DOI: https://doi.org/10.54393/pbmj.v7i07.1104

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (P): 2709-2798, (E): 2709-278X
Volume 7, Issue 7 (July 2024)

Review Article

Prevalence and Transmission Factors of Shiga Toxin 0157:H7 in Pakistan; A Review

Fiza Rafi[1], Hassan Raza[2], Bushra Nisar Khan[3*], Sarfraz Kiani[2], Shaheer Azhar[4], Tatheer Zahra[5] and Muhammad Mehmood Imran[6]

[1]Department of Biological Sciences, Superior University, Lahore, Pakistan
[2] Upscaling Green Program, Ministry of Climate Change, Islamabad, Pakistan
[3]Institute of Zoology, University of the Punjab, Lahore, Pakistan
[4]Sheikh Zayed Hospital, Lahore, Pakistan
[5]Walsall Manor Hospital, United Kingdom
[6]Service Hospital, Lahore, Pakistan

ARTICLE INFO

Keywords:
Escherichia. coli, Shiga Toxin 0157:H7, Gut Microbiome, Hemolytic Uremic Syndrome (HUS), Antibiotics

How to Cite:
Rafi, F., Raza, H., Khan, B. N., Kiani, S., Azhar, S., Zahra, T., & Imran, M. M. (2024). Prevalence and Transmission Factors of Shiga Toxin 0157:H7 in Pakistan; A Review: Prevalence and Transmission Factors of Shiga Toxin 0157:H7. Pakistan BioMedical Journal, 7(07). https://doi.org/10.54393/pbmj.v7i07.1104

***Corresponding Author:**
Bushra Nisar Khan
Institute of Zoology, University of the Punjab, Lahore, Pakistan
bushra.zool@gmail.com

Received Date: 11th June, 2024
Acceptance Date: 26th July, 2024
Published Date: 31st July, 2024

ABSTRACT

Shiga toxin-producing *E. coli* is a definitive strain of the very commensal microbe *Escherichia coli*. It is a resident of the vertebrate gut with hundreds of microbial colonies that comprise the versatile gut microbiome. Stx is a toxin, which is a protein in nature that has evolved in such a way that it can specifically target a host cell and deliver a payload inside the target cell's cytosol. These pathogens can trigger complications such as thrombotic microangiopathy and acute kidney injury. It is transmitted by food consumed in raw form (salads, milk, and curd), floods, contaminated ponds, and petting farms. Ruminants, floods, zoo fauna, and untreated sewage water were found to be the primary sources of STEC reservoirs nationally. Alarmingly, there is a high prevalence of neonatal diarrhea in Pakistan. Antibiotics are devoured in STEC-caused infections, especially. Diarrhea, as they aggravate the toxin production. Plant extracts and chemical purification methods have shown potential for shiga toxins reduction. Application of biosafety measurements can significantly reduce the chances of infection in developing countries, including Pakistan.

INTRODUCTION

Shiga-toxin-producing E. coli is the definitive strain of a very commensal microbe, Escherichia coli. As a resident of a vertebrate gut, it makes hundreds of microbial colonies that comprise the versatile gut microbiome. Kiyoshi Shiga became the name bearer of this bacterial strain in 1898 when he described S. dysenteriae, which produced Shiga toxins [1]. Later, isolation from the kidney cells of African monkeys also designated it as Vero-toxin E. coli. Over the years, the impact of gut microbes on dietary digestion, mediation of infection, and even modifying behaviour and cognition has been well proven and undeniable [2-4]. The fitness, health, and ecology of an organism are impacted by the vertebrate gut microbiome because these microbes provide a large physiological and pathophysiological contribution [4, 5]. Escherichia. coli is the widespread aerobe of the mammalian intestine that is present in soil, food, animal, and human manure [5, 6]. Despite being a part of any mammalian gastrointestinal tract's healthy microbiota [7] few strains develop virulence after attaining new genetic information. These strains are broadly classified as extra-intestinal (ExPEC) and intestinal pathogenic E. coli (InPEC). InPEC is further classified into

DOI: https://doi.org/10.54393/pbmj.v7i07.1104

individual strains as adherent-invasive E. coli (AIEC), diffuse adherent E. coli (DAEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC), and Shiga toxin-producing E. coli (STEC) [7, 8]. The unique capacity of some strains of bacteria to release toxins can lead to infections and serious illnesses, including mortality in humans. Globally, these pathogenic strains are the primary culprits of infectious mortality in hospitalised patients. In humans, a large number of microbial pathogens are transmitted via food products, known as foodborne pathogens (FBP) [9]. Stx [10,11] toxin is a key virulent factor, which is a protein in nature. It has evolved in such a way that it can specifically target a host cell and deliver a payload inside the target cell's cytosol [12]. Stx family came under the medical spotlight in 1983 because of two documented reports presented at almost the same time [3]. The most commonly studied STEC serotype is E. coli 0157:H7. This predominance is partly related to its inability to ferment sorbitol on agar plates [3]. Both shiga toxin 1 and 2 consist of two subunits: the single A plus the disease-causing B subunit, which, after passing from the Golgi apparatus and endoplasmic reticulum, inhibits protein synthesis. The degree of expression of both shiga toxin 1 and 2 determines the pathogenicity [1]. Typically, a lambdoid bacterium possesses such genes. Critically listed infectious foodborne pathogens include STEC [2]. It can be the cause of severe human illnesses that include simple diarrhoea, hemorrhagic diarrhoea, bloody diarrhoea (BD) (5–15% of which leads to the life-threatening HUS), and haemolytic uremic syndrome [13].

Hosts Species

Complex pathways of transmission are built because of the very diverse species of reservoirs and spillover hosts. Host organisms can be categorized as reservoir hosts, who are essential for maintaining pathogens in the natural environment; spillover hosts, which transmit infection to other species of interest from reservoirs; and dead-end hosts. Cattle and livestock are primary reservoirs hosts. Birds, Swine and dogs constitute spillover host while Fish and shellfish act as dead-end hosts for the shiga toxin producing Escherichia coli (STEC) in animals [14]. STEC has been characterised by wild and captive species of ruminants, including yaks, moose, antelope, and llamas. Direct transmission to humans is also reported at petting zoos, along with indirect transmission via faecal droplets in agriculture fields, water sources, and on meat [12]. E. coli reservoirs in white-tailed deer have been reported using 16S r DNA pyrosequencing [15] in the intestinal tract of domestic cows [6] and in apparently healthy captive Nilgiri Langur and colonies of laboratory Rhesus macaques [16].

Food Borne Factors

Pakistan has a voluminous consumption rate of suspected foods, including dairy products (curds, milk, and cheese), minced meat, fresh juices, vegetables, and nowadays poultry meat. Raw meat nurtures important pathogenic microbes [17], chicken in particular. Water contaminated with faeces, raw manure, or fertilizers used for washing vegetables and fruits has been a trigger for pandemics. Studies summarizing contamination methods and prevalence rates are presented here (Table 1).

Table 1: Prevalence Rate of STEC in Different Foods in Pakistan

Food Source	City	Pathotype/ Serotype	Prevalence Rate (%)	Year	Reference
Milk	Peshawar	0157:H7	8.75	2009	[18]
	Lahore	STEC	01	2016	[19]
	Khyber Pakhtunkhwa (Kpk)	STEC	05	2024	[20]
	Lahore	0157:H7	50	2013	[21]
Meat (Cattle & Goat)	Quetta	0157:H7	12	2018	[22]
	Quetta	0157:H7	10	2018	[23]
	Islamabad & Rawalpindi	0157:H7	1.33	2021	[24]
	Islamabad & Rawalpindi	STEC	43.5	2020	[25]
Chicken Meat	Northern Punjab	0157:H7	18	2023	[26]
	Southern Punjab	STEC	2.7	2021	[27]
	Lahore	0157:H7	30	2017	[28]
Sugarcane Plum, Peach & Lemonade Juice	Lahore	0157:H7	13	2014	[29]
			41		
			53		
			50		
Salads	South KPK	STEC	05	2015	[30]
Apple & Tomatoes	Lahore	STEC	17	2022	[5]
			26		

* Where rate was not present, it was calculated from sample size by using this formula. No of infected samples/No of total samples *100. Where particular serotype was not found, pathotype is mentioned.

The other major pathways associated with non-food transfer include swimming in contaminated ponds, lakes, or pools, municipal water supply, stools [31, 32] and petting farms. As of now, direct transmission via human to human is low [6]. Heavy floods all over the country caused by seasonal rains are also major sources of contamination. In the 2011–2012 floods, 200 samples were obtained to examine the presence of pathogenic E. coli. This research concluded that one-third of the total 200 samples were infected with Escherichia coli pathotypes. Fifty percent of them were enterotoxigenic strains (ETEC). There were seventy-two percent enteropathogenic strains (EPEC) and eleven percent Shiga toxin isolates carrying STX1 and STX2 genes [33]. Summer and fall are peak times for STEC infection [1], and rural areas are more at risk than urban areas [3]. In Rawalpindi and Islamabad zoos, an experimental study on 110 faecal samples of 24 species to estimate the prevalence of STEC in zoo animals reported the presence of stx1, stx2, eae, and ehxA virulence genes (with 6 different combinations) in 15 samples (2-black buck, 1-chinkara deer, 4-hog deer, 1-mouflon sheep, 4-spotted

DOI: https://doi.org/10.54393/pbmj.v7i07.1104

deer, 1-baboon monkey, 1-ostrich, and 1-tiger). The techniques used in this experiment included multiplex PCR. It indicated the zoo animals as carriers and possible causes of infection for other animals as well as humans [4]. Sewage water was investigated in 2019 for E. coli from sixteen different locations in Lahore. It provided comprehensive results with the presence of 49 serotypes of E. coli, including E35, E63, and E101, through 16S rRNA gene sequencing. Antibiotic sensitivity, biofilm formation, and shiga toxin genes (stx1, stx2, stx2c, and stx2d) were also evaluated. However, shiga toxin genes weren't detected using PCR and gel electrophoresis [34].

Clinical Infections

Multiple allelic genotype variants of stx1 and stx2 have been associated with different human diseases. Each variant is grouped on the basis of the sequence of amino acids in the Stx. Subtypes of Stx1 are Stx1a, Stx1d, and Stx1e, and subtypes of Stx2 are Stx2a to Stx21. Haemolytic uremic syndrome is caused by both Stx1 and Stx2. During bacterial lysis, Stx is released from the bacterium in the intestine [5]. STEC has two main characteristics: a pathogenicity locus with chromosomal origins that is responsible for encoding "attaching effect" producing proteins and Shiga toxins encoded by bacteriophage. These "attaching effect" proteins contain Intimin which adheres bacterium to epithelial layer of microvilli and type 3 secretion system. STEC passes through gastric fluid uninterrupted and initiate intimin & T3 System expression. Their attachment in intestinal tract results in diarrhea [9].

Haemolytic Uremic Syndrome

Symptoms of STEC-induced HUS in children include fever, abdominal pain, early illness, vomiting, and diarrhoea, followed by HUS development after a few days. Other symptoms involve bloody diarrhoea, CNS involvement, severe inflammatory colitis, cardiac dysfunction, glucose intolerance, hepatomegaly, loss of the microvilli, and leucocytosis. These disease-causing organisms can trigger a thrombotic microangiopathy called TMA, non-immune hemolytic anaemia, and azotemia. TMA is further classified as primary and secondary TMA. Primary TMA in atypical HUS presents an underlying regulatory defect in the complement system [1]. It has a high incidence in young children worldwide, with an annual prevalence of about 2–3 per 100,000 people [6]. HUS is a complex disorder that results in further organ damage. It has a major impact on renal functions. Many studies have related acute renal failure or acute kidney injury to hemolytic uremic syndrome in Pakistan. A study of 116 patients with pregnancy complications involving HUS in Sindh revealed 7% positivity. 14% developed in the first trimester, while 86% developed in the later trimester of pregnancy [35]. Children have also been shown to be a vulnerable group for HUS. In a 33-patient pediatric ward study, 3% were positive for HUS. Further analysis of their stools revealed pathogenic *E. coli* positivity [36]. Another study confirmed 10.8% HUS positivity in 74 pediatric cases that caused acute kidney injury [37]. Kidney failures have been reported in pregnancy complications and pediatric units in Pakistan, which are also linked to HUS (Table 2). A strain of E. coli, O157:H7, exhibits three genetic lineages (I, II, and I/II), further separated into nine different clades. Most of the pathogenic strains belong to clades 2, 3, 7, and 8. These clades differ from each other on account of the infections they cause and the distribution and abundance of shiga toxin genes. HUS-infected patients have higher chances of clade 8 strains, as their frequency has increased in the last half decade. Young patients under 18 years old were found to be associated with the stx2a and c genotypes of clade 8 strains [38].

Diarrhea

Around four decades ago, E. coli 0157 was acknowledged as a source of bloody diarrhea. Bloody diarrhea is caused by STEC in America and Europe, whereas S. dysentery type 1-induced shiga toxins are the main pathogenic agent in Southern Africa and Asia [1]. All around the world, Pakistan is considered to be in 26th place in the under-five death chart, with a whooping rate of 86 out of 1000 child births, making it one of the countries with 50% of mortality caused by diarrhoea or pneumonia. *Escherichia coli* has also been responsible for meningitis and urinary tract infections (UTIs) [18]. A study in Faisalabad, Pakistan, showed how important STEC prevalence is. They took 200 stool samples from children and used them to isolate and characterise STEC. They focused on genes eae, hly, and stx1 and 2, which stand for intimin, enterohemolysin, and Shiga toxin 1 and 2. The PCR results revealed 11% stx, 6.5% hly, and 8% eae positive samples, with only three samples positive for the 0157 serotype [25]. According to a research paper, the prevalence of neonatal diarrhoea in Pakistan is at its maximum in Gilgit Baltistan, with Punjab, AJK, Sindh, Baluchistan, FATA, and KP in descending order [39]. In experimental research on 37 *E. coli* isolates obtained from various laboratories in Karachi, 16 strains were found to be positive for different toxin genes. The results showed the maximum prevalence of the stx1 gene in 10 isolates, two of which were also positive for the stx2S gene [40]. In order to find the *E. coli* 0157 serotype, the latex agglutination test was used on 52 strains from the IMAM Clinic in Karachi's culture collection. Twenty samples tested positive for the 0157 strain of *E. coli* [22] (Table 2).

Table 2: Prevalence and Suspected Pathway of STEC Disease in Pakistan

Disease	No. of cases	Suspected Pathway	Isolated Agent/Genes	Prevalence (%)	Ref
HUS	33	Stools contamination	STEC and non-STEC	3	[34]
	74	Acute renal failure	STEC and non-STEC	10.8	[36]

DOI: https://doi.org/10.54393/pbmj.v7i07.1104

Diarrhea	200	Stools contamination	Stx	11	[41]
	52	Stools contamination	0157	38.15	[38]

* Where rate was not present, it was calculated from sample size by using this formula. No of infected samples/No of total samples *100

Anti-Microbial Resistance

Cattle, poultry, and humans contain widespread E. coli, making them the best source of resistance genes. After isolating 121 different types of bacteria based on their phenotypes and genotypes, commonly used antibiotics like Cefotaxime, Ceftazidime, Sulphamethoxazole-Trimethoprim combination, Nitrofurantoin, Ciprofloxacin, and Ampicillin were tested to see if they could kill the bacteria. The results indicated the resistance of E. coli isolates to sulphamethoxazole-trimethoprim [42]. During a diarrheal episode in Faisalabad, the effects of readily available cefotaxime, ampicillin, and gentamicin on shiga toxins were elucidated. STEC was confirmed in five samples. A considerable reduction in the release of toxins and cytotoxicity level was reported after exposure to minimum inhibitory concentrations of ampicillin, gentamicin, and cefotaxime, with a maximum reduction by sub-MIC of gentamicin [43]. A related study revealed the possible impact of SubMIC on 15 bacterial isolates collected from Railway General Hospital, Rawalpindi. The results showed that shiga toxin levels went up by 6.5 and 8 times when ciprofloxacin and tigecycline Sub MIC were used. On the other hand, expression levels were very low when Fosfomycin was used (2 times) and Meropenem was used (the weakest)[44].

Treatment Methods

Hydration and supportive therapy are the cornerstones of the currently advised STEC infection treatment plan. The questionable use of antibiotics has been linked to a worsening of symptoms and an increased risk of haemolytic uremic syndrome. Furthermore, by killing bacteria and causing an increase in the release of shiga toxins that may have been retained in the bacterial cell, antibiotics may exacerbate infections that are Shiga toxigenic [45]. Because there is no safe and specific therapeutic intervention, replacing intravenous fluids in infected patients and providing supportive care are the best-advised treatments. In patients with severe CNS involvement, eculizumab or plasma treatment is best recommended. When given intravenously with an isotonic solution and early parental volume expansion, the high risk of oliguria or anuria caused by renal hyper fusion is lowered [46]. Patients with suspected or confirmed STEC diarrhea should not be given antibiotics as they increase toxin production. When combined with renal replacement, supportive therapy and intensive care improvements have eventually improved the prognosis of HUS. Supportive therapy is advised to treat the following: anaemia, thrombocytopenia, fluid management, acute kidney injury and dialysis, neurological dysfunction, gastrointestinal complications, pancreatitis, and pulmonary complications [47]. Chemical additions to purification methods yielded positive treatment results. The experiment on water reservoir tanks, ground water samples, and canal water was conducted in a laboratory setting. They pasteurized the samples with the addition of ferrous salt as a coagulant and reported the highest success rate with 99% shiga toxin removal[48].

Plant Extracts

Plant extracts, particularly fruits, are a bioactive compound source for controlling *E. coli*-induced infections. It acts as either a bactericidal or bacteriostatic compound, and it could also be used as an amplifier for antibiotic activity (a synergic agent). It attenuates the pathogenic activity by acting directly on virulence characters. Fruits, either in the form of raw extracts or molecules that have been cleaned up, can help treat many different types of E. coli infections by stopping enzymes that target pathogens, preventing biofilm formation, and damaging bacterial membranes [7]. Mangosteen fruit produces α-mangostin, which in combination with β-lactams is proven useful [50]; quercetin and kemp ferol [49]; Chinese bayberry fruit; fermentation of green olives; cranberry extracts [25]; fresh and dried fruits of Chinese quince [22]; peel extract of Gabiroba; chestnut extract; grape extracts; seeds of Citrus mandarin; lemon and strawberry; cocoa beans; and grapefruit juice [12] for substantially increased efficacy; synergistic activity for amoxicillin; curing diarrhea; production of exopolysaccharides (EPS); controlling neonatal and post-weaning diarrhea; and anti-CT properties[26]. A promising study proved the efficacy of medicinal plants in combating STEC-borne pathogens. Researchers used 5 medicinal plant extracts against STEC strain 0157:H7, using extracts from Azadirachta indica, Mela azedarech, Withenia coagulans, Nigella satvia, and Calotropis procera. Calotropis procera showed the maximum inhibition zone and highest weight gain and survival rate. This enhances the potential of plant treatments without developing microbial resistance[19].

Biomedical Applications

The evolution of protein toxins, like Stx, aimed to deliver inside the cytosol of target cells while also selectively targeting host cells. These characteristics might be used to create new therapeutic proteins. The use of Stx-A and Stx-B has advanced quickly in recent years. It has been determined that Stx-A is a promising payload for creating immunotoxins that target and destroy cancer cells. Immunotoxins are fusion proteins made up of a targeting domain, which can be any protein binder that can precisely engage cell surface proteins produced in cancer cells [24,

DOI: https://doi.org/10.54393/pbmj.v7i07.1104

27]. Human volunteers are avoided because of the serious health effects associated with STEC infection, which range from neurological manifestations to death. So, the adoption of model animals, which mostly include mammals: monkeys, pigs, rabbits, and greyhounds, becomes a necessity. Some studies used chicken [20]. Greyhounds were used as test subjects for treatments. Toxin injections in these dogs caused damage to their kidneys and skin lesions. Calves and cattle had intestinal pathology. O157 infections in chickens caused intestinal pathology. Stx 1 or 2 infections in baboons caused intestinal pathology and renal damage. And streptomycin and O157 infections in ferrets caused renal damage [44].

CONCLUSIONS

This study focused on shiga toxin strains in Pakistan, specific to their transmission factors, and reported clinical infections. According to research, Shiga toxin 0157:H7 is still the most common type of STEC found around the world. Food-borne factors constitute the primary culprit of transmission especially dairy products and meat. Sewage water and floods also spread this deadly pathogen. Pakistan has seen documented outbreaks and significant clinical infections in the past decade. Pregnancy complications and neonatal vulnerability to diarrhea and HUS is primary concern in maternal and child care. This virulent pathogen is being exacerbated because of the development of anti-microbial resistance against antibiotic use. Different chemical treatments in water purification, medicinal plants, and fruit peels including Chinese bay berry fruit and cranberry extracts have shown tremendous potential in treating this emerging disease, especially in developing countries. Usage of Stx A for delivering targeted payload inside cancels cells has increased biomedical potential of protein toxins. It is recommended to increase awareness about reservoirs, transmission methods, infectious diseases, and basic preventive measures through the concerted efforts of national organizations.

Authors Contribution

Conceptualization: FR, BNK
Methodology: FR, SK
Formal analysis: SA
Writing, review and editing: HR, SK, TZ, MMI

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Joseph A, Cointe A, Mariani Kurkdjian P, Rafat C, Hertig A. Shiga toxin-associated hemolytic uremic syndrome: A narrative review. Toxins. 2020 Jan; 12(2): 67. doi: 10.3390/toxins12020067.

[2] Combrink L, Humphreys IR, Washburn Q, Arnold HK, Stagaman K, Kasschau KD et al. Best practice for wildlife gut microbiome research: A comprehensive review of methodology for 16S rRNA gene investigations. Frontiers in Microbiology. 2023 Feb; 14: 1092216. doi: 10.3389/fmicb.2023.1092216.

[3] Balaji P, Kumar KS, Vijayarani K, Vairamuthu S, Karunakaran K, Porteen K *et al.* Prevalence of Escherichia coli and Salmonella spp. in captive Niligiri langur (Trachypithecus johnii) in south India. International Journal of Current Microbiology and Applied Sciences. 2018 May; 7(6): 3119-26. doi: 10.20546/ijcmas.2018.706.366.

[4] Rasheed MB, Ahsan A, Irshad H, Shahzad MA, Usman M, Riaz A *et al.* Occurrence of Shiga toxin producing E. coli in zoo animals of Rawalpindi and Islamabad zoos. Asian Journal of Agriculture and Biology. 2023; 2: 2022080. doi: 10.35495/ajab.2022.080.

[5] Foster-Nyarko E, Pallen MJ. The microbial ecology of Escherichia coli in the vertebrate gut. FEMS Microbiology Reviews. 2022 May; 46(3): fuac008. doi: 10.1093/femsre/fuac008.

[6] Torti JF, Cuervo P, Nardello A, Pizarro M. Epidemiology and characterization of Shiga toxin-producing Escherichia coli of hemolytic uremic syndrome in Argentina. Cureus. 2021 Aug; 13(8). doi: 10.7759/cureus.17213.

[7] Mansfield KG, Lin KC, Newman J, Schauer D, MacKey J, Lackner AA *et al.* Identification of enteropathogenic Escherichia coli in simian immunodeficiency virus-infected infant and adult rhesus macaques. Journal of Clinical Microbiology. 2001 Mar; 39(3): 971-6. doi: 10.1128/JCM.39.3.971-976.2001.

[8] Pakbin B, Brück WM, Brück TB. Molecular mechanisms of shigella pathogenesis; recent advances. International Journal of Molecular Sciences. 2023 Jan; 24(3): 2448. doi: 10.3390/ijms24032448.

[9] Freedman SB, van de Kar NC, Tarr PI. Shiga toxin–producing Escherichia coli and the hemolytic-uremic syndrome. New England Journal of Medicine. 2023 Oct; 389(15): 1402-14. doi: 10.1056/NEJMra2108739.

DOI: https://doi.org/10.54393/pbmj.v7i07.1104

[10] Prado PC, Matias CL, Goulart JQ, Pinto AT. Most involved microorganisms in foodborne diseases outbreaks: A systematic review. Brazilian Journal of Development. Curitiba. 2021 Nov; 7(11): 106900-106916. doi: 10.34117/bjdv7n11-363.

[11] Liu Y, Tian S, Thaker H, Dong M. Shiga toxins: An update on host factors and biomedical applications. Toxins. 2021 Mar; 13(3): 222. doi: 10.3390/toxins13030222.

[12] Persad AK and Lejeune JT. Animal reservoirs of Shiga toxin-producing *Escherichia coli*. Enterohemorrhagic *Escherichia coli* and Other Shiga Toxin-Producing *E. coli*. 2015 May; 211-30. doi: 10.1128/9781555818791.ch11.

[13] Delgado ML, Singh P, Funk JA, Moore JA, Cannell EM, Kanesfsky J *et al.* Intestinal microbial community dynamics of white-tailed deer (Odocoileus virginianus) in an agroecosystem. Microbial ecology. 2017 Aug; 74: 496-506. doi: 10.1007/s00248-017-0961-7.

[14] Abdullah UY, Al-Sultan II, Jassim HM, Ali YA, Khorsheed RM, Baig AA. Hemolytic uremic syndrome caused by Shiga toxin-producing Escherichia coli infections: an overview. Cloning & Transgenesis. 2014 Mar; 3(2): 1-9. doi: 10.4172/2168-9849.1000125.

[15] Ismail S, Ullah K, Sunnya H, Bingjie X. Overview of O157 in Pakistan: An Important Food-Borne Disease of Public Health. The Journal of Microbiology and Molecular Genetics. 2021 Dec; 2(3): 1-21. doi: 10.52700/jmmg.v2i3.34.

[16] Feng Y, Mannion A, Madden CM, Swennes AG, Townes C *et al.* Cytotoxic Escherichia coli strains encoding colibactin and cytotoxic necrotizing factor (CNF) colonize laboratory macaques. Gut pathogens. 2017 Dec; 9: 1-5. doi: 10.1186/s13099-017-0220-y.

[17] Rehman A, Andleeb S, Ullah SR, Mustafa Z, Gul D, Mehmood K. Antibiotic-mediated expression analysis of Shiga toxin 1 and 2 in multi-drug-resistant Shiga toxigenic *Escherichia coli*. Folia Microbiologica. 2021 Oct; 66(5): 809-17. doi: 10.1007/s 12223-021-00882-0.

[18] Abid H, Ali J, Waqas M, Anwar Y, Ullah J. Microbial quality assessment study of branded and unbranded milk sold in Peshawar City, Pakistan. Pakistan Journal of Nutrition. 2009; 8(5): 704-9. doi: 10.3923/pjn.2009.704.709.

[19] Razzaq A, Shamsi S, Nawaz A, Nawaz A, Ali A, Malik KA. 12. Occurrence of Shiga toxin producing *E. coli* from raw milk. Pure and Applied Biology. 2021 Oct; 5(2): 270-6. doi: 10.19045/bspab.2016.50035.

[20] Ullah S, Khan SU, Khan MJ, Khattak B, Fozia F, Ahmad I *et al.* Multiple-drug resistant Shiga toxin-producing *E. coli* in raw milk of dairy bovine. Tropical Medicine and Infectious Disease. 2024 Mar; 9(3): 64. doi: 10.3390/tropicalmed9030064.

[21] Shahzad KA, Muhammad K, Sheikh AA, Yaqub T, Rabbani M, Hussain T *et al.* Isolation and molecular characterization of Shiga toxin producing E. coli O157. The Journal of Animal & Plant Sciences. 2013; 23(6): 1618-1621.

[22] Samad A, Abbas F, Ahmad Z, Tanveer Z, Ahmad I, Patching SG *et al.* Multiplex polymerase chain reaction detection of Shiga toxin genes and antibiotic sensitivity of Escherichia coli O157: H7 isolated from beef meat in Quetta, Pakistan. Journal of Food Safety. 2018 Dec; 38(6): e12540. doi: 10.1111/jfs.12540.

[23] Samad A, Abbas F, Ahmad Z, Pokryrshko O, Asmat TM. Prevalence of foodborne pathogens in food items in Quetta, Pakistan. Pakistan Journal of Zoology. 2018 Aug; 50:1-4. doi: 10.17582/journal.pjz/2018.50.4.sc17.

[24] Shahzad A, Ullah F, Irshad H, Ahmed S, Shakeela Q, Mian AH. Molecular detection of Shiga toxin-producing Escherichia coli (STEC) O157 in sheep, goats, cows and buffaloes. Molecular Biology Reports. 2021 Aug; 48(8): 6113-21. doi: 10.1007/s11033-021-06631-3.

[25] Irshad H, Binyamin I, Ahsan A, Riaz A, Shahzad MA, Qayyum M *et al.* Occurrence and Molecular Characterization of Shiga Toxin-Producing Escherichia coli Isolates Recovered from Cattle and Goat Meat Obtained from Retail Meat Shops in Rawalpindi and Islamabad, Pakistan. Pakistan Veterinary Journal. 2020 Jul; 40(3): 295-300. doi: 10.29261/pakvetj/2020.045.

[26] Kanwal B, Shah S, Hassan S, Shaheen F. Prevalence of Shiga Toxin Producing Enterohemorrhagic Escherichia coli O157: H7 Isolated from Chicken Meat in Northern Punjab, Pakistan. Veterinary Sciences Research and Reviews. 2023; 9(1): 50-6. doi: 10.17582/journal.vsrr/2023/9.1.50.56.

[27] Amir M, Riaz M, Chang YF, Ismail A, Hameed A, Ahsin M. Antibiotic resistance in diarrheagenic Escherichia coli isolated from broiler chickens in Pakistan. Journal of food quality and hazards control. 2021 Jun; 8(2): 78-86. doi: 10.18502/jfqhc.8.2.6472.

[28] Tanveer A, Muneer B, Mehboob K, Rafique R, Sharif F. Isolation, Identification and Characterization of Escherichia Coli O157: H7 from Poultry Meat-A Worldwide Public Health Threat!. Infectious Diseases Journal of Pakistan. 2017 Apr; 26(4): 65-71.

[29] Iqtedar ME and Yasin AY. Incidence of *E. coli* O157: H7 in fresh fruit juices of street vendors from different areas of Lahore City, Pakistan. BIOLOGIA (PAKISTAN). 2014; 60(2): 185-91.

DOI: https://doi.org/10.54393/pbmj.v7i07.1104

[30] Shah MS, Eppinger M, Ahmed S, Shah AA, Hameed A, Hasan F. Multidrug-resistant diarrheagenic *E. coli* pathotypes are associated with ready-to-eat salad and vegetables in Pakistan. Journal of the Korean Society for Applied Biological Chemistry. 2015 Apr; 58: 267-73. doi: 10.1007/s13765-015-0019-9.

[31] Shah MS, Eppinger M, Ahmed S, Shah AA, Hameed A, Hasan F. Flooding adds pathogenic Escherichia coli strains to the water sources in southern Khyber Pakhtunkhwa, Pakistan. Indian journal of medical microbiology. 2016 Oct; 34(4): 483-8. doi: 10.4103/0255-0857.195350.

[32] Bano A and Ali B. Isolation and culture dependent characterization of Escherichia coli from the sewage waste water of Lahore, Pakistan. South Asian Journal of Research in Microbiology. 2019 Nov 15;5(1):1-3. doi: 10.9734/sajrm/2019/v5i130122.

[33] Bokhari H, Shah MA, Asad S, Akhtar S, Akram M, Wren BW. *Escherichia coli* pathotypes in Pakistan from consecutive floods in 2010 and 2011. The American journal of tropical medicine and hygiene. 2013 Mar; 88(3): 519. doi: 10.4269/ajtmh.12-0365.

[34] Ansari MR, Laghari MS, Solangi KB. Acute renal failure in pregnancy: one year observational study at Liaquat University Hospital, Hyderabad. JPMA. The Journal of the Pakistan Medical Association. 2008 Feb; 58(2): 61.

[35] Ibrahim SH, Bhutta ZA, Khan IA. Haemolytic uraemic syndrome in childhood: an experience of 7 years at the Aga Khan University. Journal of Pakistan Medical Association. 1998 Apr; 48(4): 100-103.

[36] Tresa V, Yaseen A, Lanewala AA, Hashmi S, Khatri S, Ali I *et al.* Etiology, clinical profile and short-term outcome of acute kidney injury in children at a tertiary care pediatric nephrology center in Pakistan. Renal failure. 2017 Jan; 39(1): 26-31. doi: 10.1080/0886022X.2016.1244074.

[37] Baloch DG. An overview of diarrheagenic *Escherichia coli* among infants in Pakistan. Pak-Euro Journal of Medical and Life Sciences. 2021 May; 4: 11-24. doi: 10.31580/pjmls.v4iSpecial%20Is.1865.

[38] Sohail A and Mazhar E. Screening of *Escherichia coli* O157 Strain from Stool Samples in Karachi, Pakistan. RADS Journal of Biological Research & Applied Sciences. 2016 Jul; 7(2): 06-9.

[39] Khan AY, Ahmad SS, Avais M, Ashraf K. In-Vitro and In-Vivo Antimicrobial Activity of Five Medicinal Plants against Virulent *Escherichia coli* O157: H7 Strain Harboring Shiga Toxin Gene. Pakistan Journal of Zoology. 2023 Dec; 1-10. doi: 10.17582/journal.pjz/20221009171005.

[40] Phitaktim S, Chomnawang M, Sirichaiwetchakoon K, Dunkhunthod B, Hobbs G, Eumkeb G. Synergism and the mechanism of action of the combination of α-mangostin isolated from Garcinia mangostana L. and oxacillin against an oxacillin-resistant Staphylococcus saprophyticus. BMC Microbiology. 2016 Dec; 16: 1-4. doi: 10.1186/s12866-016-0814-4.

[41] Nawaz B, Ali A, Syed MN, Khan AB. Screening for toxigenic Escherichia coli in stool samples of diarrhoeal patients by polymerase chain reaction. Pakistan Journal of Pharmaceutical Sciences. 2014 Sep; 27(5): 1571-4.

[42] Dubreuil JD. Fruit extracts to control pathogenic Escherichia coli: A sweet solution. Heliyon. 2020 Feb; 6(2). doi: 10.1016/j.heliyon.2020.e03410.

[43] Siriwong S, Teethaisong Y, Thumanu K, Dunkhunthod B, Eumkeb G. The synergy and mode of action of quercetin plus amoxicillin against amoxicillin-resistant Staphylococcus epidermidis. BMC Pharmacology and Toxicology. 2016 Dec; 17:1-4. doi: 10.1186/s40360-016-0083-8.

[44] Chen JC, Chang YS, Wu SL, Chao DC, Chang CS, Li CC *et al.* Inhibition of Escherichia coli heat-labile enterotoxin-induced diarrhea by Chaenomeles speciosa. Journal of ethnopharmacology. 2007 Sep; 113(2): 233-9. doi: 10.1016/j.jep.2007.05.031.

[45] Coddens A, Loos M, Vanrompay D, Remon JP, Cox E. Cranberry extract inhibits in vitro adhesion of F4 and F18+ Escherichia coli to pig intestinal epithelium and reduces in vivo excretion of pigs orally challenged with F18+ verotoxigenic E. coli. Veterinary Microbiology. 2017 Apr; 202: 64-71. doi: 10.1016/j.vetmic.2017.01.019.

[46] Girennavar B, Cepeda ML, Soni KA, Vikram A, Jesudhasan P, Jayaprakasha GK *et al.* Grapefruit juice and its furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria. International Journal of Food Microbiology. 2008 Jul; 125(2): 204-8. doi: 10.1016/j.ijfoodmicro.2008.03.028.

[47] Badar M, Khokhar I, Batool F, Iqbal R, Ch Y. Effect of boiling on removing of shiga toxins from drinking water samples. Journal of Entomology and Zoology Studies. 2017 Feb; 5(2): 672-676.

[48] Pastan I, Hassan R, FitzGerald DJ, Kreitman RJ. Immunotoxin therapy of cancer. Nature Reviews Cancer. 2006 Jul; 6(7): 559-65. doi: 10.1038/nrc1891.

[49] Melton-Celsa A, Mohawk K, Teel L, O'Brien A. Pathogenesis of Shiga-toxin producing Escherichia coli. Ricin and Shiga Toxins: Pathogenesis, Immunity, Vaccines and Therapeutics. 2012: 67-103. doi: 10.1007/82_2011_176.