Vol. 5 No. 1 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

https://doi.org/10.5281/zenodo.19130045

Online ISSN 3007-2387

Print ISSN 3007-2379

Comparative Molecular Epidemiology and Virulence Gene Profiling of *Escherichia coli* from Clinical and Aquatic Environments: A One Health Perspective

Faiza Ahmed
Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Afshan Hussain
Department of Microbiology and Molecular Genetics, Women University Multan, Multan, Pakistan

Nimra Javaid
Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Irfan Shafaqat Ali
Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Saqib Latif
Institute of Immunology, National University of Medical Sciences, Islamabad, Pakistan

Author Details

Keywords: *Escherichia Coli*, Virulence Genes, Antimicrobial Resistance, Molecular Epidemiology, One Health

Received on 09 Feb 2026
Accepted on 12 Mar 2026
Published on 20 Mar 2026

Corresponding E-mail & Author*:

Faiza Ahmed
Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract

The *Escherichia coli* is a highly heterogeneous bacterium capable of behaving both as a commensal bacterium and a versatile pathogen causing additional intestinal and extraintestinal infections. The hypothetical purpose of the present research was to compare the molecular epidemiology, virulence gene patterns as well as antimicrobial resistance patterns of E. coli isolates in clinical and aquatic settings in a One Health paradigm. A total of 55 samples comprising of 30 water samples of filtration plants and 25 clinical isolates of urinary tract infections were collected and subjected to the procedure of bacterial isolation. Standard culture tests confirmed the isolates to be *E. coli*, and then tested them as to antibiotic susceptibility and virulence-associated genes (*stx1*, *stx2*, *eae*, *eae_y*, *bfp*, *lpfa* variants) by multiplex polymerase chain reaction (PCR). Antimicrobial testing showed a high level of sensitivity to imipenem with high levels of resistance to rifampicin and sulfamethoxazole-trimethoprim. The patterns of multidrug resistance were observed in both clinical and environmental isolates, which points to the concerns of public health. Virulence gene profiling showed that both sources carry stx1 and *stx2* with *stx2* being more common in clinical isolates. Gene related to adhesion were more common in water isolates

especially the gene *bfp* showing that there were environmental reservoirs of the enteropathogenic strains. Comparative analysis indicated that there overlapping patterns of virulence, which indicated the possibility of the transmission between the environmental and human populations. It highlights the importance of water as a possible source of pathogenic and multidrug-resistant *E. coli* and the need to integrate surveillance approaches. This paper Has emphasized the importance of the One Health approach in the study and management of the spread of E. coli in both clinical and environmental environments.

Introduction

Escherichia coli is a very heterogeneous Gram-negative bacterium, which is both a harmless commensal bacterium in the gastrointestinal tract of humans and animals and a versatile pathogen that causes many types of infections (1). Intestinal diseases *E. coli* pathogenic strains are linked to intestinal diseases like diarrhea, hemorrhagic colitis, extraintestinal infections, including urinary tract infections (UTIs), neonatal meningitis, and septicemia (2). The virulence of *E. coli* is greatly due to the expression of certain virulence factors such as adhesins, toxins, invasin and iron acquisition systems, which facilitate colonization of host tissues, escape of immune responses and disease progression of the bacterium (3).

Over the past years, there has been a growing interest in the study of the molecular epidemiology of *E. coli*, especially in the framework of its dissemination over human, animal, and environmental interfaces (4). Water sources are potential sources and pathways of pathogenic *E. coli* particularly where sanitation and water treatment systems are poor (5). The presence of virulent strains in water bodies indicates the possibility of water-borne spread of infection, which is a major threat to human health. Furthermore, comparableity of virulence gene pattern in environmental and clinical isolates indicates an interactive movement of genetic material and potential transmission routes between the ecosystems (6).

The molecular methods and especially polymerase chain reaction (PCR)-based method of genotyping has transformed the epidemiological studies of bacteria by allowing the effective and accurate identification of virulence genes *like stx1, stx2, eae,* and *bfp* (7). They are also important markers of the pathogenic strains of *E. coli*, such as E. coli that produce Shiga toxin (STEC) and *E. coli* that produce enteropathogenic (EPEC) toxin (8). The comparative study of these virulence genes between different sources of isolates is a good source of information on the diversity of strains, the potential to be pathogenic, and the epidemiological associations (9).

The onset and diffusion of antimicrobial resistance (AMR) in *E. coli* is an additional complication in its effects on public health. The resistant strains may still prevail in the clinical and environmental setting promoting their transmission in contaminated water and in human population. This emphasizes the need to have comprehensive surveillance methods that would look at different sectors at the same time (10).

The concept of One Health, which acknowledges the interconnectedness of human, animal, and environmental health, is an excellent concept that may be applied to examine the *E. coli* transmission mechanism (11). Through the combination of clinical and aquatic data, the comparative molecular epidemiological studies can reveal similar virulence patterns and possible transmission paths and therefore guide effective control measures (12).

As such, the offered study will comparatively examine the molecular epidemiology and virulence gene phenotypes of *E. coli* that can be found in clinical and aquatic environments to improve the comprehension of its transmission schemes and the impact on the human population in terms of One Health.

Materials and Methods

Study Design and Sample Collection

This cross-sectional study was conducted to investigate the molecular epidemiology and virulence gene profiles of *Escherichia coli* isolated from clinical and aquatic environments. A total of 55 samples were included, comprising 30 water samples and 25 clinical isolates. Water samples were collected from filtration plants across multiple Union Councils in Lahore using sterile 100 mL containers and transported to the laboratory under cold conditions. All samples were processed within six hours of collection. Clinical isolates were obtained from urine samples of patients diagnosed with urinary tract infections (UTIs) from tertiary care hospitals, following standard ethical guidelines (13).

Isolation and Identification of *E. coli*

Water samples were filtered through 0.45 µm membrane filters to concentrate bacterial cells, followed by suspension in phosphate-buffered saline (PBS) (14). Both water suspensions and clinical samples were cultured on MacConkey agar and incubated at 37°C for 24 hours (15). Presumptive *E. coli* colonies (pink, lactose-fermenting) were sub-cultured on Luria-Bertani (LB) agar to obtain pure cultures. Pure isolates were further propagated in LB broth for downstream analyses (16).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined using the disk diffusion method on Mueller-Hinton agar in accordance with standard guidelines. The antibiotics tested included oxytetracycline, rifampicin, imipenem, amikacin, sulfamethoxazole-trimethoprim, nalidixic acid, ciprofloxacin, piperacillin-tazobactam, and ceftazidime. After incubation at 37°C for 24 hours, zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant (17).

DNA Extraction

Genomic DNA was extracted from overnight bacterial cultures using a modified CTAB-based method. Bacterial pellets were treated with lysozyme, followed by SDS and proteinase K digestion. DNA purification involved chloroform:isoamyl alcohol and phenol:chloroform extraction steps, followed by precipitation with cold isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and resuspended in nuclease-free water (16).

PCR-Based Virulence Gene Detection

Multiplex polymerase chain reaction (PCR) was employed to detect virulence-associated genes, including *stx1*, *stx2*, *eae*, *eae_y*, *bfp*, and *lpfa* variants. Primers were divided into two multiplex sets (Complex I and Complex II) to optimize amplification efficiency (12). PCR reactions were carried out in a thermocycler under standard cycling conditions: initial denaturation at 95°C, followed by cycles of denaturation, annealing, and extension at optimized temperatures.

Agarose Gel Electrophoresis

PCR products were analyzed using 1.3% agarose gel electrophoresis in 1× TAE buffer. A 100 bp DNA ladder was used as a molecular size marker. Gels were stained with ethidium bromide and visualized under UV illumination to confirm the presence of amplified gene fragments (14).

Data Analysis

The prevalence of virulence genes and antimicrobial resistance patterns were compared between clinical and water isolates. Descriptive statistics were used to summarize the data, and comparative analysis was performed to assess differences between the two groups.

Results

Isolation and Identification of *E. coli*

Out of 30 water samples processed, *E. coli* was successfully isolated from a subset of samples, while all 25 clinical samples were confirmed as *E. coli*. On MacConkey agar, isolates produced characteristic pink to reddish colonies, indicating lactose fermentation. Subsequent subculturing on LB agar yielded pure, well-defined colonies, confirming successful isolation.

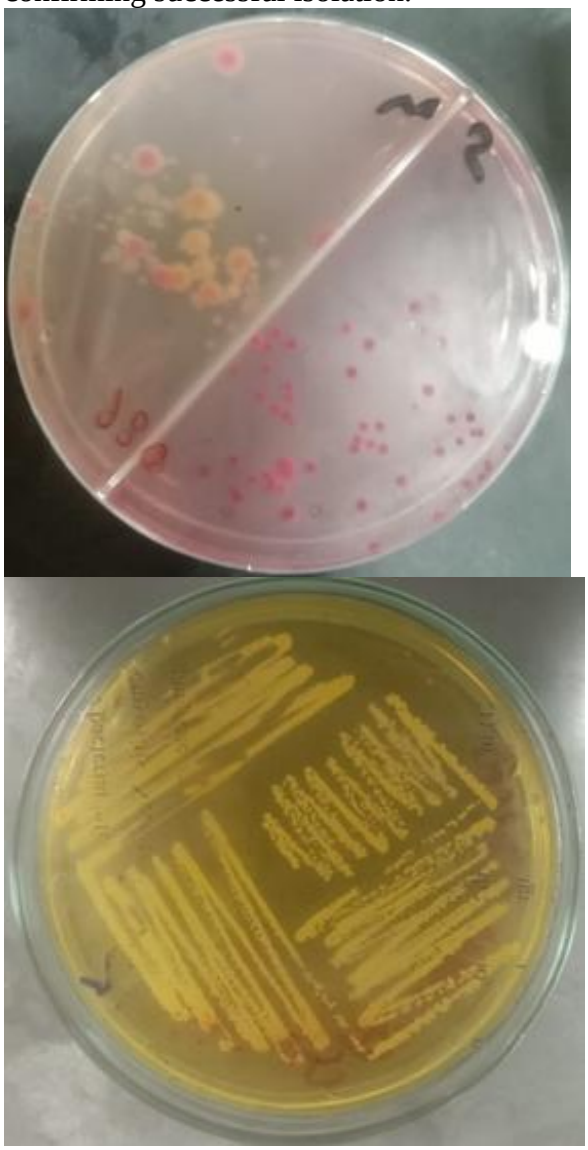

Figure 1. (a). Representative image of *E. coli* colonies on MacConkey agar
(b). Pure culture colonies on LB agar

Antimicrobial Susceptibility Patterns

Antibiotic susceptibility testing revealed variable resistance patterns among both water and clinical isolates. High sensitivity was observed against imipenem in nearly all isolates, while considerable resistance was noted against rifampicin, oxytetracycline, and sulfamethoxazole-trimethoprim.

Water isolates demonstrated higher resistance to nalidixic acid and rifampicin, whereas clinical isolates showed comparatively increased resistance to ciprofloxacin and sulfamethoxazole-trimethoprim. Multidrug resistance (MDR) patterns were observed in several isolates from both sources, indicating the widespread presence of resistant strains.

Table 1. Comparative antimicrobial susceptibility pattern of *E. coli* isolates from water (n = 30) and clinical samples (n = 25)

Antibiotic	Water Isolates Sensitive (S)	Intermediate (I)	Resistant (R)	Clinical Isolates Sensitive (S)	Intermediate (I)	Resistant (R)
CAZ (Ceftazidime)	21	5	4	20	2	3
TZP (Piperacillin–Tazobactam)	22	7	1	21	3	1
AK (Amikacin)	24	1	5	16	1	8
NA (Nalidixic Acid)	10	2	18	7	2	16
CIP (Ciprofloxacin)	19	6	5	16	3	6
IPM (Imipenem)	30	0	0	25	0	0
RD (Rifampicin)	2	4	24	4	6	15
OT (Oxytetracycline)	17	4	9	15	3	7
SXT (Sulfamethoxazole–Trimethoprim)	1	2	27	0	3	22

Antimicrobial susceptibility testing revealed variable resistance patterns among *E. coli* isolates from water and clinical sources. Imipenem showed the highest effectiveness, with 100% sensitivity in both water (30/30) and clinical isolates (25/25). In contrast, high resistance was observed against rifampicin and sulfamethoxazole–trimethoprim. Water isolates showed 80% resistance to rifampicin (24/30), while clinical isolates exhibited 60% resistance (15/25). Similarly, resistance to sulfamethoxazole–trimethoprim was observed in 90% of water isolates (27/30) and 88% of clinical isolates (22/25). Nalidixic acid also demonstrated considerable resistance in both environmental and clinical isolates.

Virulence Gene Profiling in Water Isolates

PCR-based genotyping revealed the presence of multiple virulence-associated genes among water isolates. The *stx1* gene was detected in 12% of isolates, while *stx2* was found in 16%, indicating the presence of Shiga toxin-producing *E. coli* (STEC). Notably, one isolate harbored both *stx1* and *stx2*, suggesting enhanced virulence potential.

The *bfp* gene, associated with enteropathogenic *E. coli* (EPEC), was detected in 36% of water isolates, making it one of the most prevalent virulence markers in environmental samples. Other genes, including *eae* and *lpfa* variants, were also detected at varying frequencies, reflecting genetic diversity among isolates.

Virulence Gene Profiling in Clinical Isolates

Clinical isolates also exhibited a diverse range of virulence genes. The prevalence of *stx1* and *stx2* genes was comparable to or slightly higher than that observed in water isolates, reinforcing their clinical significance. The *eae* gene, a key marker of adherence, was more frequently detected in clinical isolates, indicating its role in host colonization.

The presence of *bfp* and *lpfa* genes further highlighted the contribution of adhesion-related virulence factors in clinical infections. Some isolates carried multiple virulence genes simultaneously, suggesting increased pathogenic potential.

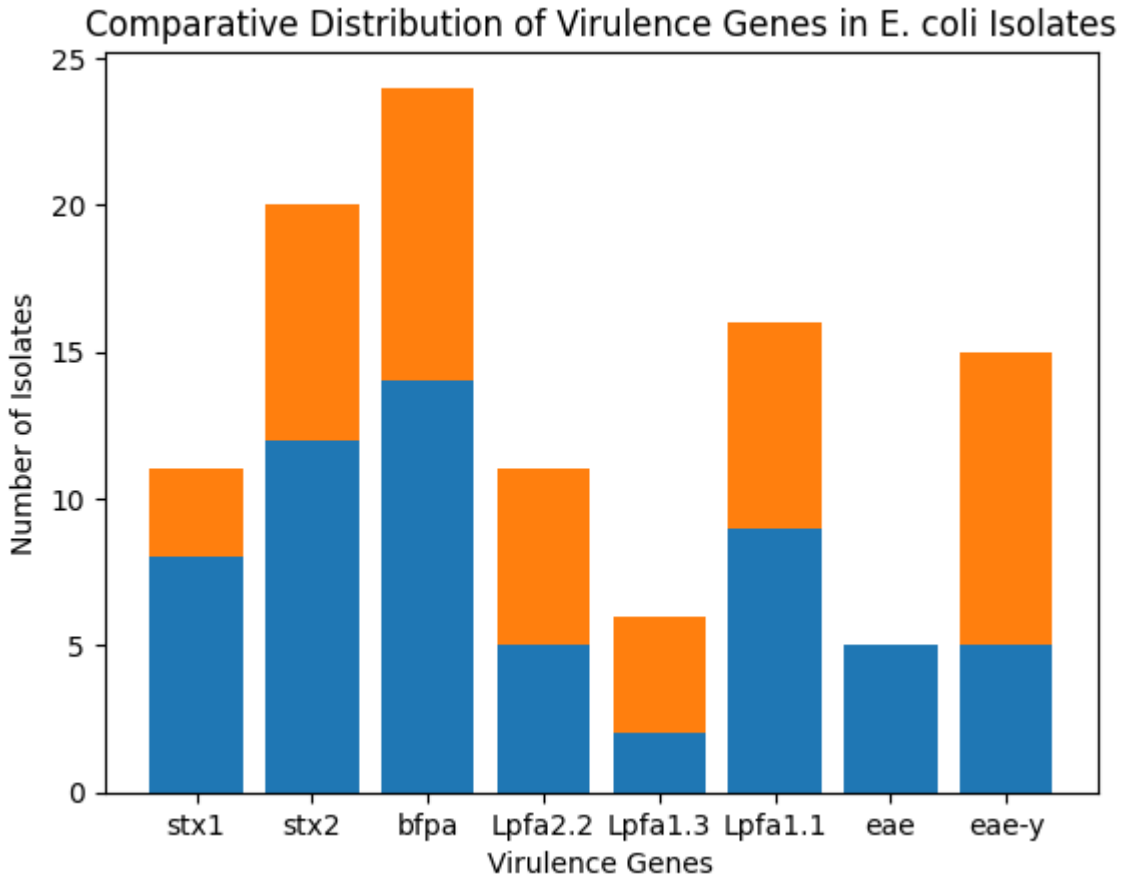

Figure 2. The bar chart represents the frequency of major virulence genes (*stx1*, *stx2*, *bfpa*, *lpfa variants*, *eae*, and *eae-y*) detected in *E. coli* isolates from water (n=30) and clinical samples (n=25). A higher prevalence of *bfpa* and *lpfa1.1* was observed in water isolates, whereas *eae-y* showed greater occurrence in clinical isolates.

Comparative Analysis of Clinical and Water Isolates

Comparative analysis showed both similarities and differences in virulence gene distribution between water and clinical *E. coli* isolates. The *stx2* gene was more prevalent in clinical isolates (20%) than in water isolates (16%), while *stx1* showed equal frequency (12%) in both groups. The *bfpa* gene was more common in water isolates (36%) compared to clinical isolates (28%), suggesting a higher environmental presence of EPEC strains.

Pathotype analysis revealed a slightly higher proportion of EHEC in clinical isolates (44%) than in water isolates (40%), whereas EPEC was more frequent in water samples (36% vs. 32%). Overall, the presence of key virulence genes in both sources indicates potential public health risks and suggests a possible link between environmental and clinical strains.

Discussion

The current paper offers comparative molecular epidemiological evaluation of *Escherichia coli* isolates in clinical and aquatic settings that demonstrate essential information about the distribution of virulence genes and antimicrobial resistance profiles (18). The effective isolation of *E. coli* in water and clinical samples confirms the widespread nature of this bacterium and points to the possible role of this organism as the source of pathogenic strains. The isolation rates observed are in line

with other reports that water bodies especially in urban centres that lack proper sanitation may harbor pathogenic E. coli that can cause infections to humans (19).

The sensitivity of imipenem was high in all the isolates, which represents its effectiveness in treating *E. coli* infections. On the other hand, high resistance to rifampicin and sulfamethoxazole and trimethoprim are in line with the global trends of increasing multidrug resistance in E. coli especially, which is a big challenge to the populace (20). It is noteworthy that, as compared to clinical isolates, environmental isolates were more resistant to nalidixic acid and rifampicin but more resistant to ciprofloxacin and sulfamethoxazole-trimethoprim. Such differences are likely the influence of different selective pressures in aquatic and clinical settings and indicate the environmental role in AMR spread (21).

Profiling of virulence genes showed that both sources have a varied repertoire with *stx1, stx2, bfp, eae* and *lpfa* variants. The observed higher frequency of detection of *stx2* in clinical (20%) versus water (16) isolates is consistent with the known association of stx2 gene with serious human disease, such as hemolytic uremic syndrome. Surprisingly, bfp gene was more common in water isolates, and it suggests that enteropathogenic *E. coli* (EPEC) is present in the environment significantly. Such results support the work of other researchers that has found water sources as possible reservoirs of virulent strains of E. coli that can genetically interact with human pathogens (22,23).

The comparative analysis highlights possible transmission connection between aquatic and clinical populations of *E. coli*. The commonality of significant virulence genes implies that the pathogenic strains might be transmitted via contaminated water, which supports the concept of One Health that embraces human, animal, environmental health. Furthermore, the finding of multidrug-resistant, virulent strains in both environments highlights the need to implement combined surveillance programs and interventions, which can reduce the effects of *E. coli* on the general population (24).

These results on the whole indicate that environmental reservoirs and clinical infections are dynamically interrelated, and both sources should be monitored to understand the epidemiology and virulence potential of *E. coli* in a holistic manner (25).

Conclusion

This paper shows that E. coli clinical and aquatic isolates have pronounced similarities in terms of virulence genes profiles, whereas there are significant distinctions in antimicrobial resistance profiles. *Stx1, stx2,* and *bfp* and other adhesion factors are present in both sources, which makes it possible that there is environmental-to-human transmission of this disease, and water bodies are repositories of pathogenic *E. coli*. These results highlight the significance of One Health methodology in monitoring and controlling the *E. coli* infection, through the union of clinical, environmental, and community health measures. Constant monitoring and responsible use of antibiotics are also necessary in order to reduce the threat of the multidrug-resistant, virulent strains.

References

Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2(2):123–140.

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev. 2013;26(4):822–880.

Gyles CL. Shiga toxin-producing Escherichia coli: an overview. J Anim Sci. 2007;85(13 Suppl):E45–E62.

Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998;11(1):142–201.
Bettelheim KA. The non-pathogenic Escherichia coli: a brief overview. Int J Food Microbiol. 2007;114(2):237–243.
Winfield MD, Groisman EA. Role of nonhost environments in the lifestyles of Salmonella and Escherichia coli. Microbiol Mol Biol Rev. 2003;67(2):189–208.
Beutin L. Emerging enterohemorrhagic Escherichia coli: an increasing public health risk. Int J Med Microbiol. 2006;296(3):133–145.
Otto P, et al. Virulence genes and antimicrobial resistance in environmental E. coli isolates. Water Res. 2015;79:97–105.
Koczura R, et al. Distribution of virulence genes among E. coli strains from water. Pol J Microbiol. 2012;61(2):123–130.
Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of Escherichia coli O157:H7 outbreaks. Epidemiol Rev. 2005;27:60–98.
Pierard D, et al. Detection of virulence genes in E. coli from environmental and clinical sources. J Appl Microbiol. 2007;102(3):775–783.
WHO. Guidelines for safe recreational water environments. Geneva: World Health Organization; 2003.
Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press; 2006.
CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press; 2001.
Paton AW, Paton JC. Detection and characterization of Shiga toxigenic Escherichia coli by multiplex PCR. J Clin Microbiol. 1998;36(2):598–602.
Hoorfar J, et al. PCR-based detection of Escherichia coli virulence genes from food and environmental samples. Appl Environ Microbiol. 2000;66(10):4478–4482.
Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008;8(3):159–166.
Ewers C, et al. Virulence genotyping of Escherichia coli isolates from human and animal sources. Vet Microbiol. 2007;123(1–3):198–206.
Dhanashree B, et al. Multidrug resistance in environmental E. coli strains: public health implications. Environ Sci Pollut Res. 2014;21(13):8132–8141.
Lopez-Saucedo C, et al. Detection of virulence genes in E. coli isolates from water and clinical samples. J Appl Microbiol. 2003;95(5):901–906.
Johnson JR, et al. Phylogenetic analysis and virulence profiling of extraintestinal pathogenic E. coli. Infect Immun. 2002;70(12):5975–5982.
Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. Infect Dis Clin North Am. 2005;19(4):523–539.
Jay-Russell MT, et al. Environmental reservoirs of Escherichia coli and their link to human disease. Microb Risk Anal. 2014;1:1–10.
Abdel-Moein KA, et al. Molecular detection of virulence genes in E. coli from water sources. J Infect Dev Ctries. 2017;11(5):379–386.