



OPEN Seasonal and environmental drivers of antibiotic resistance and virulence in *Escherichia coli* from aquaculture and their public health implications

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Aquaculture is increasingly impacted by environmental stressors such as temperature and pH fluctuations, which influence the proliferation and antibiotic resistance of *Escherichia coli* (*E. coli*). This study investigates the effects of these factors on the prevalence, virulence, and antibiotic resistance of *E. coli* isolated from aquaculture environments in Egypt, with a focus on public health implications. A total of 328 *Oreochromis niloticus* (Nile tilapia) samples were collected from Egyptian fish farms over five sampling periods, representing different seasonal conditions. *E. coli* was isolated and identified using selective culture methods and biochemical tests. Molecular characterization was conducted via polymerase chain reaction (PCR) to detect diarrheagenic *E. coli* pathotypes (*st*, *lt*, *eaeA*, *bfpA*, *stx1*, *stx2*). Additionally, PCR was utilized to screen for β -lactamase and carbapenemase resistance genes. Water parameters, including temperature and pH, were recorded, and their correlation with bacterial prevalence, virulence, and antibiotic resistance profiles were analyzed. A high prevalence of *E. coli* (92.68%) was observed, with a significant correlation between bacterial occurrence and elevated water temperatures. Diarrheagenic *E. coli* was detected in 82.1% of samples, with enterotoxigenic *E. coli* (ETEC) being the most common pathotype. Some isolates harbored multiple virulence genes, indicating hybrid strains. Resistance genes such as *bla*_{TEM}, *bla*_{CTX-M}, and *bla*_{OXA-48} were widely distributed, particularly during warmer months and at neutral pH levels. Groups with elevated water temperatures exhibited a higher prevalence of antibiotic-resistant isolates, often harboring multiple resistance genes. This study highlights the significant role of environmental stressors in influencing the prevalence, pathogenicity, and antibiotic resistance profiles of *E. coli* in aquaculture systems. The findings emphasize the need for continuous monitoring and improved biosecurity measures to mitigate the risks associated with MDR *E. coli* in aquaculture, ensuring food safety and protecting public health.

Keywords Seasonal variations, *E. coli*, Virulence genes, Antibiotic resistance genes, Fish farms, Egypt

Climate change has become a critical and widely discussed global issue¹, with significant implications for various sectors, including aquaculture². It impacts aquaculture by altering water quality, threatening species viability, and risking sustainable production³. Higher global temperatures are directly linked to increased water temperatures, which elevate mortality rates in aquatic species⁴. Water quality fluctuations, such as CO₂-induced acidification, further compromise fish health by disrupting pH balance⁵. Combined with oxygen deprivation, these stressors reduce fish growth, reproduction, and survival^{6,7}. Climate change also exacerbates existing aquaculture stressors and contributes to the emergence of zoonotic diseases⁸. The World Health Organization has recognized that these environmental changes contribute to the spread of infectious diseases, posing risks not only to aquatic species but also to human health through water-related diseases, which are projected to rise due to climate change^{9,10}.

Escherichia coli poses a significant threat to aquaculture, leading to economic losses and public health risks. Pathogenic *E. coli* strains cause symptoms such as sores, hemorrhages, and slowed growth rates in fish¹¹, with

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infections influenced by environmental changes like rising water temperatures and altered precipitation patterns, which favor bacterial proliferation in aquatic systems^{1,12}. In aquaculture, *E. coli* enters through contaminated water or feed, forms biofilms, invades host tissues, and releases toxins, leading to intestinal damage and systemic infections¹¹. The implications of *E. coli* infections extend to public health, as contamination can cause foodborne illnesses ranging from mild gastrointestinal symptoms to severe conditions like hemolytic uremic syndrome (HUS), particularly affecting vulnerable populations such as young children and the elderly¹³. Pathogenic *E. coli* strains are classified into different pathotypes based on their virulence factors, infection mechanisms, and clinical effects. Key diarrheagenic *E. coli* (DEC) pathotypes include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and Shiga toxin-producing *E. coli* (STEC), each possessing specific genes that facilitate adhesion, toxin production, and disease development¹⁴. Virulence factors such as adhesins (fimbriae, intimin), toxins (Shiga-like toxins, hemolysins), invasins, and siderophores enable *E. coli* to colonize fish, disrupt cellular functions, and cause infections^{15–17}.

Enterotoxigenic *Escherichia coli* (ETEC) produces heat-stable (ST) and heat-labile (LT) enterotoxins, leading to watery diarrhea, traveler's diarrhea, and infantile diarrhea in developing countries. Shiga toxin-producing *E. coli* (STEC), also known as enterohemorrhagic *E. coli* (EHEC), is characterized by the *stx* and *eaeA* genes, which encode Shiga toxins (Stx1 and Stx2) associated with severe outcomes like HUS^{15–17}. Enteropathogenic *E. coli* (EPEC) expresses the *eaeA* gene, crucial for attachment and effacement lesions, and the *bfpA* gene, encoding the bundle-forming pilus (BFP) essential for intestinal adherence^{15–17}. EPEC is further classified into typical (TEPEC) and atypical (aEPEC) based on the presence of the adherence factor plasmid (pEAF)¹⁸. Temperature plays a pivotal role in regulating the transcription of virulence genes in *E. coli*, influencing its pathogenicity under varying environmental conditions¹⁹.

Antimicrobial-resistant (AMR) in aquaculture is a significant public health concern, primarily driven by the excessive use of antibiotics in fish farming, which fosters the emergence of multidrug-resistant (MDR) strains²⁰. Intensive aquaculture systems act as reservoirs for these bacteria, threatening fish health and enabling their transmission to humans through the food chain, leading to hard-to-treat illnesses^{20–22}. Rising temperatures due to climate change significantly contribute to the proliferation of antibiotic resistance in ecosystems by increasing selective pressure on bacteria and facilitating horizontal gene transfer of resistance genes^{10,23}. Extended-spectrum β -lactamases (ESBLs) and carbapenemases represent significant challenges in managing antibiotic resistance in *E. coli*²⁴. ESBLs, such as *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, and *bla*_{OXA-1}, hydrolyze a broad range of β -lactam antibiotics²⁵, while carbapenemases, including *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48} and *bla*_{VIM}, confer resistance to carbapenems, which are often used as a last-line treatment for MDR Gram-negative infections²⁶.

Recent studies have often neglected the influence of environmental factors on aquaculture habitats and the organisms residing within them. Specifically, there is a lack of comprehensive knowledge regarding how environmental variables such as temperature and pH affect the distribution of antibiotic-resistant genes in *E. coli* isolates from Egyptian fish farms. This study addresses this gap by investigating the combined impact of environmental stressors such as temperature and pH on the prevalence of *E. coli* pathotypes, their virulence factors, and antibiotic-resistance genes in fish farms in Egypt. Additionally, the study evaluates the potential zoonotic risks posed by consuming fish contaminated with resistant pathogenic *E. coli*.

Materials and methods

Sample collection and processing

A total of 328 Nile Tilapia (*Oreochromis niloticus*) were randomly collected from two fish farms with similar environmental conditions across five sampling periods: winter, spring, early summer, summer, and autumn. Environmental parameters, including water temperature and pH levels, were routinely monitored onsite in the fishponds using specialized portable devices. Water temperature was measured using aquarium thermometers with LCD panels for precise readings, while pH levels were determined using a Fisher Scientific pH meter (Waltham, Massachusetts, USA), following the manufacturer's guidelines. Measurements were taken from multiple locations within each pond to ensure representative and reliable data, which were subsequently analyzed for seasonal trends.

The samples were grouped into five categories based on seasonal environmental conditions: Group 1 (winter: 22 °C, pH 7.5), Group 2 (spring: 28 °C, pH 8), Group 3 (early summer: 35 °C, pH 6.5), Group 4 (summer: 39 °C, pH 5.5), and Group 5 (autumn: 37 °C, pH 6). These samples were further organized into 82 pools, each consisting of four fish. The seasonal distribution of the pools included 15 pools in winter, 18 in spring, 18 in early summer, 16 in summer, and 15 in autumn. The samples were transported under sterile and refrigerated conditions to prevent contamination and maintain their suitability for laboratory analysis.

For each pool, the skin surface, intestinal tract, and muscle tissues of the fish were examined. Skin samples were collected by swabbing various areas of the fish's surface with sterile cotton swabs. To ensure sterility, a red-hot scalpel was used to disinfect the exterior of the fish before excising portions of the gut and muscle tissue for analysis.

In this study, tilapia fish were euthanized using rapid cooling in water below 4 °C to minimize distress. Following euthanasia, the fish were disposed of by incineration.

Isolation and identification of *E. coli*

The collected pooled samples were placed into a sterile tube containing fresh tryptic soy broth (HiMedia, India) and incubated overnight at 37 °C, after enrichment a loopful of the broth was streaked onto MacConkey agar (Oxoid, Hampshire, UK), and Eosin methylene blue agar (EMB) (Oxoid, Hampshire, UK) and incubated aerobically at 37 °C for 24–48 h. Colonies exhibiting characteristic pink coloration on MacConkey agar and metallic green shiny on EMB were selected and sub-cultured to obtain pure isolates. *E. coli* identification was based on colonial morphology and Gram staining, followed by further characterization using conventional

biochemical tests, including the IMViC test (Indole, Methyl Red, Voges-Proskauer, and Citrate), according to methods described by Tille²⁷.

Extraction of the genomic DNA

Genomic DNA was extracted from the *E. coli* isolates using a conventional boiling process according to Lesiani et al.²⁸. The DNA concentration and purity of each sample were determined using a NanoDrop Spectrophotometer. The extracted DNA was preserved at -20 °C until ready for use.

Molecular identification and characterization of virulence genes in *E. coli* isolates

All *E. coli* isolates were analyzed using multiplex PCR to identify six virulence genes (*eaeA*, *bfpA*, *stx1*, *stx2*, *st*, *lt*) according to Prakasan et al.²⁹ and Samir et al.³⁰. Bacterial DNA was amplified in a 25 µL PCR reaction with 12.5 µL of Emerald Amp MAX PCR master mix (Takara, Japan), 3 µL of template DNA from each isolate, 0.5 µL of each primer (10 pmol/µL; Metabion, Germany), and PCR-grade water to reach the final volume. The sequences of the primers, amplicon sizes, and PCR conditions are demonstrated in Table 1.

Diarrhoeagenic *E. coli* strains were categorized into different pathotypes based on the presence of these virulence genes. The ETEC pathotype expresses the genes *st* and *lt*. The EPEC pathotype expresses *eaeA* and *bfpA* genes, whereas STEC expresses *stx1*, *stx2* genes. Strains that tested positive for genes expressed by various pathotypes were categorized as hybrids³¹.

Molecular detection of β -lactamase and carbapenem resistance-encoding genes

Multiplex-PCR was performed to detect β -lactamase-encoding genes *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, and *bla*_{OXA-1} employing specific primers mentioned in Table 1. The reaction was carried out following Monstein et al.³² and Djefal et al.³³.

Additionally, both multiplex and uniplex PCR assays were employed to identify carbapenemase-encoding genes in *E. coli* isolates. Uniplex PCR was used to screen for the presence of the *bla*_{OXA-48} and *bla*_{VIM} genes, following the protocols outlined by Dallenne et al.³⁴ and Li et al.³⁵. Meanwhile, multiplex PCR was performed to identify the *bla*_{KPC} and *bla*_{NDM} genes according to Mohammed et al.³⁶. The PCR mixtures were run in a total volume of 25 µL, with 5 µL of template DNA from each isolate, 12.5 µL of Emerald Amp MAX PCR master mix (Takara, Japan), 0.5 µL of each primer (10 pmol/µL; Metabion, Germany), and PCR-grade water to get the volume up to 25 µL. The specific oligonucleotide of each primer and PCR amplification conditions are detailed in Table 1.

Target gene (bp)	Primer sequence (5'–3')	Cycling condition	References
Virulence genes of <i>E. coli</i>			
<i>eaeA</i> (891 bp)	F: GTGGCGAATACTGGCGAGACT R: CCCCATTCTTTTCACCGTCG	94 °C for 1 min;30 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min), 72 °C for 10 min	29
<i>bfpA</i> (326 bp)	F: AATGGTGCTTGCGCTTGCTGC R: GCCGCTTTATCCAACCTGGTA		
<i>stx1</i> (370 bp)	F:AAATCGCCATTCTGTTGACTACTTCT R:CAGTCGTCACCTACTGGTTTCATCA		
<i>stx2</i> (283 bp)	F:TGCCATTCTGGCAACTCGCGATGCA R: GGATCTTCTCCCCACTCTGACACC		
<i>st</i> (190 bp)	F: ATTTTCTTTCTGTATTGTCTT R: CACCCGGTACAAGCAGGATT		30
<i>lt</i> (450 bp)	F: GGC GAC AGA TTA TAC CGT GC R: CGG TCT CTA TAT TCC CTG TT		
β lactamase (ESBLs)-encoding genes			
<i>bla</i> _{TEM} (445 bp)	F:CGCCGCATACACTATTCTCAGAATGAR:ACGCTCACC GGCT CCAGATT TAT	95 °C for 5 min; 30 cycles (94 °C for 30 s, 62 °C for 90 s, 72 °C for 60 s), 72 °C for 10 min	32,33
<i>bla</i> _{SHV} (237 bp)	F:CTTTATCGGCCCTCACTCAA R:AGGTGCTCATCATGGGAAAG		
<i>bla</i> _{CTX-M} (593 bp)	F:ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSACCAGAAYC AGC GG		
<i>bla</i> _{OXA-1} (813 bp)	F:ACA CAA TAC ATA TCA ACT TCG C R:AGT GTG TTT AGA ATG GTG ATC		
Carbapenemase-encoding genes			
<i>bla</i> _{KPC} (882 bp)	F:ATG TCA CTG TAT CGC CGT CT R: TTT TCA GAG CCT TAC TGC CC	95 °C for 5 min; 30 cycles (94 °C for 1 min, 55 °C for 1 min , 72 °C for 2 min), 72 °C for 10 min	36
<i>bla</i> _{NDM} (621 bp)	F:GGT TTG GCG ATC TGG TTT TC R:CGG AAT GGC TCA TCA CGA TC		
<i>bla</i> _{VIM} (261 bp)	F:AGT GGT GAG TAT CCG ACAG R:ATG AAA GTG CGT GGA GAC	95 °C for 5 min; 35 cycles (94 °C for 30 Sec, 55 °C for 30 Sec, 72 °C for 1 min), 72 °C for 10 min	35
<i>bla</i> _{OXA-48} (283 bp)	F :GCTTGATCGCCCTCGATT R:GATTGCTCCGTGGCCGAAA	94 °C for 10 min; 30 cycles (94 °C for 40 Sec, 60 °C for 40 Sec, 72 °C for 1 min), 72 °C for 7 min	34

Table 1. The sequence of oligonucleotide primers and their PCR conditions used in the current study.

Different environmental groups	Sampling period	No. of Examined fish	Positive for <i>E. coli</i>	
			No	%
Group 1	Winter	15	11	73.33
Group 2	Spring	18	17	94.44
Group 3	Early Summer	18	17	94.44
Group 4	Summer	16	16	100
Group 5	Autumn	15	15	100
Total	-	82	76	92.68

Table 2. Seasonal distribution and occurrence of *E. coli* in fish across different environmental groups.

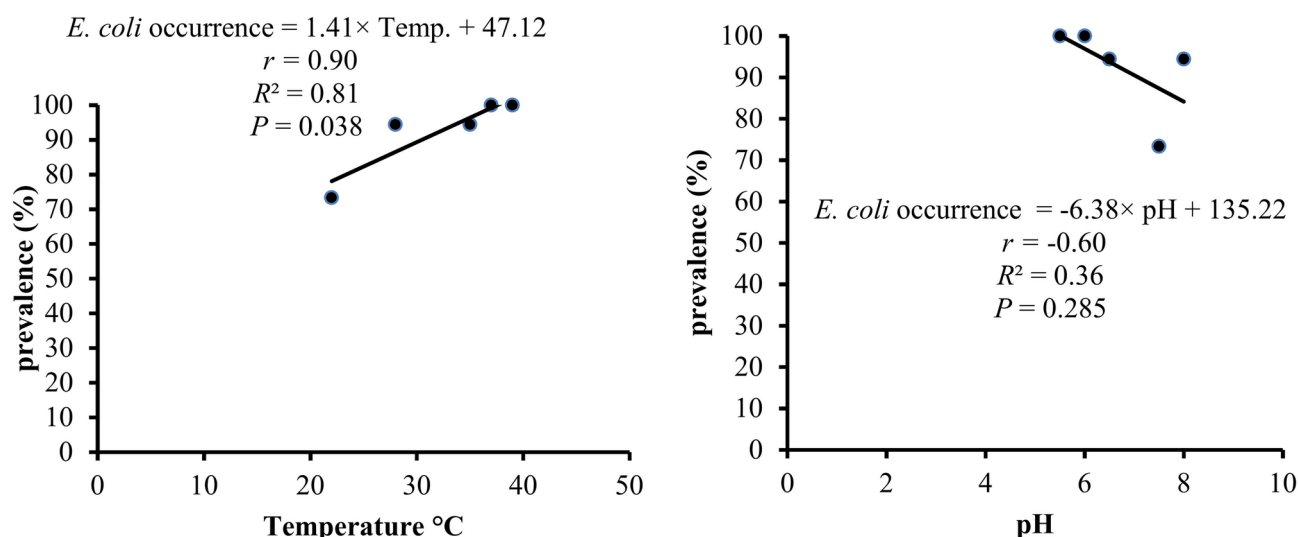


Fig. 1. Relation between occurrence of *E. coli* and fishpond temperature and water pH.

Statistical analysis

Pearson's correlation coefficient (r) and regression analysis (R^2) were used to assess the relationship between the variables under study. All statistical tests were performed using PASW Statistics, Version 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as a P value less than 0.05.

Results

Occurrence of *E. coli* in different environmental fish groups

A total of 76 confirmed *E. coli* isolates were obtained from 82 pooled fresh fish samples collected from two fish farms in Egypt, resulting in an overall prevalence of 92.68%. The occurrence of *E. coli* varied somewhat throughout the seasons. The seasonal variation in the frequency of *E. coli* is summarized in Table 2.

The relationship between the occurrence of *E. coli* and fishpond temperature and water pH is illustrated in Fig. 1. A strong positive correlation was observed between the occurrence of *E. coli* and fishpond temperature ($r = 0.90$, $P = 0.038$). For every 1 °C increase in water temperature, the occurrence of *E. coli* increased by 1.41 ($P = 0.038$, $R^2 = 0.81$). Conversely, a moderate negative association was noted between the occurrence of *E. coli* and water pH ($r = -0.60$, $P = 0.285$), with a decrease in pH associated with a 6.38 increase in the occurrence of *E. coli* ($P = 0.285$, $R^2 = 0.36$).

Occurrence of diarrhoeagenic *E. coli* pathotypes and their associated virulence genes

A total of 69 DEC isolates were obtained from 82 pooled fresh fish samples collected from two fish farms in Egypt, resulting in an overall prevalence of 84.1%. The occurrence of DEC pathotypes and their associated virulence genes is summarized in Table 3.

Enterotoxigenic *E. coli* (ETEC) was widely distributed across all seasons, with particularly high prevalence in warmer conditions. PCR analysis of virulence genes revealed that the most frequently detected gene was *st*, with no strains carrying only the *lt* gene; instead, it was found in combination with *st* across all groups.

Additionally, other pathotypes such as STEC, aEPEC, and tEPEC were identified. STEC showed low prevalence in winter but increased during the summer and autumn, with the *stx2* gene being more prevalent than *stx1*. Similarly, aEPEC was predominantly found in summer while tEPEC was rare, with only one isolate containing the tEPEC gene identified in summer.

Groups (sampling period)	Diarrhoeagenic <i>E. coli</i> (+)	ETEC		STEC		tEPEC		aEPEC		ETEC + STEC	ETEC + aEPEC	ETEC + STEC + aEPEC
		<i>st</i>	<i>st + lt</i>	<i>stx 1</i>	<i>stx 2</i>	<i>eaeA + bfpA</i>		<i>eaeA</i>	<i>bfpA</i>			
Group 1 (Winter)	9	8/11		1/11		0/11		2/11		0	2	0
		6	2	0	1	0		0	2			
Group 2 (Spring)	12	11/17		3/17		0/17		1/17		3	0	0
		8	3	0	3	0		1	0			
Group 3 (Early Summer)	17	17/17		7/17		0/17		3/17		7	3	0
		7	10	2	5	0		2	1			
Group 4 (Summer)	16	15/16		8/16		1/16		5/16		8	5	0
		14	1	1	7	1		2	3			
Group 5 (Autumn)	15	15/15		11/15		0/15		3/15		9	1	2
		13	2	3	8	0		1	2			

Table 3. Distribution of diarrhoeagenic *E. coli* Pathotypes and associated virulence genes across different environmental groups.

Groups (sampling period)	Isolates carry β -lactamase-encoding genes (ESBLs)				Isolates carry carbapenemase-encoding genes			
	<i>bla</i> _{TEM}	<i>bla</i> _{SHV}	<i>bla</i> _{CTX-M}	<i>bla</i> _{OXA-1}	<i>bla</i> _{KPC}	<i>bla</i> _{NDM}	<i>bla</i> _{VIM}	<i>bla</i> _{OXA-48}
Group 1 (Winter)	8/11				3/11			
	5 (45.45%)	2 (18.18%)	2 (18.18%)	0 (0%)	1 (9.09%)	0 (0%)	1 (9.09%)	2 (18.18%)
Group 2 (Spring)	12/17				6/17			
	8 (47.06%)	3 (17.65%)	3 (17.65%)	0 (0%)	2 (11.76%)	3 (17.65%)	3 (17.65%)	2 (11.76%)
Group 3 (Early Summer)	12/17				13/17			
	9 (52.94%)	7 (41.18%)	3 (17.65%)	0 (0%)	2 (11.76%)	7 (41.18%)	7 (41.18%)	9 (52.94%)
Group 4 (Summer)	16/16				7/16			
	16 (100%)	10 (62.5%)	3 (18.75%)	0 (0%)	1 (6.25%)	3 (18.75%)	2 (12.5%)	5 (31.25%)
Group 5 (Autumn)	15/15				12/15			
	13 (86.67%)	10 (66.67%)	5 (33.33%)	0 (0%)	3 (20%)	6 (40%)	6 (40%)	5 (33.33%)

Table 4. The occurrence of β lactamase (ESBLs) and carbapenemase—encoding genes of all *E. coli* isolates from fish farms in Egypt across different environmental groups.

Hybrid pathotypes were also observed, with some *E. coli* isolates exhibiting characteristics of two distinct pathotypes. Notably, Group 5, representing autumn with environmental conditions of 37 °C and pH 6, exhibited a combination of three *E. coli* pathotypes: ETEC, STEC, and aEPEC.

Distribution of β -lactamase and carbapenemase-encoding genes

The distribution of carbapenemase-encoding and β -lactamase-encoding genes (ESBLs) in *E. coli* isolates is summarized in Table 4, Figs. 2, and 3. The results indicate that the summer season (39 °C, pH 5.5) and autumn season (37 °C, pH 6) exhibited the highest prevalence of *E. coli* isolates carrying β -lactamase genes. Additionally, early summer (35 °C, pH 6.5), summer (39 °C, pH 5.5), and autumn (37 °C, pH 6) showed the highest proportions of *E. coli* isolates harboring carbapenemase genes.

Among the β -lactamase (ESBL)-encoding genes, *bla*_{TEM} was significantly more prevalent during the summer season, while *bla*_{SHV} and *bla*_{CTX-M} showed variable distributions, with their highest abundance recorded in Group 5 (autumn). Notably, *bla*_{OXA-1} was absent across all seasons. Groups 4 (summer) and 5 (autumn), characterized by elevated water temperatures, also had the largest proportion of isolates carrying multiple β -lactamase-encoding genes, as illustrated in Fig. 2.

For carbapenemase-encoding genes, *bla*_{OXA-48}, *bla*_{VIM}, and *bla*_{NDM} were most frequently detected during early summer (Group 3), while *bla*_{KPC} was less common but observed across all seasons, with the highest prevalence in autumn (Group 5). Group 3 (early summer) exhibited the highest occurrence of isolates carrying multiple carbapenemase-encoding genes, as depicted in Fig. 3.

The distribution of isolates co-harboring both β -lactamase- and carbapenemase-encoding genes across the different environmental fish groups is shown in Table 5. As water temperatures increased, Groups 3 (early summer), 4 (summer), and 5 (autumn) displayed a variety of gene combinations.

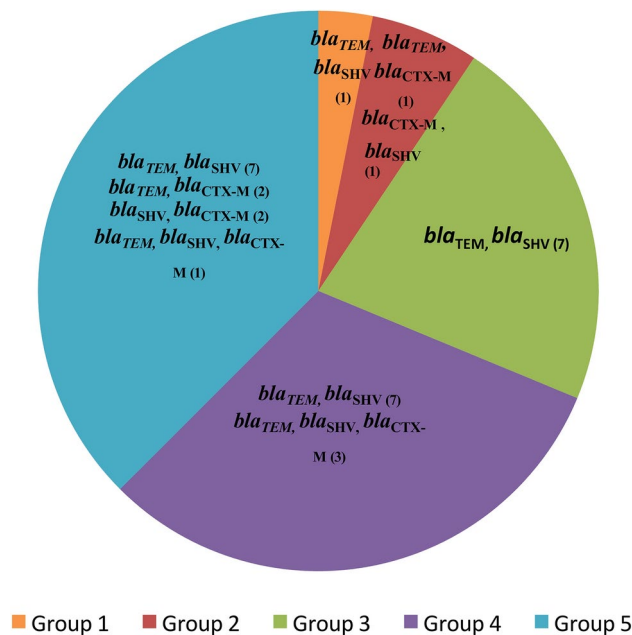


Fig. 2. The occurrence of mixed β lactamase (ESBLs)—encoding genes among *E. coli* isolates from fish farms in Egypt across different environmental groups.

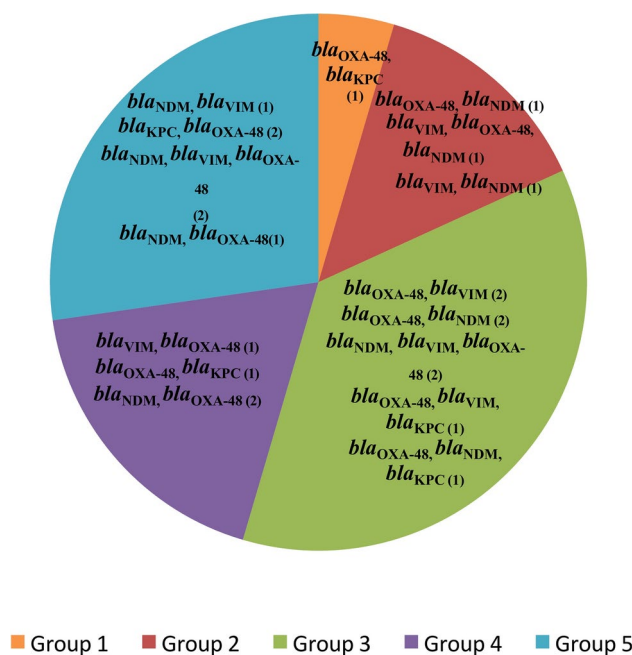


Fig. 3. The occurrence of mixed Carbapenemase-encoding genes among *E. coli* isolates from fish farms in Egypt across different environmental groups.

The occurrence of pathotypes and antibiotic resistance genes among *E. coli* isolates

EPEC was the most frequently identified *E. coli* pathotype across all seasons, demonstrating extensive resistance to various β -lactamase-encoding genes and peaking during the summer season (Table 6). STEC displayed similar resistance patterns and was often detected in conjunction with EPEC. Additionally, aEPEC showed resistance to multiple β -lactamase genes and maintained a consistent association with EPEC.

Interestingly, one isolate from Group 4 (summer), characterized by the highest water temperature, was identified as tEPEC and linked to a β -lactamase-encoding gene. In Group 5 (autumn), under conditions optimal for *E. coli* growth (37 °C and neutral pH), some isolates exhibited a unique combination of three pathotypes, accompanied by one or more carbapenemase- or β -lactamase-producing genes.

Different environmental fish groups	No. of isolates	Mixed β lactamase and carbapenemase- encoding genes
Group 1 (Winter)	1	<i>bla</i> _{CTX-M} , <i>bla</i> _{VIM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{OXA-48}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{KPC}
Group 2 (Spring)	1	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{KPC}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{VIM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{CTX-M} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{NDM}
Group 3 (Early Summer)	1	<i>bla</i> _{OXA-48} , <i>bla</i> _{VIM}
	1	<i>bla</i> _{CTX-M} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{CTX-M} , <i>bla</i> _{VIM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
Group 4 (Summer)	2	<i>bla</i> _{TEM} , <i>bla</i> _{SHV}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{KPC}
	2	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM} , <i>bla</i> _{OXA-48}
Group 5 (Autumn)	1	<i>bla</i> _{TEM} , <i>bla</i> _{VIM}
	4	<i>bla</i> _{TEM} , <i>bla</i> _{SHV}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{NDM} , <i>bla</i> _{CTX-M}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{VIM} , <i>bla</i> _{CTX-M}
	2	<i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{CTX-M}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM}
	2	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{KPC}
	1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM}

Table 5. The occurrence of mixed β lactamase and carbapenemase- encoding genes among *E.coli* isolates from fish farms in Egypt across different environmental groups.

Discussion

This study highlights the profound impact of climate change on the prevalence, virulence, and antibiotic resistance of *E. coli* in aquatic ecosystems, particularly in aquaculture settings. The observed high prevalence of *E. coli* (92.68%) in farmed fish samples underscores the growing health risks associated with aquaculture, consistent with findings by Akter et al.³⁷ in Bangladesh. Rising global temperatures and altered rainfall patterns disrupt ecological stability, creating conditions that favor the survival, proliferation, and virulence of *E. coli*³⁸. The positive correlation between water temperature and *E. coli* occurrence emphasizes the critical role of temperature in shaping bacterial dynamics in fishpond environments. These findings align with Iqbal et al.³⁹, who reported increased *E. coli* concentrations in warmer environments, highlighting the global significance of temperature-driven bacterial proliferation. Elevated temperatures have been linked to weakened immune responses in fish, creating favorable conditions for bacterial adaptation and spread, as previously documented by Lawlor⁴⁰ and others⁴¹. Furthermore, warmer conditions not only accelerate bacterial growth but also enhance the pathogenic potential of *E. coli*, which thrives near its optimal growth temperature of 37 °C^{42,43}.

The study also reveals a negative association between water pH and *E. coli* occurrence, suggesting that pH fluctuations may influence bacterial survival and virulence. These variations could be driven by factors such as biological activity, feeding practices, and daily environmental changes within controlled culture systems. Although this relationship was less pronounced compared to temperature, it aligns with the findings of Moussa et al.⁴⁴, who reported significant impacts of pH changes on bacterial populations. The adaptability of *E. coli* to varying environmental conditions, including pH stress, underscores its resilience and ability to persist in diverse aquatic habitats⁴⁵.

Different environmental fish groups	No. of isolates	<i>E. coli</i> pathotypes and antibiotic resistance genes
Group 1 (Winter)	3	ETEC, <i>bla</i> _{TEM}
	1	STEC, <i>bla</i> _{SHV}
	1	ETEC, <i>bla</i> _{CTX-M}
	1	ETEC, <i>bla</i> _{CTX-M} , <i>bla</i> _{VIM}
	1	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{OXA-48}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{KPC}
Group 2 (Spring)	1	ETEC, <i>bla</i> _{TEM}
	1	ETEC, <i>bla</i> _{CTX-M}
	2	ETEC, STEC, <i>bla</i> _{TEM}
	1	ETEC, <i>bla</i> _{VIM}
	1	ETEC, STEC, <i>bla</i> _{KPC}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{KPC}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
	1	ETEC, <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
Group 3 (Early Summer)	1	ETEC, <i>bla</i> _{CTX-M} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{NDM}
	1	ETEC, <i>bla</i> _{CTX-M}
	1	ETEC, STEC, <i>bla</i> _{OXA-48}
	1	ETEC, <i>bla</i> _{OXA-48} , <i>bla</i> _{VIM}
	1	ETEC, STEC, <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM}
	1	ETEC, STEC, <i>bla</i> _{CTX-M} , <i>bla</i> _{VIM}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{VIM} , <i>bla</i> _{KPC}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM} , <i>bla</i> _{KPC}
	1	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{VIM} , <i>bla</i> _{NDM}
Group 4 (Summer)	1	tEPEC, <i>bla</i> _{TEM}
	3	ETEC, STEC, <i>bla</i> _{TEM}
	1	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV}
	2	ETEC, STEC, <i>bla</i> _{TEM}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48}
	1	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM}
	1	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} , <i>bla</i> _{KPC}
	2	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM} , <i>bla</i> _{OXA-48}
Group 5 (Autumn)	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{KPC}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{VIM}
	1	ETEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV}
	2	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{NDM} , <i>bla</i> _{CTX-M}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{VIM} , <i>bla</i> _{CTX-M}
	1	ETEC, STEC, <i>bla</i> _{SHV} , <i>bla</i> _{VIM} , <i>bla</i> _{CTX-M}
	1	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM}
	2	ETEC, STEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{KPC} , <i>bla</i> _{OXA-48}
	1	ETEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	ETEC, STEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{NDM} , <i>bla</i> _{VIM} , <i>bla</i> _{OXA-48}
	1	ETEC, STEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM} , <i>bla</i> _{OXA-48}
	1	ETEC, STEC, aEPEC, <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M} , <i>bla</i> _{NDM} , <i>bla</i> _{OXA-48}

Table 6. The pattern of pathotypes and antibiotic resistance genes among *E. coli* isolates.

The pathogenic potential of *E. coli* is largely attributed to its virulence genes, which play critical roles in causing intestinal disorders⁴⁶. This study identifies the dominance of *st*/*lt* gene combination observed during early summer. Despite the heat-labile nature of the LT toxin, these findings suggest a complex interplay between environmental factors and strain pathogenicity⁴⁷. Rising temperatures appear to regulate virulence through mechanisms involving heat-shock proteins and stress-responsive pathways, enhancing gene expression⁴⁸. Similarly, the prevalence of Shiga toxin-producing *E. coli* (STEC) peaked during warmer months, with the *stx2* gene being more common than *stx1*, indicating higher virulence potential⁴⁹. The temperature-regulated virulence of enteropathogenic *E. coli* (EPEC), particularly atypical EPEC (aEPEC), further emphasizes the role of environmental factors in modulating pathogenicity. This study reveals that aEPEC strains are more prevalent than typical EPEC (tEPEC), with their occurrence peaking during summer at 39 °C. The activation of virulence genes in aEPEC, attributed to the locus of enterocyte effacement (LEE), involves temperature-sensitive regulatory cascades, such as those mediated by the Ler protein⁵⁰.

The emergence of hybrid *E. coli* pathotypes, which harbor virulence markers from multiple pathotypes, presents significant challenges for disease management due to their enhanced pathogenic potential and resistance to treatment. The genetic flexibility of *E. coli* facilitates horizontal transfer of virulence factors, leading to hybrid strains that defy traditional classification^{51,52}. Climate change exacerbates this phenomenon by fostering bacterial proliferation, transmission, and horizontal gene transfer under elevated temperatures, as documented by Zhao et al.⁵³.

Aquatic environments are recognized as critical reservoirs for antibiotic-resistant bacteria (ARB), with improper antibiotic use, contamination, and aquaculture practices driving AMR development^{21,54}. Rising temperatures amplify these issues by promoting bacterial adaptation and stress responses⁵⁵. Bacteria develop antibacterial resistance through various mechanisms, including enzyme production (e.g., β -lactamases and carbapenemases) that degrade antibiotics, efflux pumps that expel drugs, and target site modifications that prevent antibiotic binding. Changes in membrane permeability reduce drug entry, while biofilm formation protects bacteria from antimicrobials and immune responses. Additionally, resistance genes spread via horizontal gene transfer through plasmids, transposons, or bacteriophages, making treatment more challenging⁵⁶.

This study highlights the detection of extended-spectrum β -lactamase (ESBL)-encoding genes in *E. coli* isolates from aquaculture, particularly in warmer waters. The predominance of the *bla*_{TEM} gene over other ESBL genes, such as *bla*_{SHV} and *bla*_{CTX-M}, aligns with previous reports⁵⁷. Interestingly, *bla*_{SHV} exhibited a temperature-dependent prevalence, while *bla*_{CTX-M} showed variable distribution across different environmental conditions, with the highest rate observed in autumn. Additionally, isolates from summer and autumn frequently harbored multiple ESBL genes, consistent with findings by Silago et al.⁵⁸, who documented the co-occurrence of *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV} in bacterial isolates.

Carbapenemase-encoding genes, including *bla*_{OXA-48}, *bla*_{NDM}, and *bla*_{VIM}, were prominent during early summer, summer, and autumn. Among them, *bla*_{OXA-48} was the most frequently detected, aligning with previous reports from Iraq⁵⁹. The prevalence of *bla*_{NDM} and *bla*_{VIM} significantly increased in early summer at 35 °C and in autumn at 37 °C, suggesting that elevated temperatures create favorable conditions for bacterial survival and proliferation⁶⁰. Notably, the highest frequency of *bla*_{KPC} was recorded at 37 °C in autumn, further emphasizing the role of increased temperatures in promoting the clonal spread of carbapenemase genes.

The coexistence of β -lactamase and carbapenemase genes in *E. coli* isolates represents a significant challenge for treatment, as this combination confers resistance to a wide range of antibiotics, potentially leading to multidrug-resistant infections⁶¹. The diversity of resistance gene combinations observed, particularly during warmer months, underscores the influence of environmental factors such as temperature and pH on the development of antibiotic resistance in aquaculture.

These findings underline the urgency of addressing climate change to mitigate the rapid dissemination of antibiotic-resistant genes in aquatic ecosystems. Environmental factors contribute to the spread of antimicrobial-resistant *E. coli*, increasing health risks associated with consuming contaminated fish⁶².

The findings also reveal a strong association between *E. coli* pathotypes, such as ETEC, aEPEC, and STEC, and resistance genes. These pathotypes frequently co-occurred, particularly during warmer months. Such combinations not only complicate the treatment of infections but also pose a risk of zoonotic transmission through the food chain or direct contact with contaminated water⁶³.

Escherichia coli serves as a key indicator of water quality, highlighting potential sewage contamination and fecal pollution⁶⁴. The presence of pathogenic and antibiotic-resistant strains in water bodies poses significant risks to both aquatic life and public health. Regular monitoring and strict water quality management are essential to mitigate these risks and prevent the spread of resistant infections^{65,66}. Additionally, further studies on water samples are recommended to better understand their impact on fish health and public safety.

Conclusion

This study provides critical evidence that environmental factors, particularly rising temperatures and pH fluctuations, significantly influence the prevalence, virulence, and antibiotic resistance of *E. coli* in aquaculture. The identification of diverse diarrheagenic *E. coli* pathotypes, hybrid strains, and their associated virulence genes underscores the growing public health threat posed by these bacteria, especially in warmer conditions. Additionally, the widespread presence of β -lactamase- and carbapenemase-encoding genes highlights the alarming escalation of antibiotic resistance, with the coexistence of these resistance determinants complicating treatment and fostering multidrug-resistant strains.

These findings emphasize the urgent need for continuous surveillance of aquaculture environments, stricter regulations on antibiotic use, and proactive measures to mitigate climate change's impact on aquatic ecosystems.

Addressing these challenges is crucial for safeguarding food safety, public health, and the sustainability of aquaculture.

Data availability

All the data generated or analyzed in this study are included in this published article.

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References

- Duchenne-Moutien, R. A. & Neetoo, H. Climate change and emerging food safety issues: A review. *J. Food Prot.* **84**(11), 1884–1897 (2021).
- Wang, P. & Mendes, I. Climate resilience in aquaculture: A cluster analysis of Chinese coastal regional production and farming practices in the face of climatic shifts 2017–2021. *J. Infrastruct. Policy Dev.* **8**(9), 6052 (2024).
- Matolia, J., Khan, M. A., Verma, S., & Prasad, A. The interplay of climate change and water chemistry: Impacts on freshwater aquaculture (2024).
- Combe, F. J. et al. Density and climate effects on age-specific survival and population growth: Consequences for hibernating mammals. *Anim. Conserv.* **26**(3), 317–330 (2023).
- Anyanwu, C. N., Amadi-Eke, A. S. & Nwaka, D. E. Climate change, effects and mitigation strategies on aquaculture: A review. *Agric. Forestry Fisheries* **9**(1), 70–72 (2015).
- Islam, M. & Yasmin, R. Impact of aquaculture and contemporary environmental issues in Bangladesh. *Int. J. Fish. Aquat. Stud.* **5**, 100–107 (2017).
- Okon, E. M., Oyesiji, A. A., Okeleye, D. E., Kanonuhwa, M., Khalifa, N. E., Eissa, E. S. H., & Abdelnour, S. A. The escalating threat of climate change-driven diseases in fish: Evidence from a global perspective: A literature review. *Environ. Res.* 120184 (2024).
- Mishra, V., Aadhar, S. & Mahto, S. S. Anthropogenic warming and intraseasonal summer monsoon variability amplify the risk of future flash droughts in India. *Npj Climate Atmos. Sci.* **4**(1), 1 (2021).
- Romanello, M. et al. The 2022 report of the Lancet Countdown on health and climate change: health at the mercy of fossil fuels. *Lancet* **400**(10363), 1619–1654 (2022).
- Fernández Salgueiro, M., Cernuda Martínez, J. A., Gan, R. K. & Arcos González, P. Climate change and antibiotic resistance: A scoping review. *Environ. Microbiol. Rep.* **16**(5), e70008 (2024).
- Wanja, D. W. Occurrence, antimicrobial susceptibility and risk factors for the transmission of fish bacteria in small-scale farms in Kirinyaga County, Kenya (2018).
- Hassan, M. A., Abd Allah, N. A. & Mabrok, M. Inevitable impact of some environmental stressors on the frequency and pathogenicity of marine vibriosis. *Aquaculture* **536**, 736447 (2021).
- Abebe, E., Gugsu, G. & Ahmed, M. Review on major food-borne zoonotic bacterial pathogens. *J. Trop. Med.* **2020**(1), 4674235 (2020).
- Ohmura-Hoshino, M., Miyaki, Y., & Yashima, S. A one-step multiplex PCR-based assay for simultaneous detection and classification of virulence factors to identify five diarrheagenic *E. coli* pathotypes. *Heliyon*, **8**(8) (2022).
- Makarova, M. A. A modern view of diarrheagenic *Escherichia coli*: A causative agent of acute intestinal infections. *J. Microbiol. Epidemiol. Immunobiol.* **100**(4), 333–344 (2023).
- Kaur, P., & Dudeja, P. K. Pathophysiology of enteropathogenic *Escherichia coli*-induced Diarrhea. Newborn (Clarksville, Md.), **2**(1), 102 (2023).
- Alhadlaq, M. A. et al. Overview of pathogenic *Escherichia coli*, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982–2024) and food safety criteria. *Gut Pathogens* **16**(1), 57 (2024).
- Carlino, M. J. et al. Quantitative analysis and virulence phenotypes of atypical enteropathogenic *Escherichia coli* (EPEC) acquired from diarrheal stool samples from a Midwest US hospital. *Gut Microbes* **12**(1), 1824562 (2020).
- Sy, B. M. & Tree, J. J. Small RNA regulation of virulence in pathogenic *Escherichia coli*. *Front. Cell. Infect. Microbiol.* **10**, 622202 (2021).
- Raharjo, H. M. et al. The first evidence of blaCTX-M-55, QnrVC5, and novel insight into the genome of MDR *Vibrio vulnificus* isolated from Asian sea bass (*Lates calcarifer*) identified by resistome analysis. *Aquaculture* **571**, 739500 (2023).
- Milijasevic, M., Veskovic-Moracanic, S., Milijasevic, J. B., Petrovic, J. & Nastasijevic, I. Antimicrobial resistance in aquaculture: Risk mitigation within the One Health context. *Foods* **13**(15), 2448 (2024).
- Thaotumpitak, V. et al. Insights into antimicrobial and multidrug resistance of *Escherichia coli* in hybrid red tilapia cultivation. *Aquaculture* **588**, 740927 (2024).
- Nasrollahian, S., Graham, J. P., & Halaji, M. A review of the mechanisms that confer antibiotic resistance in pathotypes of *E. coli*. *Front. Cell. Infect. Microbiol.* **14**, 1387497 (2024).
- Widodo, A., Effendi, M. H. & Khairullah, A. R. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. *Syst Rev Pharm* **11**(7), 382–392 (2020).
- Sivaraman, G. K. et al. Antibiotic resistance profiles and molecular characteristics of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from shrimp aquaculture farms in Kerala, India. *Front. Microbiol.* **12**, 622891 (2021).
- Nakayama, T. et al. Isolation of carbapenem-resistant *Enterobacteriaceae* harbouring NDM-1, 4, 5, OXA48 and KPC from river fish in Vietnam. *Food Control* **133**, 108594 (2022).
- Tille, P. M. Bailey & Scott's. *Diagnostic microbiology*, 14th edn (Elsevier, 2017)
- Lesiani, B. R., Abror, Y. K., Merdekawati, F. & Djuminar, A. Analysis of purity and concentration *Escherichia coli* DNA by boiling method isolation with addition of proteinase-K and RNase, Indonesian. *J. Med. Lab. Sci. Technol.* **5**(2), 160–171 (2023).
- Prakasan, S. et al. Occurrence, pathogroup distribution and virulence genotypes of *Escherichia coli* from fresh seafood. *Food Control* **133**, 108669 (2022).
- Samir, A., Abdel-Moein, K. A. & Zaher, H. M. Predominance of enterotoxigenic *Escherichia coli* among ESBL/plasmid-mediated AmpC-producing strains isolated from diarrheic foals: A public health concern. *Acta Vet. Scand.* **66**(1), 54 (2024).
- Wolde, D., Eguale, T., Alemayehu, H., Medhin, G., Haile, A. F., Pirs, M., & Starčić Erjavec, M. Antimicrobial susceptibility and characterization of extended-spectrum β -lactamase-producing *Escherichia coli* isolated from stools of primary healthcare patients in Ethiopia. *Antibiotics*, **13**(1), 93 (2024).
- Monstein, H. J. et al. Multiplex PCR amplification assay for the detection of blaSHV, bla_{TEM} and bla_{CTX-M} genes in *Enterobacteriaceae*. *APMIS* **115**(12), 1400–1408 (2007).
- Djeffal, S. et al. Prevalence and clonal relationship of ESBL-producing *Salmonella* strains from humans and poultry in northeastern Algeria. *BMC Vet. Res.* **13**, 1–9 (2017).
- Dallenne, C., Da Costa, A., Decré, D., Favier, C. & Arlet, G. Development of a set of multiplex PCR assays for the detection of genes encoding important β -lactamases in *Enterobacteriaceae*. *J. Antimicrob. Chemother.* **65**(3), 490–495 (2010).

35. Li, J., Hu, Z. & Hu, Q. Isolation of the first IMP-4 metallo- β -lactamase producing *Klebsiella pneumoniae* in Tianjin, China. *Braz. J. Microbiol.* **43**, 917–922 (2012).
36. Mohammed, R., Nader, S. M., Hamza, D. A. & Sabry, M. A. Public health concern of antimicrobial resistance and virulence determinants in *E. coli* isolates from oysters in Egypt. *Sci. Rep.* **14**(1), 26977 (2024).
37. Van de Vuurst, P. & Escobar, L. E. Climate change and infectious disease: A review of evidence and research trends. *Infect. Dis. Poverty* **12**(1), 51 (2023).
38. Akter, M., Abedin, M., Mosharaf, M. P., Islam, M. A. & Hassan, Z. Prevalence and distribution of antimicrobial resistance profile of *Escherichia coli* isolated from various local fish markets in Dhaka city, Bangladesh. *J. Bangladesh Acad. Sci.* **46**(1), 9–18 (2022).
39. Iqbal, M. S., Ahmad, M. N. & Hofstra, N. The relationship between hydro-climatic variables and *E. coli* concentrations in surface and drinking water of the Kabul river basin in Pakistan. *AIMS Environ. Sci.* **4**(5), 690–708 (2017).
40. Lawlor, E. Interaction of disease and temperature on the aerobic scope of freshwater fish and implications for changing climates (Doctoral dissertation, Murdoch University) (2016).
41. Hassan, A. & Ali, S. Epidemiology and molecular confirmation of *E. coli* isolated from diseased fish in Muzaffargarh, Punjab, Pakistan. *BioSci. Rev.* **6**(1), 1–15 (2024).
42. Shamsudin, S. N., Rahman, M. H. F., Taib, M. N., Razak, W. R. W. A., Ahmad, A. H., & Zain, M. M. Analysis between *Escherichia coli* growth and physical parameters in water using Pearson correlation. In *2016 7th IEEE Control and System Graduate Research Colloquium (ICSGRC)* (pp. 131–136). IEEE (2016).
43. Chatreman, N., Seecharran, D. & Ansari, A. A. Prevalence and distribution of pathogenic bacteria found in fish and fishery products: A review. *J. Fisheries Life Sci.* **5**(2), 53–65 (2020).
44. Boubacar Moussa, M., Abdourhamane Toure, A., Lartiges, B., Kergoat, L., Robert, E., Mamane, A., & Grippa, M. *Escherichia coli* in the Niger River: Links to environmental variables and anthropogenic activities in Niamey, Niger (2024).
45. Roufou, S. et al. Identification of genes used by *Escherichia coli* to mitigate climatic stress conditions. *Gene Rep.* **36**, 101998 (2024).
46. Denamur, E., Clermont, O., Bonacorsi, S. & Gordon, D. The population genetics of pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.* **19**(1), 37–54 (2021).
47. Ma, A. Characterization of heat resistant *Escherichia coli* isolates associated with human infection (2021).
48. Shapiro, R. S. & Cowen, L. E. Thermal control of microbial development and virulence: Molecular mechanisms of microbial temperature sensing. *MBio* **3**(5), 10–1128 (2012).
49. Irshad, H. et al. Genetic diversity and zoonotic potential of shiga toxin-producing *E. coli* (STEC) in cattle and buffaloes from Islamabad, Pakistan. *Agriculture* **14**(9), 1537 (2024).
50. Umanski, T., Rosenshine, I. & Friedberg, D. Thermoregulated expression of virulence genes in enteropathogenic *Escherichia coli*. *Microbiology* **148**(9), 2735–2744 (2002).
51. Méndez-Moreno, E. et al. Characterization of diarrheagenic *Escherichia coli* strains isolated from healthy donors, including a triple hybrid strain. *Antibiotics* **11**(7), 833 (2022).
52. Lee, W. et al. Genome-based characterization of hybrid Shiga toxin-producing and enterotoxigenic *Escherichia coli* (STEC/ETEC) strains isolated in South Korea, 2016–2020. *Microorganisms* **11**(5), 1285 (2023).
53. Zhao, J., Chen, K., Palsson, B. O., & Yang, L. StressME: Unified computing framework of *Escherichia coli* metabolism, gene expression, and stress responses (2024).
54. Lajqi Berisha, N., Poceva Panovska, A. & Hajrulai-Musliu, Z. Antibiotic resistance and aquatic systems: Importance in public health. *Water* **16**(17), 2362 (2024).
55. Gomes, M. P. The convergence of antibiotic contamination, resistance, and climate dynamics in freshwater ecosystems. *Water* **16**(18), 2606 (2024).
56. Belay, W. Y. et al. Mechanism of antibacterial resistance, strategies and next-generation antimicrobials to contain antimicrobial resistance: A review. *Front. Pharmacol.* **15**, 1444781 (2024).
57. Rashid, R. Epidemiology and molecular characterization of multidrug resistant *Escherichia coli* isolated from fish (doctoral dissertation, Chattogram Veterinary & Animal Sciences University, Khulshi, Chattogram) (2023).
58. Silago, V. et al. Existence of multiple ESBL genes among phenotypically confirmed ESBL producing *Klebsiella pneumoniae* and *Escherichia coli* concurrently isolated from clinical, colonization and contamination samples from neonatal units at Bugando Medical Center, Mwanza, Tanzania. *Antibiotics* **10**(5), 476 (2021).
59. Gatyia Al-Mayahie, S. M., Al-Guranie, D. R. D. T., Hussein, A. A. & Bachai, Z. A. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic *Escherichia coli* phylogroup B2 isolates from outpatients in Wasit Province/Iraq. *PLoS ONE* **17**(1), e0262984 (2022).
60. Pepi, M. & Focardi, S. Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the Mediterranean area. *Int. J. Environ. Res. Public Health* **18**(11), 5723 (2021).
61. El-Shaer, S., Abdel-Rhman, S. H., Barwa, R. & Hassan, R. Genetic characterization of extended-spectrum β -lactamase- and carbapenemase-producing *Escherichia coli* isolated from Egyptian hospitals and environments. *PLoS ONE* **16**(7), e0255219 (2021).
62. Mumbo, M. T. et al. Antimicrobial resistance profiles of *Salmonella* spp. and *Escherichia coli* isolated from fresh Nile tilapia (*Oreochromis niloticus*) fish marketed for human consumption. *BMC Microbiol.* **23**(1), 306 (2023).
63. Silva, A. et al. Antimicrobial resistance and clonal lineages of *Escherichia coli* from food-producing animals. *Antibiotics* **12**(6), 1061 (2023).
64. Catherine N, A., Claudia, S., Savino, A., Edgar, M. M., Rogers, K., Julius, L. B., & Andreas, T. Antibiotic resistance of *E. coli* isolates from different water sources in Mbarara, Uganda. *J. Water Health*, **22**(9), 1579–1593 (2024).
65. Okeke, E. S. et al. Antibiotic resistance in aquaculture and aquatic organisms: a review of current nanotechnology applications for sustainable management. *Environ. Sci. Pollut. Res.* **29**(46), 69241–69274 (2022).
66. Dimpoy, J. J., Lucky, O. P., Kwarkye, D. F., Watts, S., Oguayo, C. P., Ojewole, C. O., & Kusi, J. Identifying spatiotemporal patterns and drivers of fecal indicator bacteria in an urban lake for water quality assessment and management. *Heliyon*, **11**(1) (2025).

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Author contributions

AE performed the collection of samples, and the molecular detection of the target genes. AE, DH, ZA, and MS performed the analysis and interpretation of the data and the writing of the manuscript. All authors read and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This study followed the ethical guidelines from the Faculty of Veterinary Medicine at Cairo University, Egypt, and was approved by the Institutional Animal Care and Use Committee (VET-CU-IACUC-131020241011). All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Cairo University, Egypt. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

All authors have read the manuscript and consent to publish.

Additional information

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