Vol. 15(7), pp. 401-407, July, 2021
DOI: 10.5897/AJMR2021.9548
Article Number: B56502A67421
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Isolation, identification and antibiogram of verotoxin producing *Escherichia coli* from raw salad vegetables at Jashore, Bangladesh

Nigar Sultana Meghla[1*], Debashish Mridha[2], Md. Sohel Rana[1], Md. Ahosanul Haque Shahid[2] and Md. Muket Mahmud[2]

[1]Department of Microbiology, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore, Bangladesh.
[2]Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Received 3 June, 2021; Accepted 23 July, 2021

Verotoxin-producing *E. coli* are getting an emergence for human health and day by day, it is also becoming more alarming in the fact that, they are acquiring multidrug-resistant profiles. This study aimed at isolation and identification of verotoxigenic *E. coli* followed by the antibiogram of the species from salad vegetables at Jashore district in Bangladesh. A total of 119 raw vegetable salad samples were collected from different areas of the Jashore district. The collected samples were initially enriched in nutrient broth and then used for streak plating on MacConkey and followed by Eosin Methylene Blue (EMB) agar media. *Escherichia coli* species were isolated and identified following observation of the cultural, microscopic, and biochemical characteristics and further analyzed to determine the presence of verotoxin (VT) producing genes through polymerase chain reaction (PCR) using specific primers (VT1, VT2, and Eae). A panel of antibiotics were tested following the disc diffusion method for determining the antibiotic profile. Total 55 (46.22%) *E. coli* was isolated phenotypically. 6 (10.9%) isolated *E. coli* showed positive by amplifying verotoxin-producing gene *vt1,* but none of the samples were positive *vt2* and *eaeA* gene. Antibiogram for 55 *E. coli* isolates showed the highest resistance to Erythromycin (83.64%), Streptomycin (78.18%), Ceftriaxone (70.91%). On the other hand, the most heightened susceptibility was observed for Nalidixic acid (76.36%). Moreover, six *vt1* positive isolates showed a multidrug-resistant profile. Thus, the presence of toxigenic genes in the isolates suggests the proper maintenance and regular monitoring of raw salad items should be increase to ensure healthy life in this region.

Key words: Vegetable salad, *Escherichia coli*, verotoxin, antimicrobial resistant, polymerase chain reaction (PCR).

INTRODUCTION

Escherichia coli is one of the most common bacteria on the planet. Theodor Escherich, a German bacteriologist, was the first to discover it in 1885. As a result, this bacteria was named after him. *E. coli* is a Gram-negative

*Corresponding author. E-mail: nigar@just.edu.bd.

facultative anaerobe that does not generate spores (Parvej et al., 2018). The cells are generally rod-shaped, with a diameter ranging from 0.2 to 1 µm and a length of around two µm. Flagella-containing strains are motile, and the flagella are frequently arranged in a peritrichous pattern. It is typically found in animal excrement, mammalian lower intestines, and on the outskirts of hot springs. They thrive at 37°C. These bacteria live mainly in the intestines of warm-blooded mammals. *E. coli* contains a broad spectrum of strains with actions ranging from harmful to helpful (Law, 2000).

Verotoxin-producing *E. coli* (VTEC) is an unusual *E. coli* strain. O26:H11 was the most prevalent VTEC isolated from sporadic instances of hemolytic uremic syndrome (HUS) (Foxman, 2010). Verotoxins, also known as Shiga toxin, initially found in *Shigella* dysentery. However, the genes for their manufacture are easily spread across *E. coli* strains by toxin-encoding bacteriophages (Herold et al., 2004). The antigenically unique toxins VT1 and VT2 are encoded by two individual special lysogenic lambdoid bacteriophages (933J and 933W) introduced into the chromosome of *E. coli* 933 (Nazmul et al., 2008). VT phages have also been found in strains belonging to serogroups O111, O119, O128, and O157 (Ranjbar et al., 2017). Although this virulence factor (VT) may be found in a variety of serotypes, only a few well-characterized bio-serotypes and clones, especially O157:H7 and O26:H11, are the most significant hosts for the verotoxin phage(s) and are distributed globally (Nazmul et al., 2007).

Infections produced by isolates of VTEC serotypes other than the O157 have received more attention in recent years. Non-O157 VTEC infections are becoming more common in several countries. Many non-O157:H7 isolates linked to outbreaks do not have *eae* or the pO157 plasmid (Gyles, 2007), suggesting more non-O157 VTEC strains are not yet non-pathogenic. As a result, enhancing our understanding of these organisms requires efficient detection, isolation, and characterization of non-O157 VTEC isolates. The universality of the Polymerase Chain Reaction (PCR) test for amplification of *vt1* and *vt2* gene sequences has been proven, with both toxin genes being identified and distinguished across a wide range of verotoxigenic *E. coli*. The PCR approach allows for the quick, reliable, and low-cost identification of these critical toxin genes in clinical samples and potentially contaminated foods (Hamed et al., 2017).

Recently all over the world, food safety issues are given much concern because of the increased number of foodborne illnesses (WHO, 2002; Peattie, 2006). According to World Health Organization (WHO), Foodborne illness is referred to as hazardous for human health when the pathogen-associated contaminated food enters the human body (WHO, 2002). VTEC isolates are commonly present in food. Predictions are made of the possible increase in problems associated with these emerging pathogens (Younus et al., 2020; Parvej et al., 2018). As time passed, the food habits of ordinary people have changed rapidly in Bangladesh. Nowadays it is more popular in this country to take a meal in a nearby hotel or restaurant rather than eating homemade food. Here in Bangladesh, Restaurant and hotels are not only just food places but also assessed as refreshment sites. In restaurants, hotels, roadside food carts, along with different fast or Bengali foods, salad are common for all types of dishes. Mainly seasonal vegetables are used for making this salad, such as tomato, carrot, cucumber, coriander leaf, radish, and green chili are very common among them (Younus et al., 2020).

Jashore is the third-largest city in the Khulna Division and one of the most populous cities in Bangladesh's southwestern area. It is the second most developed city in the Khulna Division and Bangladesh's most industrious and progressive city. According to the Jashore municipality's records, it has a population of around 0.298 million people. There are various roadside food stores or restaurants that offer a range of foods with a vegetable salad throughout the city. A vast variety of microorganisms, particularly verotoxin-producing *E. coli*, can be found in vegetables. This organism has been associated with outbreaks connected to fresh vegetable intakes, such as lettuce, spinach, carrots, sprouts, and alfalfa (Younus et al., 2020). According to outbreak research, *E. coli* may live and thrive in a variety of minimally processed vegetable*s* (Kabir et al., 2014). It can also withstand freezing temperatures and extreme environmental conditions. Still now there is no study on the microbiological aspect of this vegetable salad in this region. It is necessary to evaluate the food safety issue of this served vegetable salad from a microbial aspect. So, this study was conducted for isolation, identification, and antibiogram of verotoxin-producing *E. coli* from raw salad vegetables at Jashore, Bangladesh.

MATERIALS AND METHODS

Sample collection

In this study, raw vegetables were selected as they are often eaten raw without any heat treatment, sometimes without washing and peeling. Total 119 vegetable samples (25 carrots, 22 cucumbers, 27 tomatoes, 20 radishes, 15 coriander leaves, and 10 green chilies) were collected from different locations at Jeshore in Bangladesh. Sterile zip bags (wiped with 70% ethanol) were used to collect salad vegetables, and the samples were carried to the laboratory without any delay.

Isolation and identification

After processing the collected samples, the nutrient broth was used for preliminary enrichment of the bacteria where the incubation temperature was 37°C for overnight. Overnight incubated test tubes then steaked on MacConkey and Eosin Methylene Blue (EMB) (Both were HiMedia, India) agar media for isolation of the

Table 1. Oligonucleotide sequences used in this study to detect verotoxin producing *E. coli*.

Primer	Target gene	Primer sequence	Annealing Tm (°C)	Amplification size (KB)	Reference
VT1	*vt1*	5'-CAAGAGTCCGTGGGATTACG-3' 5'-AGCGATGCAGCTATTAATAA-3'	55	130	Lindeberg et al. (2018)
VT2	*vt2*	5'-ACCGTTTTTCAGATTTACACATA-3' 5'-TACACAGGAGCAGTTTCAGACAGT-3'	56	298	
Eae	*eaeA*	5'-CACACGAATAAACTGACTAAAATG-3' 5'-AAAAACGCTGACCCGCACCTAAAT-3'	58	376	

Table 2. Prevalence of *E. coli* in different vegetable samples.

Types of vegetables	No. of examined vegetables	No. of vegetable with culture-positive *E. coli*	% of vegetables
Carrot	25	13	52
Cucumber	22	7	31.81
Tomato	27	10	37.04
Radish	20	12	60
Coriander leaf	15	8	53.33
Green chili	10	5	50
Total	119	55	46.22

suspected organism. *E. coli* produced a pinkish colony on MacConkey agar and a green metallic sheen containing colony on EMB agar. To confirm the microscopic examination, a loop of bacteria from the suspected colony was taken for Grams stain and placed the slide under 100X microscopic view (Punom et al., 2020).

Biochemical tests

Oxidase test and citrate utilization test were performed with culture-positive isolates according to the methods described in the Microbiology Laboratory Manual (Cheesbrough, 2006). Different sugar fermentation tests such as Dextrose, Mannitol, Lactose, Maltose, and Sucrose were done to confirm the organism (Punom et al., 2020).

Molecular detection

Genomic DNA was extracted by the conventional crude boiling method (Mishra et al., 2020). In short, 2-3 fresh cultured colonies were taken in a sterile 1.5 ml microcentrifuge tube and added 200 µl sterile Milli-Q water. Vortexed thoroughly and heated the microcentrifuge tube at 99°C for 10 min, followed by rapidly frozen at −20°C and centrifuged at 13,000 rpm for 5 min. Finally, 100 µl supernatant was collected and used as the DNA template in a PCR. The oligonucleotide primers used to amplify the genes by PCR are mentioned in Table 1, and the PCR reaction mixture was prepared as the author described in their study. The PCR products were visualized on a gel documentation system (Biometra, Jena, Germany) after electrophoresis with 1.5% agarose gel (SeaKem® LE Agarose from Lonza) as described by other researcher (Shahid et al., 2021).

Antimicrobial susceptibility test

The antimicrobial susceptibility was carried out for all the phenotypically positive isolates using the standardized agar disc diffusion method recommended by the Clinical Laboratory and Standards Institute (CLSI, 2018). Commercially available disc and Mueller Hinton Agar (Both were HiMedia, India) were used for the antimicrobial assay. Total 10 antibiotics disc from a different group of antimicrobial agents (Amoxicillin, 10 µg; Azithromycin, 15 µg; Ceftriaxone, 30 µg; Chloramphenicol, 30 µg; Ciprofloxacin, 5 µg; Erythromycin, 15 µg; Gentamycin, 10 µg; Nalidixic acid, 30 µg; Streptomycin, 10 µg and Tetracycline, 30 µg) were used in this study. Interpretation of the test results was made according to the CLSI guidelines and recorded in an excel file.

RESULTS AND DISCUSSION

Out of 119 vegetable samples, 55 samples were positive for the presence of *E. coli*. These positive samples were phenotypically confirmed where cultural characteristics on different agar media, microscopic examination, and biochemical test results were the parameters. The present study showed that the overall prevalence of *E. coli w*as 46.22% (n=55/119). The highest and lowest incidence of *E. coli* were 60% and 31.81% found in the radish and cucumber, respectively (Table 2). *E. coli* produced small, round, smooth, and greenish colonies onto EMB agar and pink-colored colonies on Mac-Conkey agar. They were Gram-negative and small rod-

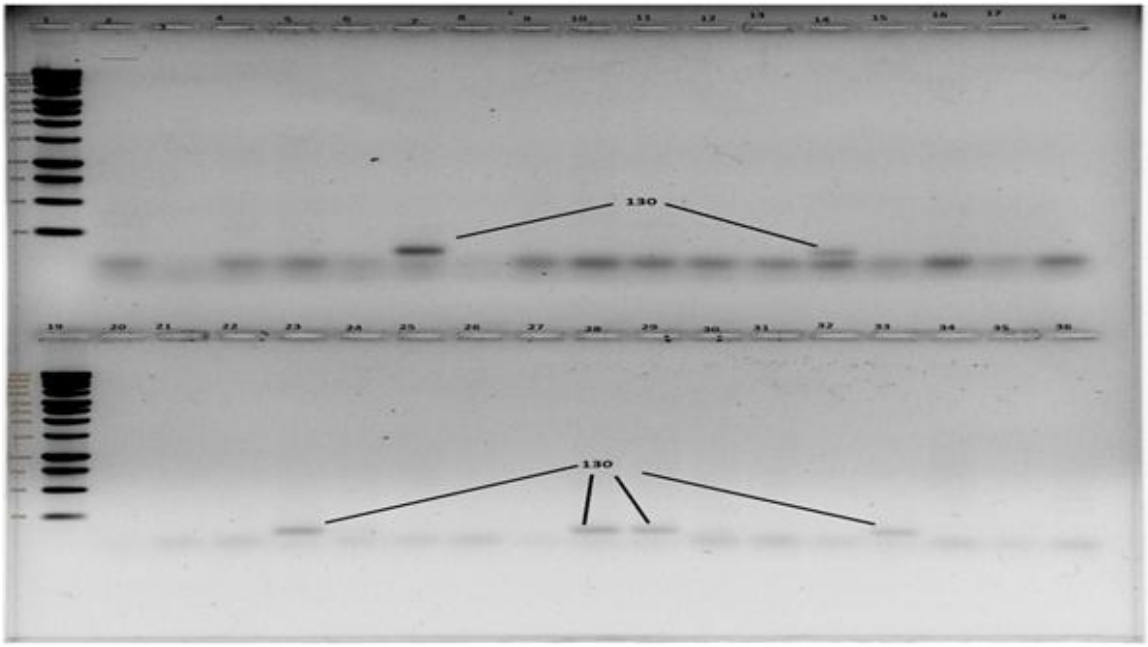

Figure 1. PCR amplification of *Vt1* gene of verotoxin-producing *E. coli*; Lane 1 and 19 are 1kb DNA ladder; Lane 7, 14, 23, 28, 29, and 33 are the positive *E. coli* isolates where they amplify verotoxin-producing *Vt1* gene at 130 bp.

Table 3. Antibiotic susceptibility profile of *E. coli* isolates.

Antibiotic name	Antibiotic sensitivity pattern (%) 0f *E. coli*		
	Susceptible % (n/N)	Intermediate % (n/N)	Resistant % (n/N)
Azithromycin	61.82 (34/55)	14.55 (8/55)	23.64 (13/55)
Ceftriaxone	12.73 (7/55)	16.64 (9/55)	70.91 (39/55)
Nalidixic acid	76.36 (42/55)	12.73 (7/55)	10.91 (6/55)
Chloramphenicol	58.18 (32/55)	25.45 (14/55)	16.36 (9/55)
Gentamycin	25.45 (14/55)	36.36 (20/55)	38.18 (21/55)
Erythromycin	0	16.36 (9/55)	83.64 (46/55)
Streptomycin	0	21.82 (12/55)	78.18 (43/55)
Ciprofloxacin	52.72 (29/55)	27.27 (15/55)	20 (11/55)
Amoxicillin	69.09 (38/55)	18.18 (10/55)	12.73 (7/55)
Tetracycline	63.64 (35/55)	20 (11/55)	16.36 (9/55)

shaped under microscopic view. Oxidase and Citrate tests were both negative for these isolates. All the suspected isolates fermented the sugars without producing any gas. The findings of microscopic exam and biochemical results were similar to the previously described in Punom et al. (2020), Younus et al., (2020) and Hasan et al. (2018).

A total of 6 isolates (cucumber-6a, cucumber-15c, carrot-11a, radish-4a, coriander leaf-13d, and coriander leaf-14a) were confirmed verotoxin producing *E. coli* by amplification of the *vt1* gene (Figure 1), but none of the samples were positive for *vt2* and *eaeA* verotoxin producing genes. Antibiogram study of all *E. coli* isolates revealed that the highest was 83.64% (46), 78.18% (43), and 70.91% (39) isolates found resistant to Erythromycin, Streptomycin, and Ceftriaxone, respectively. 76.36% (42) and 69.09% (38) isolates showed susceptibility to Nalidixic acid and Amoxicillin antibiotics (Table 3 and Figure 2). Isolates of verotoxin-producing *E. coli* were found multidrug-resistant.

For maintaining a healthy life there is no alternative to take fresh fruit and vegetables. Because of their low fat and high vitamin, mineral contents, day by daily total consumption ratio is increasing (Rekhy and McConchie,

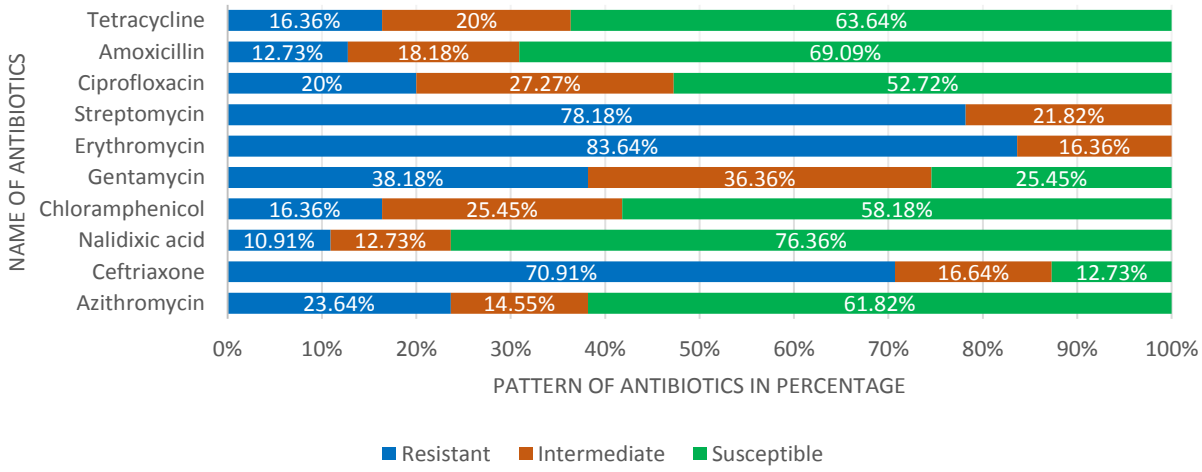

Figure 2. Pattern of antibiotics for the *E. coli* isolates from raw vegetable salad samples.

2014). Moreover, fresh fruit and vegetables are also prescribed to cardiovascular, cancer, diabetes-like diseased patients (Wang et al., 2014). But it must be ensured first that the consumable fruit and vegetables are free from all types of contamination. In this study, it had been found that Vegetable salad is working as a potential source of *E. coli*. It is predicted that foods are primarily contaminated during post-harvest handling and unhygienic processing environment. (Chowdhury et al., 2014; Alam et al., 2015). Moreover, the excess amount of use of antibiotics in farm areas is increasing the number of MDR organisms. It has been found that pesticide residue, which is used for agricultural purposes, may affect the body homeostasis of animals (Kobir et al., 2020).

During this investigation, one hundred nineteen raw vegetable samples were collected from a different market in Jashore, Bangladesh. Out of the 119 samples, *E. coli* was isolated from 55 samples, with a prevalence of 46% for this investigation. Isolation of *E. coli* from raw vegetables is also published in Dhaka city by several researchers (Islam et al., 2015; Kabir et al., 2014). But the prevalence ratio is relatively low. It might be the knowledge gap on the community level in this region, where farmers do not maintain the necessary hygienic measures during the production and harvesting of their products. The use of untreated wastewater and manure as fertilizers for producing fruits and vegetables is a major contributing factor to contamination (Rai and Tripathi, 2007). Also, restaurants, and hotel kitchens' hygienic condition is responsible for being so high prevalence in this area. Another source might be the middle man who is unconscious about the hygienic state, which plays a crucial role in contamination.

The *vt* gene was also detected in *E. coli* utilizing a PCR methodology and its molecular characterization in this investigation. In all, 55 *E. coli* isolates were tested for the presence of the *vt* gene using three sets of primers (VT1, VT2, and Eae). 6 (10.9%) *E. coli* isolates were discovered to possess the *vt1* gene, but no isolates carried the *vt2* and *eaeA* gene. According to these findings, the *vt1* gene is the most frequent verotoxin found in vegetable strains. In bovine isolates, the *vt1* gene is the most frequent toxin, while in porcine isolates, the *vt2* gene is the most prevalent (Gyles, 2007). The presence of just the *vt1* gene in this study might be related to the geographical dispersion of the isolates or members of a single clone that has spread globally.

The antibiotic susceptibility test yielded that the isolates exhibited resistance to Erythromycin 83.64% (46), Streptomycin 78.18% (43), Ceftriaxone 70.91% (39). Nalidixic acid 76.36% (42), Amoxicillin 69.09% (38), Tetracycline 63.64% (35) antibiotics showed the highest susceptibility to the isolates. Antibiotic pattern normally depends on the use of antibiotics on an area. For example, in the Chittagong district, one study says the *E. coli* isolates from salad samples are highly resistant to Gentamycin, Ampicillin, and Streptomycin, where our isolate showed only 38.18% (21) resistant to Gentamycin. Their isolate showed 100% sensitivity to Ciprofloxacin. But in our study, we found only 52.72% (29) susceptible to this antibiotic (Nipa et al., 2011). For any specific region, a class of antibiotics may be successful in any outbreak, whereas it may work as resistant in another region. To eradicate or successfully treat any outbreak, along with genetic information, location-specific information (data containing which antibiotic is mostly resistant or susceptible in any location) is compulsory (Sarker et al., 2021). These developments of antibiotic resistance can be deadly to both humans and other animals. In the investigation, the highest resistance was found against Erythromycin and Streptomycin. Uncontrolled usage of antibiotics in the treatment of animals and their integration in animal feeds has been believed to account for the increase in antibiotic resistance (Kundu et al., 2021; Hassan et al., 2017;

Woolhouse et al., 2015). In Bangladesh, all open markets are more or less in the same arrangement or style. There all types of raw vegetables, fish, meat are sold nearby. In this unhygienic and damp environment of the open market, flies play a vital role in contaminating raw vegetables or other food products. Resistance of *E. coli* to the Penicillin group of antibiotics has been on the higher side and is increasing day by day in different parts of the world. Nalidixic acid, the most efficient antibiotic against the isolates, prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome.

Conclusion

It might be concluded from this study that the raw salad vegetables collected from different areas are contaminated with antibiotic-resistant *E. coli*. The highest resistance was found against Erythromycin 83.64% (46), Streptomycin 78.18% (43), Ceftriaxone 70.91% (39) drug discs. They also contain the *vt* gene where *vt1* was the most common verotoxin among the *E. coli* strains isolated from vegetables in Jashore, Bangladesh. *vt1* gene locates on *E. coli,* might play an important role in horizontal transfer of *vt* gene. Above all, we must be aware of proper washing of salad vegetables.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to hotel and restaurant owners for giving us access to collect samples during the study and this research was funded by R & D (Research and development) project of MOST (Ministry of Science and Technology), Bangladesh.

REFERENCES

Alam M, Chowdhury M, Hossain MS, Rahman MM, Rahman MA, Gan SH, Khalil M (2015). Detection of residual levels and associated health risk of seven pesticides in fresh eggplant and tomato samples from Narayanganj District, Bangladesh. Journal of Chemistry. http://eprints.usm.my/38639/1/Detection_of_Residual_Levels_and_Associated_Health_Risk_of_Seven_Pesticides_in_Fresh_Eggplant.pdf

Cheesbrough M (2006). District Laboratory Practice in Tropical Countries. London English Language Book, pp. 100-194.

Chowdhury AZ, Hasan M, Karim N, Fakhruddin ANM, Hossain S, Chowdhury AA, Alam K (2014). Contamination and health risk assessment of pesticide residues in vegetables from agricultural fields of Gazipur District, Bangladesh. Sigma 2(4).

Clinical Laboratory Standards Institute (CLSI) (2018). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, VET08, Clinical and Laboratory Standards Institute, Wayne, PA.

Foxman B (2010). The epidemiology of urinary tract infection. Nature Reviews Urology 7(12):653-660.

Gyles CL (2007). Shiga toxin-producing *Escherichia coli*: an overview. Journal of Animal Science 85(13):45-62.

Hamed OM, Sabry MA, Hassanain NA, Hamza E, Hegazi AG, Salman MB (2017). Occurrence of virulent and antibiotic-resistant Shiga toxin-producing *Escherichia coli* in some food products and human stool in Egypt. Veterinary World 10(10):1233.

Hassan J, Nazir KNH, Parvej MS, Kamal T, Rahman MT (2017). Molecular based prevalence of shigatoxigenic Escherichia coli in rectal swab of apparently healthy cattle in Mymensingh district, Bangladesh. Journal of Advanced Veterinary and Animal Research 4(2):194-199.

Herold S, Karch H, Schmidt H (2004). Shiga toxin-encoding bacteriophages–genomes in motion. International Journal of Medical Microbiology 294(2-3):115-121.

Islam Z, Sultana S, Rahman MM, Rahman SR, Bari ML (2015). Effectiveness of different sanitizers in inactivating E. coli O157: H7 in Tomato and Cucumber. Journal of Food and Nutrition Sciences *3*(1-2):60-64.

Kabir A, Das AK, Kabir MS (2014). Incidence of antibiotic resistant pathogenic bacteria in vegetable items sold by local and super shops in Dhaka city. Stamford Journal of Microbiology *4*(1):13-18.

Kobir MA, Akther L, Hasan I, Shahid MAH, Haque Z, Karim MR (2020). Effects of Imidacloprid-Contaminated Feed Exposure on Hematological Parameters in Adult Rabbits (Oryctolagus Cuniculus). Research in Agriculture Livestock and Fisheries *7*(3):439-444.

Kundu T, Rumi NA, Hossain MK, Rahman MS, Hossain MMK, Halder J (2021). Isolation of multidrug-resistant *Escherichia coli* from turkeys in Dinajpur, Bangladesh, and their antibiogram profile. Journal of Advanced Veterinary and Animal Research 8(1):64.

Law D (2000). The history and evolution of *Escherichia coli* O157 and other Shiga toxin-producing E. coli. World Journal of Microbiology and Biotechnology 16(8):701-709.

Lindeberg YL, Egedal K, Hossain ZZ, Phelps M, Tulsiani S, Farhana I, Jensen PKM (2018). Can *Escherichia coli* fly? The role of flies as transmitters of E. coli to food in an urban slum in Bangladesh. Tropical Medicine and International Health 23(1):2-9.

Mishra P, Mahmud MM, Shahid MAH, Hasan A, Yadav VK, Hasan M (2020). Molecular detection of methicillin resistant staphylococcus aureus (MRSA) from a clinical case of myiasis wound: a case report. *Authorea Preprints*. http://researcherslinks.com/current-issues/Molecular-Detection-of-Methicillin-resistant-Staphylococcus-aureus-MRSA-from-a-Clinical-Case-of-Myiasis-Wound/18/25/3044/html.

Nazmul MHM, Salmah I, Jamal H, Ansary A (2008). Molecular characterization of verotoxin gene in enteropathogenic *Escherichia coli* isolated from Miri Hospital, Sarawak, Malaysia. Biomedical Research 19(1):9-12.

Nazmul MHM, Salmah I, Jamal H, Ansary A (2007). Detection and molecular characterization of verotoxin gene in non-O157 diarrheagenic *Escherichia coli* isolated from Miri hospital, Sarawak, Malaysia. Biomedical Research 18(1):39-43.

Nipa MN, Mazumdar RM, Hasan MM, Fakruddin MD, Islam S, Bhuiyan HR, Iqbal A (2011). Prevalence of multi drug resistant bacteria on raw salad vegetables sold in major markets of Chittagong city, Bangladesh. Middle-East Journal of Scientific Research 10(1):70-77.

Parvej MS, Mamun M, Hassan J, Mahmud MM, Rahman M, Rahman MT, Nazir KNH (2018). Prevalence and characteristics of Shiga-toxin producing *Escherichia coli* (STEC) isolated from beef slaughterhouse. Journal of Advanced Veterinary and Animal Research 5(2):218-225.

Peattie K (2006). Corporate social responsibility and the food industry-Ken Peattie discusses why developing a CSR agenda is so important. Food Science Technology-Information Quarterly of the Institute of Food Science and Techn 20(2):46-48.

Punom SA, Khan MSR, Pritha ST, Hassan J, Rahman S, Mahmud MM, Islam MS (2020). Isolation and molecular-based identification of bacteria from unhatched leftover eggs of ducks in selected mini-hatcheries of Kishoreganj, Bangladesh. Journal of Advanced Veterinary and Animal Research 7(1):164.

Rai PK, Tripathi BD (2007). Microbial contamination in vegetables due to irrigation with partially treated municipal wastewater in a tropical city. International Journal of Environmental Health Research *17*(5):389-395.

Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E (2017). Shiga (Vero)-toxin producing *Escherichia coli* isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. Antimicrobial Resistance and Infection Control 6(1):1-11.

Rekhy R, McConchie R (2014). Promoting consumption of fruit and vegetables for better health. Have campaigns delivered on the goals?. Appetite *79*:113-123.

Sarker MSA, Shahid MAH, Hoque MN, Sarker MA, Rahman MB, Islam SS (2021). The The Rich Mapping: Be a Supplementary Approach for Anthrax Control at Community Level. Journal of Advanced Veterinary Research 11(1):41-46.

Shahid MAH**,** Nazir KHMNH, Zowalaty MEE, Kabir A, Sarker MSA, Siddique MP, Ashour HM (2021). Molecular Detection of Vancomycin Resistance and Methicillin Resistance in *Staphylococcus aureus* from Food Processing Environments. One Health 100276.

Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, Hu FB (2014). Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. British Medical Journal P 349.

World Health Organization (WHO) (2002). WHO global strategy for food safety: safer food for better health. WHO, Geneva, Switzerland, 2002.

Woolhouse M, Ward M, van Bunnik B, Farrar J (2015). Antimicrobial resistance in humans, livestock and the wider environment. Philosophical Transactions of the Royal Society B: Biological Sciences 370(1670):20140083.

Younus MI, Sabuj AAM, Haque ZF, Sayem SM, Majumder S, Parvin MS, ... Saha S (2020). Microbial risk assessment of ready-to-eat mixed vegetable salads from different restaurants of Bangladesh Agricultural University campus. Journal of advanced veterinary and animal research 7(1):34.