and effacing (A/E) lesion formation is instigated when STEC disrupts which in proceedings enables insinuate adherence of the bacterium to epithelial cells of host intestine. Another remarkable pathogen factor of STEC is enterohaemolysin, encoded by ehxA gene, simultaneously its existence in HC and HUS case isolates implies the consequential application of ehxA in the pathological process (Mccarthy et al., 2021).

Ruminants may preferentially harbor different Stx variants (Shridhar et al., 2017). The subtypes Stx2d and Stx1c are firmly related to sheep (Alonso et al., 2016). The capability of STEC to adhere and colonize the host’s lower gastrointestinal tract is a threatening factor in community and persistence of STEC in ruminants (La Ragione et al., 2009).

**Infectious Dose**

The infectious dose of the organism on a level that initiates disease symptoms in a person has been stated at the lowest possible level as 4 to 24 organisms (Howie et al., 2003). Ruminants are claimed to be the source when cattle have been taken into consideration as the fundamental origin of infection following goats, sheep (Mersha et al., 2010) and buffaloes which assist in the spread of the pathogen, exempted camels (Irshad et al., 2020). Animals like pigs (Ateba et al., 2008; Ojo et al., 2010) and pigeons have been articulated to entail virulent strains of E. coli. As in polluted water, fish has stated to also have STEC O157:H7 (Tuyet et al., 2006; Lupindu et al., 2018).
Transmission

The most persistent way of the spread of STEC O157:H7 is to consume polluted water, food, marine sediments and by inanimate objects such as soil, manure, air (Chigor et al., 2010; Hutchinison et al., 2005; WHO, 2015), but it can also be dispersed straight from human to human, from hands to mouth, from animal to human, through the fecal-oral route (Cleary et al., 2021) and peculiarly in day-care facilities. Several reports have documented the human infection amalgamation with dairy farms and grounds where cattle have been grazing (Heuvelink et al., 2002). STEC being a zoonotic pathogen of public health (Jaros et al., 2014), contaminated food such as beef products (Frost et al., 2005; Grys et al., 2005), mutton, raw milk, fermented sausage, mayonnaise (Elmali et al., 2005) and chicken.

Aside from 350 outbreaks documented by the CDC from 1982-2002, the resolute transference ways were food-related (52%), ambiguous (21%), through people (14%), water-related (9%) besides animal junction (3%). Isolation of pathogen from shellfish for a reason of contamination of the marine environment has inferred a great threat (Gansheroff et al., 2000). Inadequately cooked or processed food heads up for the dissemination of pathogens. Natural habitats for the E. coli O157:H7 are ruminants including cattle, buffalos, sheep (Tanveer et al., 2017), shed the pathogen in their wastes/feces (Jaros et al., 2014). Goat cannot colonize E. coli O157:H7 but due to its natural behavior, it may imply a major part in spreading infection either direct or indirect (Mian, 2021). The regular channel for epidemics is ground beef contaminated with E. coli. The products can get contaminated by the time of slaughter and minced beef may pass on the pathogen into the meat, consequently, survival of the pathogen is probable if it is cooked halfway (Samad et al., 2018; Ishaq et al., 2020).

Moreover, an array of contaminated food vectors have been allied to the E. coli O157:H7 incidences apart from beef, incorporated raw milk, potable water, salami, fresh juices, vented raw vegetables, fresh lettuce, radish sprout, and apple cider. The
immense epidemic was pursued by radish sprout contamination at Osaka, Japan in 1996, in which 7,966 persons were inspected with confirmed infection (Michino et al., 1999). STEC O157:H7 has a potency to survive in unfavorable conditions like low pH, poor personal hygiene after contact and handling animals before eating rises a huge risk to public health. These investigations call for the examination of sterility after exposure to animals, assumed areas during food preparation (Crump et al., 2002; Smith et al., 2004).

III. STEC: Occurrence World Wide

Human diseases interlinked with water and food-borne pathogens are as ancient as the Universe. Worldwide waves of waterborne and foodborne diseases epidemics have been reported. food-borne illnesses are more frequent in third-world states than progressed ones. But it doesn't indicate any region or state free of any disorders (Javed, 2016). *Escherichia coli* O157:H7 of all times is a more constantly marked out STEC serotype. Likewise, hundred STEC serogroups have been kindred to human diseases; “serogroup O157” is the well-known reason for STEC diseases in the US. “Center of Diseases Control and Prevention” (CDC) has documented that “STEC O157” may solely cause approximately 73,000 illnesses (Mohsin et al., 2007), 2,200 are placed for medical care and 60 fatalities occur per annum in the United States (Tack et al., 2021).

In 2015, STEC O157 was taken into consideration for 36.6% and 41% of infections in the United States and European Union, sequentially (CDC, 2017; EFSA and ECDC, 2018). In 2011 in European Union and New Zealand STEC infection rate was 1.93 and 3.5 cases per lac populace, respectively. In 2010, 1.78 cases of STEC illnesses per lac population were reported in the United States. Between 2002 and 2005, in Argentina, only 4 reports of HUS were recorded that were associated with food intake. In 2012, Australia reported 0.5 per lac populace cases of STEC contamination (Mian, 2021).
In 1982, 2 drastic epidemics of HUS arose in Michigan and Oregon. The leading cause of the disease was STEC O157:H7 isolated from the samples of the stool of the patients. Stx assembly differed from the toxins isolated from S. dysenteriae and 3 E. coli insulates from the epidemic (O’Brien et al., 1983). As of the date, STEC O157 has expeditiously appeared as the central obstacle in the food industry and clinical esthetic (Kim et al., 2020). In 2016 and 1998, the Western Pacific Region (WPR) and European region (EUR) reported 176 and 35 outbreaks, respectively, which is far-reaching than the number of outbreaks in America (FAO/WHO, 2018). In 1999 The immense epidemic recorded at any time happened in Japan (Michino et al., 1999).

Most STEC infections are not correlated to known outbreaks but the statistical features acquired through investigation of outbreaks postulate salient data for an understanding of spreading modes and other paths that have been illustrated in varied periods (Heiman et al., 2015).

Remarkably, though non-O157 illness is almost twofold common like O157 illness, non-O157 source fewer outbursts than the O157 (Scallan et al., 2011). This could be a reason for greater infection of O157 or problems with the subtyping methods as it is problematic to subtype the non-O157 type strains (Gould et al., 2013). The epidemic surveillance statistics from CDC stated that E. coli O157:H7 diseases are reducing following the climax in the year 1999. However, massive epidemics and irregular cases pursue to come about (Tack et al., 2021).

IV. STEC: Occurrence in Pakistan

In Pakistan, investigation data about the bacterium is very scarce. However, several reports have demonstrated the pathogenic organism (Mian, 2021). In Pakistan, there is an intensive consumption of raw fresh vegetables, raw fruit juices (apple, cider), alfalfa sprouts, beef meat, dairy products (cheese), dry curd, poultry meat (Iqtedar & Yasin, 2014) particularly chicken, largely consumed throughout the world. In
the last few decades, the demand for poultry meat has elevated. Pakistan has made great strides in the industry of poultry by producing 0.652 million tons of meat per year, constituting the 20 to 25% of the total meat production in the country. Raw meat may harbor many important pathogenic microbes producing meat a significant danger for human health. Particularly chicken that is largely consumed throughout the world (Sunniya et al., 2017). Sliced fruits and chopped salad trading is a common practice. Pandemics usually occur when the vegetables and fruits are washed with contaminated water that transmits through fecal, raw manure, or sewage fertilizers (Razzaq et al., 2014).

**Peshawar:** In the year 2009, a study stated the occurrence of STEC O157:H7 in raw milk sold in Peshawar City, posing a great hazard to public health (Abid et al., 2009).

**Lahore:** A report documented the existence of *E. coli* O157:H7 in sugarcane (13%), plum juices (41%), peach (53%), lemonade juices (50%) of total study and slush revealed countless contamination in various areas of Lahore City (Iqtedar & Yasin, 2014). Tanveer (2017) analyzed *E. coli* O157:H7 existence in minced chicken meat (30%).

**Quetta:** An investigation from discrete areas of Quetta city examined food-borne pathogens in chicken, beef meat, raw milk, and vegetables. The study results revealed 8% of the total samples in which beef, milk, and salad samples exhibited the higher percentage (12%) of *E. coli* O157:H7. The report illustrated the high prevalence of STEC in the local markets of Quetta (Samad et al., 2018). In his other study, Samad stated 10% in beef meat and observed the season-wise diversification manifested the incidence of *E. coli* O157:H7 particularly in the summer months (Samad et al., 2018). Another study from Quetta by Tahira inspected the incidence of *E. coli* O157:H7 in raw milk (Tahira et al., 2017).
Kohat: Afreen et al. (2013) investigated the presence of high rates of multi-drug resistant enteropathogen which are correlated with infantile diarrhea, one of the serious public health matters in Pakistan. Samad et al. (2018) also demonstrated resistance to a range of regular antibiotics emphasizing the global warning to public health.

V. Safety Measurements

The food industry and competent authorities have done many efforts to ensure the safety of food and consumers, still, it remains a noteworthy public wellbeing challenge. In Pakistan, there exists no routine investigation and monitoring organization for various STEC particularly of zoonotic status. Microbial toxins should be addressed to the general influence of a worldwide food system and current food-processing technology on microbial food safety. In such an attempt to meet new trends in food consumption and preparation, alternative procedures and technologies such as high hydrostatic pressure, ultrasound, strong light pulses, supercritical CO₂, cold plasma, and pasteurization have risen to replace existing and established heat treatments. These technological advances are less harmful to food, but they may also be harmful to resident bacteria and their toxins (Rajkovick et al., 2019). Sunniya (2017) advises in her investigation that the food safety regulations should be properly followed in line with HACCP to reduce food-borne diseases and maintain the microbial burden under check (Hazard analysis critical control point).

References


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