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Review Article

Shiga Toxin-Producing *Escherichia coli*: A Focused Review of the Middle East Countries

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ABSTRACT

The Middle East and Gulf region has been identified as high-risk for Shiga toxinproducing E. coli (STEC) infections due to the various outbreaks and cases reported in recent years. This research presents an overview of the virulence, epidemiology and clinical symptoms regarding this region's most frequently identified STEC strains. In addition to O157:H7, the six strains of concern in the Middle East and Gulf region are O26, O111, O103, O145, O45 and O121. These strains are known to generate Shiga toxins that cause severe gastrointestinal symptoms and lead to potentially fatal outcomes, including haemolytic uremic syndrome. The virulence of these strains stems from their ability to colonise the human gut and produce toxins such as adhesins, intimin and other toxins, inducing severe illnesses in the human gastrointestinal system. The prevalence of each STEC strain varies throughout Middle Eastern and Gulf countries, with O157:H7 being the most frequently identified strain, followed by O26:H11 and O103:H2. The epidemiology of STEC infections is complex, with several factors influencing strain distribution and transmission in the Middle Eastern and Gulf countries. Risk factors include contact with animals and their environment, ingesting contaminated food and drink and person-to-person transmission. In addition, lack of hygiene, poor food safety regulations and limited surveillance and reporting all contribute to the high prevalence of STEC infections in this region. The top six STEC strains discovered in the Middle East and Gulf region pose a significant public health risk due to their ability to cause major infections and illnesses. It is essential to comprehend the epidemiology, clinical symptoms and virulence characteristics of these strains to design efficient preventive and control measures. Further research and monitoring are needed to better understand the dynamics of STEC infections in this area and to implement appropriate policies to reduce their impact on public health.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) presents a serious public health issue (Glassman et al., 2022), as it is a significant cause of foodborne illnesses worldwide. The attendant illnesses range from moderate diarrhoea to fatal haemolytic uremic syndrome (HUS). Although *E. Coli*, a facultative, symbiotic Gram-negative anaerobe present in human intestines, is a relatively innocuous bacterium extensively utilised as a marker for faecal adulteration and hygiene breaches, the STEC are pathogenic variants (Figure-1). They represent several *E. coli* strains that have become virulent, allowing them to acclimatise to new surroundings and, in rare cases, cause severe sickness (Oliveira et al., 2023). These zoonotic (they exist as typical gut flora in ruminant animals, notably cattle) pathogenic variants spread from animals to

people through contaminated food, drinks, faeces or contact with infected plants and animals (Nada et al., 2023). This pathogenic subgroup of *E. coli* includes diffusely adherent *E. coli*, enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (Kaper et al., 2004; Bryan et al., 2015). The gastrointestinal illnesses caused by STEC are due to the powerful Shiga toxins (Stx) encoded in the bacteriophage on the EHEC chromosome (CDC, 2019). The term 'Stx' comes from the Japanese scientist Kiyoshi Shiga (1870-1957), who also gave his name to the genus *Shigella* since the toxin formed by *Shigella dysenteriae* type 1 is strikingly analogous to the compounds Stx1 and Stx2 formed by STEC (Glassman et al., 2022). The World Health Organization (WHO) has identified O157:H7 and another six serogroups of STEC



AUSMR



Figure-1. The main six categories of pathoginic *E. coli*.

Here, the interaction of each category with a typical target cell is schematically represented. $\mathbf{a} \mid$ EPEC adhere to small bowel enterocytes, but destroy the normal microvillar architecture, inducing the characteristic attaching and effacing lesion. $\mathbf{b} \mid$ EHEC also can induce the attaching and effacing lesion in the colon only. $\mathbf{c} \mid$ ETEC adhere to small bowel enterocytes and results watery diarrhoea by the secretion of heat-labile and/or heat-stable enterotoxins. $\mathbf{d} \mid$ EAEC usually adheres to bowel (small and large) epithelia in a thick biofilm and elaborates secretory cytotoxins and enterotoxins. $\mathbf{e} \mid$ EIEC invades the colonic epithelial cell, lyses the phagosome and moves through the cell by nucleating actin microfilaments. $\mathbf{f} \mid$ DAEC elicits a characteristic signal transduction effect in small bowel enterocytes that manifests as the growth of long finger-like cellular projections. Source: (Kaper et al., 2004).

most frequently linked with infections in humans worldwide (Panel et al., 2020). These are O45, O104, O26, O145, O111 and O103. The *E. coli* causing the most concern in the dairy sector is O157:H7, a STEC serotype with high potential virulence; it can cause illness even at minimal doses of 5–50 cells. Outbreaks caused by STEC-contaminated food are on the rise, and in instances involving food produced and distributed on a large scale, they can affect significant numbers of individuals (EFSA, 2019).

Reactive oxygen species (ROS), growth phase, antibiotics, temperature and quorum sensing are all environmental signals influencing Stx expression. There is currently no effective therapy or prophylactic for HUS. Since antibiotics induce Stx formation and their efficacy in treating EHEC infections is debatable, researchers are intensely investigating new treatments (Navarro-Gonzalez et al., 2020). Due to the typical clinical characteristic of bloody stools, STEC was formerly known as EHEC, an acronym still used today (Shridhar et al., 2017). Although EHEC is a disease-causing agent in humans, it is a symbiont in the gastrointestinal (GI) tract of cattle, and cattle faeces is a significant cause of EHEC contamination and transmission. Many researchers have focused on eradicating EHEC from ruminant GI tracts to minimise infections from this enteric disease-causing agent (Huang et al., 2020). Due to its cytotoxic activity on the kidney cell lining of Vero monkeys, STEC is also characterised as verocytotoxin-producing or 'verotoxin-producing' *E. coli* (VTEC).

The most common STEC serotype linked with human sickness and the principal source of HUS is the STEC strain, O157 (Lee et al., 2016). Cattle cause most of the global zoonotic STEC infections, and they are the primary reservoir for O157 STEC and several key non-O157 STEC strains, such as O113, O103, O111 and O26 (Figure-2). Infection by STEC typically causes diarrhoea marked by bloody stools (known as bloody diarrhoea) and usually resolves on its own (Figure 1) (Alharbi et al., 2022). However, around 5%-7% of patients develop HUS, the most toxic complication of STEC infection, which involves thrombocytopenia, haemolytic anaemia and abrupt renal failure (Smith et al., 2016; Tunsjo et al., 2023); the young and the elderly are more susceptible to STEC complications. In children with STEC infection, HUS frequently occurs shortly after the advent of diarrhoea. It is a potentially fatal illness with a 5% fatality rate and is caused by the discharge of Stx. However, other STEC strains, such as O26, O111 and O103, have also been



Figure 2. The common sources of infections associated with STEC infections. Source: (Alharbi et al., 2022)

linked to significant human illnesses worldwide. Most STEC serotypes do not cause disease in cattle; however, a few serotypes, including O157, O113, O5 and O26, cause diarrhoea, especially in young calves (Figure-2). The infections are transmitted to people through raw or poorly cooked beef (or beef products), vegetables polluted with cow excrement, raw or improperly pasteurised milk (or milk products) and direct exposure to host animals or their natural derivatives (Lee and Tesh, 2019).

Bacteria that evolve to become antimicrobial-resistant are a vital public health concern, with approximately 10 million projected deaths per year by 2050. Worldwide, STEC isolates with genes that produce enzymes extended-spectrumlactamase (ESBL) have been discovered in both humans and animals. They are resistant to an extensive range of lactam antibiotics widely utilised in clinical and veterinary treatment. Moreover, clinical E. coli, which produce carbapenemase segregates in humans, are snowballing worldwide (Kintz et al., 2017). The discovery that STEC serotypes other than O157 were connected to haemorrhagic colitis and diarrhoea has accelerated the designing of laboratory testing protocols to identify the more than 150 STEC non-O157 strains that have since been discovered. The proportion of STEC serotypes sequestered from ruminants and sick people varies widely, emphasising the need for clinical laboratories to identify common endemic serotypes while also looking for less frequent or imported serotypes in unusual diseases (WHO, 2019). Other non-O157 STEC also harbour and express Stx, existing on bacteriophages in the STEC genome, causing haemorrhagic colitis, diarrhoea and HUS-like STEC O157 infections (Espinosa et al., 2018).

The ingestion of raw vegetables, water, unpasteurised or poorly pasteurised juices or milk polluted with STEC from cattle excrement are the common infection vectors. Direct transmission from human to human and animal to human occurs frequently worldwide (Byrne et al., 2014). In the following sections, STEC, EHEC, and VTEC are all abbreviations used interchangeably.

Methods for Detecting STEC

In general, culture-dependent approaches are used to count and isolate foodborne pathogens. These techniques are time consuming, but they are accurate, affordable and enable qualitative and quantitative assessment of the microorganisms present in a sample. One amino acid differentiates Stx1 from the Shiga toxin of the serotype *S. dysenteriae*, and Stx2 has an approximate 60% amino acid resemblance to Stx1. The genetic sequences of Stx1 and Stx2 variations are known, and a single STEC bacteria can create several variations (WHO, 2019). Identifying the O157 strain in stool specimens is limited by its inability to ferment sorbitol quickly. Therefore, O157 is grown on Sorbitol-MacConkey agar (SMAC).

However, most non-O157 STEC ferment sorbitol and cannot be distinguished from *E. coli* strains that are non-pathogenic on SMAC agar. Consequently, to diagnose non-O157 STEC, testing for Stx or the genes that produce them is necessary. Since non-O157 STEC are not commonly discovered in clinical laboratories using traditional stool culture techniques, their impact as pathogens is largely unknown (Withenshaw et al., 2022). Comprehensive laboratory testing has progressed, meaning diagnosing this illness from stool specimens is now standard. In addition, the clinician must engage with the clinical microbiology laboratory to ensure proper specimen gathering



Figure 3. Estimated prevalence of E. coli O157 in cattle in different countries. Source: (Islam et al., 2014)

and transportation and that cultures are appropriately prepared to retrieve and separate STEC O157 from stool samples submitted for other bacterial cultures (Gioia-Di Chiacchio et al., 2018). The benchmark for verifying the diagnosis is segregating a viable STEC culture from the stool samples (Ferraro et al., 2023).

Scientists are working on new and faster methods to identify food infections. Some techniques rapidly detect and quantify *Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus*, and *E. coli* that generate Stx in food samples, including immunologically based methods, nucleic acid-based tests and biosensors (Valderrama et al., 2016). The most common molecular detection techniques, such as polymerase chain reaction (PCR) and real-time PCR are time consuming, meaning there is an urgent need for a rapid screening assay that can be used in agri-food contexts. In one study, an amplification-free multiplex electrochemical sensor for the simultaneous detection of the Stx1 and Stx2 genes was developed using six interdigitated gold microelectrode sensors on a silicon-based device (Wasiewska et al., 2023).

Modern analytical techniques to identify STEC in foods frequently use PCR screening of the enrichment medium. However, when testing for DNA sequences linked to the disease, the PCR inhibitors found in food enrichments might result in misleading negative results. To avoid false-negative findings in enrichment screening, including DNA extraction procedures that effectively eliminate PCR inhibitors from various foods is desirable. This extraction procedure uses Bio-Rad InstageneTM Matrix according to the Canadian STEC standard, MFLP-52 (Bouvier et al., 2023). A rapid test is created based on amplifying two Stx genes by isothermal recombinase polymerase in a single reaction. On a single lateral flow paper strip, the outcomes of the amplification response for both Stxs are shown simultaneously. This approach is used to broadly detect Stx-producing bacterial species, explicitly targeting the DNA encoding Stx1 and Stx2. With a 10 CFU/mL detection limit, this approach may produce results in around 35 minutes. However, this sensitive and selective method can only detect Stx-producing bacteria (Petrucci et al., 2022). Instagene Matrix with Beckman Coulter Ampure XP Beads, a Qiagen Gentra Puregene Yeast/Bact. Kit and Qiagen DNeasy Blood and Tissue were used in three distinct DNA extraction methods to identify the Stx genes using PCR. Bean sprouts, blackberries, blue cheese, cilantro, cocoa powder, coleslaw, cream of mushroom dry soup mix, cream of vegetable dried soup mix, flaxseed, guacamole, peanut butter, soft cheese, soy butter, spinach, walnuts and wheat flour were some of the foods that had been contaminated (McMahon et al., 2023). The researcher's findings indicate that the IC-Protocol is a reliable technology for isolating STEC across various cheeses (Miszczycha et al., 2023).

Global Outbreaks

At present, STEC outbreaks are a global public health concern. Several significant STEC outbreaks have occurred in various countries worldwide in the last few years (Figure 2) (Islam et al., 2014) (Figure-3). The Middle East region, including Saudi Arabia, has also experienced several outbreaks of STEC, highlighting the necessity for improved scrutiny and control (Mir et al., 2019). The discovery of STEC O157 as the cause of HUS outbreaks in children in the 1980s made it a public health issue. In the 1990s, epidemiological and microbiological surveillance systems were designed in the United Kingdom to tackle this public health hazard, and they are continually being improved. In 2009, Public Health England (PHE) created the National Enhanced STEC Surveillance System (NESSS) to collect epidemiological data on all STEC O157 cases in England and Wales. Local diagnostic laboratories send STEC O157 isolates to PHE's Gastrointestinal Bacteria Reference Unit (GBRU) for validation (Treacy et al., 2019).

According to the WHO, in 2010, STEC infection caused over a million illnesses and 100 deaths. Between 1998 and 2016, the European and Western Pacific regions (EUR and WPR) documented 211 STEC incidences, far fewer than the 708 outbreaks recorded in the Americas (EFSA, 2019). Egypt is part of the Middle East region with the world's maximum annual rates of STEC infections in humans (Kim et al., 2020). In the United States (USA) and Canada, E. coli strains with various Oantigenic serotypes related to bloody diarrhoea and postdiarrhoeal haemorrhagic colitis have been detected in children. Over the last 45 years, there has been much consideration of a single STEC O-antigen serotype, which is prevalent in documented outbreaks and reports of isolated cases in the USA and other regions, but not all over the world (Havelaar et al., 2015). In Germany, for instance, STEC O91 is the most common serotype found in mature patients, while STEC O157 was suspected of causing a diarrhoea outbreak in the USA in 1982 and an outbreak in Japan in 1996, afflicting approximately 6,000 youngsters, of which three died. In 1982, two severe HUS outbreaks occurred in Oregon and Michigan in the USA and were connected to infected meat supplied by a fast-food restaurant chain, resulting in four deaths. The pathogen was isolated from the patients' faeces and identified as E. coli O157. A year later, the toxins extracted from three E. coli segregates from the outbreaks were compared with S. dysenteriae to validate the synthesis of Stx (Heiman et al., 2015; Kim et al., 2020).

In May 2011, a large outbreak of diarrhoea triggered by EAEC occurred in Germany, and it was linked to a significant number of HUS cases. By the end of the pandemic in late July 2011, 782 HUS cases with 29 fatalities and 3,128 non-HUS cases involving 17 deaths had been recorded, making it the largest HUS outbreak in history. The consumption of fenugreek sprouts was linked to the outbreak. In 2018, a STEC epidemic in the USA resulted in more than 200 cases spread across 36 states. The outbreak was linked to romaine lettuce (Halsby et al., 2017). These outbreaks demonstrate the importance of STEC surveillance and control measures worldwide. Rapid detection, investigation and containment of outbreaks are crucial to reducing the spread and limiting the number of illnesses and deaths (Bannon et al., 2016). Increasing awareness among both the public and healthcare professionals about the risk factors and preventive measures for STEC infections is essential. Furthermore, the appropriate implementation of investigation and control measures is necessary to contain and end STECrelated issues, reducing the disease burden on public health (Jajarm et al., 2017).

Laws and Policies

Several laws and policies have been instituted to prevent and respond adequately to STEC outbreaks. The detection of pathogenic *E. coli*, explicitly O157, by the Food and Drug Administration (FDA) in the USA between 2018–2020 in leafy greens within mutually dependent geographical areas and problems associated with the production of cattle and migrant birds in neighbouring lands led to the formulation of the Leafy Greens STEC Action Plan (LGAP) (Lacombe et al., 2022). The LGAP is the FDA's response to the repeated outbreaks connected with leafy greens; it is a regulatory method for licensing commercially available sanitisers that may be used in irrigation waters to battle STEC, especially *E. coli* O157. It was developed alongside the USA's Environmental Protection Agency (EPA). However, the protocol has significant realworld restrictions and economic implications (FDA, 2015). The FDA thoroughly examined the reasons for persistent STEC outbreaks in collaboration with the Center for Disease Control (CDC) and local administrators. The investigations revealed three recurring characteristics in the STEC contamination of leafy greens: the incidence of pathogenic *E. coli*, similar geographical locations and difficulties with neighbouring land operations. The epidemic-causing bacterial strain were traced to cow faeces samples obtained from surrounding properties (Lacombe et al., 2022).

The Food Safety Modernization Act (FSMA) instituted by the FDA created standards to guarantee that soil additives, water and food contact exteriors do not lead to crosscontamination. The Produce Safety Rule (PSR) establishes the minimum requirements for cultivating, picking, packaging and storing vegetables and fruits for human use. This rule also covers agricultural water standards, the use of biological soil additives, the avoidance of adulteration by rearing and working animals, workers' health, cleanliness standards and farm designs (Fonseca et al., 2020). Following the adoption of the PSR in 2015, the Leafy Green Products Handler Marketing Agreements (LGMA) were amended in 2017 to align with the rule. The recurring epidemics connected with eating leafy greens prompted the FDA to adopt defensive steps by issuing the 2020 Food Safety Modernization Act (Singh and Greene, 2017). The FDA has established several laws and policies to guarantee the safety and well-being of food commodities and prevent the spread of STEC. The zero-tolerance policy for STEC O157 in beef is one example. Before being supplied to customers, all raw beef products must be tested for the presence of STEC O157. If STEC O157 is found in a product, the product must be recalled and destroyed (Bottichio et al., 2020).

In addition to the zero-tolerance policy, the FDA has created production requirements for beef products. The use of hazard analysis and critical control points (HACCP) and good manufacturing practices, methods that ensure the quality and safety of foods while they are being manufactured, are part of these standards. All USA meat and poultry processing facilities must employ HACCP systems (Thomas, 2019). In addition, the FDA has created criteria for cultivating produce. These standards include the usage of good agricultural practices (GAPs), procedures that assure product safety and quality during manufacturing and good health and good handling practices (GHPs) that ensure product safety and quality during handling and storage (Tozzoli et al., 2014). Although GAPs and GHPs are optional, many producers and handcrafters use them (Maguire et al., 2021). Other regulatory bodies, such as the US Department of Agriculture (USDA) and the Environmental Protection Agency (EPA), in addition to the FDA, have enacted regulations and policies concerning STEC. The former is responsible for guaranteeing the safety of meat and poultry products, whereas the EPA oversees water source safety. Similar to the FDA's requirements for beef products, the USDA has set rules for the manufacturing of meat and poultry products, while the EPA has created drinking water safety requirements, including monitoring for STEC in water sources (Bottichio et al., 2020).

STEC Breakouts and Related Research Studies in Different Countries

Gastrointestinal illnesses caused by STEC are a significant concern in the Middle East. Due to dietary and lifestyle changes, STEC infections have become more common in this region over the past few decades. There have been STEC outbreaks in several Middle Eastern nations, including Saudi Arabia, Egypt, Kuwait and Iran (Al-Ajmi et al., 2020). The absence of adequate surveillance and reporting mechanisms is among the significant obstacles to managing STEC outbreaks in the Middle East; it is difficult to determine the disease's exact impact due to underreporting (Havelaar et al., 2019). The symptoms and available treatments for STEC infections are also unknown among both the general population and healthcare experts (Lang et al., 2023).

In recent years, there have been several STEC outbreaks in Saudi Arabia. The number of STEC illnesses reported countrywide in 2012 exceeded 14,000, making it one of the most severe epidemics. The incident was linked to consuming contaminated milk from a nearby dairy farm, which was subsequently closed, as it led to the loss of numerous lives. Saudi Arabia has since taken numerous measures to develop STEC surveillance and control systems (Hessain et al., 2015; Nada et al., 2020). Further research on the prevalence and characteristics of the six common STEC serogroups is necessary, given the growth in STEC infections in Saudi Arabia over time (Elafify et al., 2020).

The *Saudi Journal of Biological Sciences* published an article on the molecular and serotyping characterisation of STEC associated with food collected from Saudi Arabia. The researchers aimed to detect and characterise *E. coli* bacteria in raw meat and milk samples and look for Stx and intimin genes. A total of 540 milk samples were gathered from five dairy farms, while 150 raw meat samples were acquired from several abattoirs in Riyadh, Saudi Arabia. To identify and describe the *E. coli strains*, the researchers employed a mix of microbiological, biochemical and molecular approaches (Alsayeqh et al., 2023). The findings revealed that 11 (2.04%) of the 540 milk samples and 23 (15.33%) of the 150 raw meat samples tested positive for STEC strains. The bacterial strains, O157 and O26, were the most prevalent serotypes in both the milk and the meat samples.

The researchers also discovered that all STEC strains isolated from food samples included at least one Stx gene (Stx1 or Stx2), with some strains carrying both. Furthermore, all STEC strains harboured the intimin gene (eaeA), linked to adhering and effacing lesions in the intestine. According to the study, raw meat and milk products in Saudi Arabia are possible sources of STEC infections in humans. The authors advocated for effective hygienic measures to be implemented throughout food and handling to avoid manufacturing, processing contamination (Alsayegh et al., 2023). Al-Zogibi et al. (2015) provided significant information on the prevalence and characteristics of STEC strains in Saudi food items. Their study emphasised the significance of appropriate food safety measures to avoid foodborne diseases caused by E. coli and other pathogens. Finally, this paper provided insights into the occurrence of Stx in E. coli connected with Saudi Arabian cuisine.

Al-Humam and Mohamed (2022) investigated the likely prevalence of *Staphylococcus aureus, E. coli*, and *Salmonella spp*. in fast-food restaurants in Al-Ahsa Province in Saudi Arabia, as well as their potential risk of human infection and antimicrobial resistance. The study gathered 100 samples of shawarma poultry meat from various locations around the province and utilised traditional, commercial VITEK[®] 2 and molecular methods to identify isolates and detect antibiograms. It was found that *Campylobacter jejuni, E. coli* O157, *S. aureus, Listeria monocytogenes* and *Bacillus cereus* are the cause of most foodborne infections. The study discovered that 60% of the samples obtained from fast-food restaurants in Al-Ahsa Province were infected with S. aureus. The highest contamination was found in samples collected from Hofuf City (80%), followed by Al-Mubarraz City (70%), while the contamination was the lowest in the samples from Al-Qarah City (40%). However, none of the samples were contaminated with E. coli or Salmonella spp. The study also found that all S. aureus isolates were resistant to penicillin G but sensitive to vancomycin. Moreover, 90% of the isolates were resistant to ampicillin/sulbactam and cefoxitin but sensitive to linezolid. The study highlighted the potential risk associated with consuming fast food contaminated with S. aureus in Al-Ahsa Province and recommended continuous monitoring of food safety and hygiene practices to prevent the spread of foodborne diseases and antimicrobial resistance (Karmali et al., 2017).

In Egypt, Mansour et al. (2023) tested E. coli strains for antimicrobial sensitivity to 11 antimicrobial agents. According to the documented results, the STEC isolate was shown to be extremely sensitive to nalidixic acid (76.19%, 77.7%) and doxycycline (90.5%, 88.89%) while being only moderately susceptible to ampicillin (52.3%, 44.4%) and erythromycin (47.6%, 44.4%) in the food and faeces, respectively. In addition, there was considerable resistance to cephalexin (81.0%, 77.7%) and vancomycin (76.19%, 77.7%). Using real-time PCR and the genes for Stx1, Stx2, eaeA, and hylA, the recovered E. coli isolates from the investigated materials, such as chicken products or faeces, were successfully molecularly characterised. Despite the relatively low percentage of EPEC isolation, it was concluded from the data that Alexandria's retail food products pose an infection risk to people. Further investigation found that all STEC isolates were in eaeA, Stx1 and Stx2 genes (Rivas et al., 2023). In addition, Ochieng et al. identified ehxA and saa virulence genes in certain strains (2023). The researchers also used pulsed-field gel electrophoresis (PFGE) to evaluate the genetic diversity of the STEC isolates. The isolates showed a wide genetic variety; no dominant PFGE pattern was observed. The primary food chain information (FCI) and post-mortem inspection (PMI) results and the frequency of public health concerns by slaughtering turkeys in Finland were the focus of an investigation by Blomwall et al. (2023). The study involved 82 fattened turkey flocks from Finland. The FCI records of PMI data showed that Salmonella spp., Campylobacter spp., E. coli generating ESBL/AmpC, STEC, Listeria monocytogenes, and enteropathogenic Yersinia spp., were all present in the faecal swab samples. Around 45% of farmers with slaughtered turkey flocks reported FCI abnormalities (Blomwall et al., 2023). Another critical modelling study provided information on the growth of E. coli O157 and non-O157 STEC in ground beef, aiding the safety determination of commercial ground beef products (Walker et al., 2023).

A study conducted in Bangladesh determined the frequency and genetic characterisation of STEC isolates from butchered animals (Islam et al., 2008). Rectal contents were collected shortly after the animals were slain, with 1,000 samples gathered from cattle, goats and sheep at three separate slaughterhouses in Dhaka. The samples were examined using microbiological and molecular techniques to identify and characterise STEC. According to the analysis, STEC was present in 4.9% of the samples, with 70% of the positive samples coming from the cattle, 20% from the goats and 10% from the sheep. The researchers discovered that the STEC isolates belonged to the serogroups, O26, O111, or O157, which were associated with life-threatening human diseases.

The researchers suggested certain measures Bangladesh could take to reduce the risk of STEC contamination in meat products. This entails improving the hygiene standards in slaughtering and processing facilities, creating monitoring systems to track the prevalence of STEC in animals and meat products and warning consumers about the risks of consuming contaminated meat. The findings emphasised the importance of improved awareness and action to lower the risk of STEC (Islam et al., 2008).

In Korea, the genetic diversity of the STEC strains and the differences between strains acquired throughout time were emphasised by multilocus sequence typing and pulsed-field gel electrophoresis patterns. According to antimicrobial susceptibility testing, multidrug resistance grew from 12% in 2010 to 42% in 2011. It is possible that seasonal fluctuations or the extensive slaughtering carried out in Korea to contain an early 2011 epidemic of foot and mouth disease caused differences between the isolates collected in 2010 and 2011. However, to better comprehend the processes, further epidemiologic investigations will be required. More public health initiatives are needed to reduce STEC infection spread through dairy products and the incidence of these bacteria in dairy animals (Kang et al., 2014).

Challenges and Solutions

Despite the laws and policies of the regulatory authorities, preventing and controlling STEC remains challenging. One difficulty pertains to detecting the presence of STEC in food products. The current testing methods are unreliable and sometimes lead to false-negative results. Consequently, contaminated products enter markets, causing illnesses in unwary consumers. Researchers are developing new STEC testing techniques to address this problem. One such method involves next-generation sequencing, an advanced technique that can sequence an organism's whole genome, enabling the identification of specific STEC strains. This strategy may improve the precision of STEC testing while reducing the incidence of false-negative results.

The spread of STEC from animal-to-human sources is another problem. Cross-contamination can occur during transit or at retail outlets, even though there are strict regulations for producing meat and other food products. Scientists are looking at cutting-edge ways to prevent cross-contamination. One such strategy involves using bacteriophages, viruses that attack and kill bacteria. Before cattle and vegetables are transported or sold to consumers, scientists are exploring utilising bacteriophages to eradicate STEC.

Determining the source of STEC outbreaks is another issue. Locating and controlling the source of contamination in STEC outbreaks is challenging. Whole-genome sequencing is an advanced tool for determining the genetic signatures of STEC strains. This strategy allows for identifying the source of contamination and stopping further outbreaks.

2. Conclusion

The prevalence of the STEC strains, O157, O45, O26, O111, O103, O145 and O121, pose a serious threat in the Middle East and Gulf region. These virulent strains cause severe GI symptoms such as HUS and bloody diarrhoea. Ingesting contaminated food and water, touching animals and inadequate hygiene standards compound the risk of STEC infections. Assessing the prevalence of STEC infections and

implementing efficient prevention and control measures are more challenging without adequate surveillance and reporting systems. More studies and efforts are needed to better comprehend the epidemiology of STEC infections in this region, including identifying potential contamination sources and transmission pathways. More effective food safety regulations, cleanliness requirements and surveillance systems are needed to stop the spread of these illnesses and protect public health. In addition, in the Middle East and Gulf region, education and awareness campaigns directed at the public and healthcare professionals could aid in preventing and managing STEC infections.

This study highlights the importance of locating the six major STEC strains in the Middle East and Gulf region and the necessity for concerted action to address this public health issue. Implementing appropriate measures to prevent and manage STEC infections may reduce their impact on public health. Understanding the virulence factors, clinical symptoms and epidemiology of these strains is critical to meeting public health objectives.

Conflicting Interests

The authors have declared that no conflicting interests exist.

References

- [1] Al-Ajmi, D., Rahman, S., & Banu, S. (2020). Occurrence, virulence genes, and antimicrobial profiles of Escherichia coli O157 isolated from ruminants slaughtered in Al Ain, United Arab Emirates. *BMC microbiology*, 20(1), 1-10.
- [2] Alharbi, M.G.; Al-Hindi, R.R.; Esmael, A.; Alotibi, I.A.; Azhari, S.A.; Alseghayer, M.S.; Teklemariam, A.D. The "Big Six": Hidden Emerging Foodborne Bacterial Pathogens. *Trop. Med. Infect. Dis.* 2022, 7, 356. https://doi.org/10.3390/tropicalmed7110356
- [3] Al-Humam, N. A., & Mohamed, A. F. (2022). Monitoring of Escherichia coli, Salmonella spp. and Staphylococci in Poultry Meat-Based Fast Food in Saudi Arabia. *Advances in Microbiology*, 12(3), 159-176.
- [4] Alsayeqh, A. F., Mohamed, A. S., Mohamed, R. E., Ibrahim, N. A., Hamdy, E., & Alnakip, M. E. (2023). PREVALENCE OF MULTIDRUG RESISTANT SHIGA TOXIN PRODUCING E. coli IN THE MILK OF CATTLE, BUFFALOES, AND CAMEL. Slovenian Veterinary Research, 60.
- [5] Al-Zogibi, O. G., Mohamed, M. I., Hessain, A. M., El-Jakee, J. K., & Kabli, S. A. (2015). Molecular and serotyping characterization of shiga toxogenic Escherichia coli associated with food collected from Saudi Arabia. *Saudi Journal of Biological Sciences*, 22(4), 438-442
- [6] Bannon, J., Melebari, M., Jordao Jr, C., Leon-Velarde, C. G., & Warriner, K. (2016). Incidence of Top 6 shiga toxigenic Escherichia coli within two Ontario beef processing facilities: Challenges in screening and confirmation testing. *Aims Microbiol*, 2, 278-291.
- [7] Bouvier, M., Canizares, M., Hamadou, B. *et al.* Evaluation of Larger Test Portion Sizes for *Escherichia coli* Shiga Toxin Producer (STEC) on the Detection by Immunomagnetic Separation and Real-Time PCR in Meat and Vegetables. *Food Anal. Methods* 16, 1271–1282 (2023).
- [8] Blomvall, L., Kaukonen, E., Kurittu, P., Heikinheimo, A., & Fredriksson-Ahomaa, M. (2023). Food chain information and post-mortem findings in fattening Turkey flocks. *Food Control*, 150, 109739.

- [9] Elafify, M., Khalifa, H. O., Al-Ashmawy, M., Elsherbini, M., El Latif, A. A., Okanda, T., ... & Abdelkhalek, A. (2020). Prevalence and antimicrobial resistance of Shiga toxinproducing Escherichia coli in milk and dairy products in Egypt. *Journal of Environmental Science and Health, Part B*, 55(3), 265-272
- [10] EUROPEAN FOOD SAFETY AUTHORITY, & European Centre for Disease Prevention and Control. (2021). The European Union one health 2019 zoonoses report. *Efsa Journal*, 19(2), e06406.
- [11] Blomvall, L., Kaukonen, E., Kurittu, P., Heikinheimo, A. & Fredriksson-Ahomaa, M. (2023). Food chain information and post-mortem findings in fattening Turkey flocks. Food Control, 150, p.109739.
- [12] BOTTICHIO, L., KEATON, A., THOMAS, D., FULTON, T., TIFFANY, A., FRICK, A., MATTIOLI, M., KAHLER, A., MURPHY, J. & OTTO, M. 2020. Shiga toxin-producing Escherichia coli infections associated with romaine lettuce – United States, 2018. *Clinical Infectious Diseases*, 71, e323-e330.
- [13] BRYAN, A., YOUNGSTER, I. & MCADAM, A. J. 2015. Shiga toxin producing Escherichia coli. *Clinics in laboratory medicine*, 35, 247-272.
- [14] BYRNE, L., VANSTONE, G. L., PERRY, N. T., LAUNDERS, N., ADAK, G. K., GODBOLE, G., GRANT, K. A., SMITH, R. & JENKINS, C. 2014. Epidemiology and microbiology of Shiga toxin-producing Escherichia coli other than serogroup O157 in England, 2009–2013. *Journal* of medical microbiology, 63, 1181-1188.
- [15] CDC 2019. Outbreak of E. coli infections linked to Romaine lettuce. Centre for Disease Control and Prevention.
- [16] Deliephan, A., Dhakal, J., Subramanyam, B. & Aldrich, C.G. (2023) . Use of organic acid mixtures containing 2hydroxy-4-(methylthio) butanoic acid (HMTBa) to mitigate Salmonella enterica, Shiga toxin-producing Escherichia coli (STEC) and Aspergillus flavus in pet food kibbles. *Animals*, 13(5), p.877.
- [17] ESPINOSA, L., GRAY, A., DUFFY, G., FANNING, S. & MCMAHON, B. J. 2018. A scoping review on the prevalence of Shiga-toxigenic Escherichia coli in wild animal species. *Zoonoses and public health*, 65, 911-920.
- [18] FDA, U. 2015. FSMA final rule on produce safety. Internet site: http://www.fda.gov/food/guidanceregulation/fsma/ucm334114.

http://www.juligo/joodygulanteregulation/jsma/uenso4114.

- [19] Ferraro, L. (2023). Notes from the Field: An Outbreak of Shiga Toxin-Producing Escherichia coli O157: H7 Associated with a Farming Camp-Tennessee, 2022. MMWR. Morbidity and Mortality Weekly Report, 72.
- [20] FONSECA, J. M., RAVISHANKAR, S., SANCHEZ, C. A., PARK, E. & NOLTE, K. D. 2020. Assessing the food safety risk posed by birds entering leafy greens fields in the US southwest. *International Journal of Environmental Research* and Public Health, 17, 8711.
- [21] GIOIA-DI CHIACCHIO, R. M., CUNHA, M. P. V., DE SA, L. R. M., DAVIES, Y. M., PEREIRA, C. B. P., MARTINS, F. H., MUNHOZ, D. D., ABE, C. M., FRANZOLIN, M. R. & DOS SANTOS, L. F. 2018. Novel hybrid of typical enteropathogenic Escherichia coli and Shiga-toxinproducing E. coli (tEPEC/STEC) emerging from pet birds. *Frontiers in Microbiology*, 9, 2975.
- [22] GLASSMAN, H., FERRATO, C. & CHUI, L. 2022. Epidemiology of Non-O157 Shiga Toxin-Producing Escherichia coli in the Province of Alberta, Canada, from 2018 to 2021. *Microorganisms*, 10, 814.

- [23] HALSBY, K., TWOMEY, D., FEATHERSTONE, C., FOSTER, A., WALSH, A., HEWITT, K. & MORGAN, D. 2017. Zoonotic diseases in South American camelids in England and Wales. *Epidemiology & Infection*, 145, 1037-1043.
- [24] HAVELAAR, A. H., KIRK, M. D., TORGERSON, P. R., GIBB, H. J., HALD, T., LAKE, R. J., PRAET, N., BELLINGER, D. C., DE SILVA, N. R. & GARGOURI, N. 2015. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS medicine*, 12, e1001923.
- [25] HEIMAN, K. E., MODY, R. K., JOHNSON, S. D., GRIFFIN, P. M. & GOULD, L. H. 2015. Escherichia coli O157 outbreaks in the United States, 2003–2012. *Emerging infectious diseases*, 21, 1293.
- [26] HESSAIN, A. M., AL-ARFAJ, A. A., ZAKRI, A. M., EL-JAKEE, J. K., AL-ZOGIBI, O. G., HEMEG, H. A. & IBRAHIM, I. M. 2015. Molecular characterization of Escherichia coli O157: H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences, 22, 725-729.
- [27] HUANG, L., HUANG, R., PANG, F., LI, A., HUANG, G., ZHOU, X., LI, Q., LI, F. & MA, X. 2020. Synthesis and biological evaluation of dehydroabietic acid-pyrimidine hybrids as antitumor agents. *RSC advances*, 10, 18008-18015.
- [28] ISLAM, M. A., MONDOL, A. S., DE BOER, E., BEUMER, R. R., ZWIETERING, M. H., TALUKDER, K. A. & HEUVELINK, A. E. 2008. Prevalence and genetic characterization of shiga toxin-producing Escherichia coli isolates from slaughtered animals in Bangladesh. *Applied* and environmental microbiology, 74, 5414-5421.
- [29] Islam MZ, Musekiwa A, Islam K, Ahmed S, Chowdhury S, et al. (2014) Regional Variation in the Prevalence of E. coli O157 in Cattle: A Meta-Analysis andMeta-Regression. PLoS ONE 9(4): e93299. doi:10.1371/journal.pone.0093299 (PDF) Regional Variation in the Prevalence of E. coli O157 in Cattle: A Meta-Analysis and Meta-Regression.
- [30] Jajarmi, M., Fooladi, A.A.I., Badouei, M.A. & Ahmadi, A. (2017). Virulence genes, Shiga toxin subtypes, major Oserogroups, and phylogenetic background of Shiga toxinproducing Escherichia coli strains isolated from cattle in Iran. Microbial pathogenesis, 109, pp.274-279.
- [31] Kang, E., Hwang, S.Y., Kwon, K.H., Kim, K.Y., Kim, J.H. & Park, Y.H. (2014). Prevalence and characteristics of Shiga toxin-producing Escherichia coli (STEC) from cattle in Korea between 2010 and 2011. Journal of veterinary science, 15(3), pp.369-379.
- [32] Karmali, M.A. (2017). Emerging public health challenges of Shiga toxin-producing Escherichia coli related to changes in the pathogen, the population, and the environment. Clinical Infectious Diseases, 64(3), pp.371-376.
- [33] Kaper, J., Nataro, J. & Mobley, H. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* **2**, 123–140 (2004).
- [34] KIM, J.-S., LEE, M.-S. & KIM, J. H. 2020. Recent updates on outbreaks of Shiga toxin-producing Escherichia coli and its potential reservoirs. *Frontiers in Cellular and Infection Microbiology*, 10, 273.
- [35] KINTZ, E., BRAINARD, J., HOOPER, L. & HUNTER, P. 2017. Transmission pathways for sporadic Shiga-toxin producing E. coli infections: A systematic review and meta-analysis. *International journal of hygiene and environmental health*, 220, 57-67.
- [36] LACOMBE, A., QUINTELA, I. A., LIAO, Y.-T. & WU, V. C. 2022. Shiga Toxin-Producing Escherichia coli

Outbreaks in California's Leafy Greens Production Continuum. Frontiers in Food Science and Technology, 2, 45.

- [37] Lang, C., Fruth, A., Campbell, I. W., Jenkins, C., Smith, P., Strockbine, N., ... & Flieger, A. (2023). O-Antigen Diversification Masks Identification of Highly Pathogenic Shiga Toxin-Producing Escherichia coli O104: H4-Like Strains. *Microbiology Spectrum*, 11(3), e00987-23
- [38] LEE, M.-S. & TESH, V. L. 2019. Roles of Shiga toxins in immunopathology. *Toxins*, 11, 212.
- [39] LEE, M.-S., KOO, S., JEONG, D. G. & TESH, V. L. 2016. Shiga toxins as multi-functional proteins: Induction of host cellular stress responses, role in pathogenesis and therapeutic applications. *Toxins*, 8, 77.
- [40] MAGUIRE, M., KASE, J. A., ROBERSON, D., MURUVANDA, T., BROWN, E. W., ALLARD, M., MUSSER, S. M. & GONZÁLEZ-ESCALONA, N. 2021. Precision long-read metagenomics sequencing for food safety by detection and assembly of Shiga toxinproducing Escherichia coli in irrigation water. *PLoS One*, 16, e0245172.
- [41] Mansour, A.M., Shehab, S.A., Nossair, M.A., Ayyad, A.S., Tawfik, R.G., El-Lami, S.A. & Eskander, M. (2023). Molecular Characterization of Shiga Toxin-producing Escherichia coli Isolated from Some Food Products as well as Human Stool in Alexandria, Egypt. Journal of Advanced Veterinary Research, 13(6), pp.1004-1010.
- [42] McMahon T, Abdelmesih M, Gill A. (2023) Evaluation of DNA extraction methods for the detection of Shiga toxin producing Escherichia coli in food by polymerase chain reaction. Int J Food Microbiol. 2023 Jul 9;404:110317.
- [43] Milani, G., Daza, M.V.B., Cortimiglia, C., Bassi, D. & Cocconcelli, P.S. (2023). Genome engineering of Stx1-and Stx2-converting bacteriophages unveils the virulence of the dairy isolate Escherichia coli O174: H2 strain UC4224. Frontiers in Microbiology, 14.
- [44] Mir, R.A. and Kudva, I.T., (2019). Antibiotic-resistant Shiga toxin-producing Escherichia coli: An overview of prevalence and intervention strategies. Zoonoses and Public Health, 66(1), pp.1-13.
- [45] Miszczycha, S.D., Mazuy-Cruchaudet, C., Thollet, C. & Sergentet-Thevenot, D. (2023). Comparison of two Shiga toxin-producing Escherichia coli (STEC) isolation protocols in raw cow's milk cheese enrichment broths: direct STEC isolation versus techniques based on immuno-concentration. Journal of Food Protection, p.100128.
- [46] Nada, Hanady G., et al. "Detection of multidrug-resistant Shiga toxin-producing Escherichia coli in some food products and cattle faeces in Al-Sharkia, Egypt: one health menace." *BMC microbiology* 23.1 (2023): 127.
- [47] NAVARRO-GONZALEZ, N., WRIGHT, S., AMINABADI, P., GWINN, A., SUSLOW, T. & JAY-RUSSELL, M. 2020. Carriage and subtypes of foodborne pathogens identified in wild birds residing near agricultural lands in California: a repeated cross-sectional study. *Applied and Environmental Microbiology*, 86, e01678-19.
- [48] Ochieng, J.B., Powell, H., Sugerman, C.E., Omore, R., Ogwel, B., Juma, J., Awuor, A.O., Sow, S.O., Sanogo, D., Onwuchekwa, U. & Keita, A.M. (2023). Epidemiology of Enteroaggregative, Enteropathogenic, and Shiga Toxin– Producing Escherichia coli Among Children Aged< 5 Years in 3 Countries in Africa, 2015–2018: Vaccine Impact on Diarrhea in Africa (VIDA) Study. *Clinical Infectious Diseases*, 76(Supplement_1), pp.S77-S86.
- [49] OLIVEIRA, A., DIAS, C., OLIVEIRA, R., ALMEIDA, C., FUCIÑOS, P., SILLANKORVA, S. & OLIVEIRA, H. 2023.

Paving the way forward: Escherichia coli bacteriophages in a One Health approach. *Critical Reviews in Microbiology*, 1-18.

- [50] PANEL, E. B., KOUTSOUMANIS, K., ALLENDE, A., ALVAREZ-ORDÓÑEZ, A., BOVER-CID, S., CHEMALY, M., DAVIES, R., DE CESARE, A., HERMAN, L. & HILBERT, F. 2020. Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. *Efsa Journal*, 18, e05967.
- [51] Petrucci, S.; Dikici, E.; Daunert, S.; Deo, S.K. Isothermal Amplification and Lateral Flow Nucleic Acid Test for the Detection of Shiga Toxin-Producing Bacteria for Food Monitoring. *Chemosensors* 2022, *10*, 210.
- [52] Rivas, M., Pichel, M., Colonna, M., Casanello, A.L., Alconcher, L.F., Galavotti, J., Principi, I., Araujo, S.P., Ramírez, F.B., González, G. & Pianciola, L.A. (2023). Surveillance of Shiga toxin-producing Escherichia coli associated bloody diarrhea in Argentina. Revista Argentina de Microbiología.
- [53] SHRIDHAR, P. B., SIEPKER, C., NOLL, L. W., SHI, X., NAGARAJA, T. & BAI, J. 2017. Shiga toxin subtypes of non-O157 Escherichia coli serogroups isolated from cattle feces. *Frontiers in Cellular and Infection Microbiology*, 7, 121.
- [54] SINGH, H. & GREENE, H. M. 2017. Food Safety Reforms in the United States: The Food Safety Modernization Act (FSMA). *Food Safety and Protection*. CRC Press.
- [55] SMITH, R., POLLITT, W. & PAIBA, G. 2016. A longitudinal study of risk factors for shedding of VTEC O157 by young cattle in herds with known E. coli O157 carriage. *Epidemiology & Infection*, 144, 1818-1829.
- [56] Thomas, C. L. (2019). The effect of antimicrobial interventions on quality and safety characteristics of blade tenderized beef, and veal and goat carcasses (Doctoral dissertation, University of Georgia)
- [57] Tozzoli, R., Grande, L., Michelacci, V., Ranieri, P., Maugliani, A., Caprioli, A. and Morabito, S. (2014). Shiga toxin-converting phages & the emergence of new pathogenic Escherichia coli: a world in motion. Frontiers in cellular and infection microbiology, 4, p.80.
- [58] TREACY, J., JENKINS, C., PARANTHAMAN, K., JORGENSEN, F., MUELLER-DOBLIES, D., ANJUM, M., KAINDAMA, L., HARTMAN, H., KIRCHNER, M. & CARSON, T. 2019. Outbreak of Shiga toxin-producing Escherichia coli O157: H7 linked to raw drinking milk resolved by rapid application of advanced pathogen characterisation methods, England, August to October 2017. Eurosurveillance, 24, 1800191.
- [59] Tunsjo, H.S., Ullmann, I.F. & Charnock, C. (2023) . A preliminary study of the use of MinION sequencing to specifically detect Shiga toxin-producing Escherichia coli in culture swipes containing multiple serovars of this species. Scientific Reports, 13(1), p.8239.
- [60] Valderrama, W.B., Dudley, E.G., Doores, S. & Cutter, C.N. (2016). Commercially available rapid methods for detection of selected food-borne pathogens. Critical reviews in food science and nutrition, 56(9), pp.1519-1531.
- [61] Walker, L., Sun, S. & Thippareddi, H. (2023). Growth comparison and model validation for growth of Shiga toxin-producing Escherichia coli (STEC) in ground beef. LWT, 182, p.114823.
- [62] Wasiewska, L.A., Diaz, F.G., Teixeira, S.R., Burgess, C.M., Duffy, G. & O'Riordan, A. (2023). Amplification-free, highly sensitive electrochemical DNA-based sensor for simultaneous detection of stx1 and stx2 genes of Shiga toxin-producing *E. coli* (STEC). Electrochimica Acta, 441, p.141814.

⁹ The American Journal of Science and Medical Research.2023; 9(3)

- [63] WITHENSHAW, S. M., SMITH, R. P., DAVIES, R., SMITH, A. E., GRAY, E. & RODGERS, J. 2022. A systematized review and qualitative synthesis of potential risk factors associated with the occurrence of non-O157 Shiga toxin-producing Escherichia coli (STEC) in the primary production of cattle. *Comprehensive Reviews in Food Science and Food Safety*, 21, 2363-2390.
- [64] WORLD HEALTH ORGANIZATION, (WHO) 2018. Shiga Toxin-producing Escherichia Coli (STEC) and Food: Attribution, Characterization and Monitoring, World Health Organization.