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Research Article

Occurrence of Diarrheagenic *Escherichia coli* in aquaculture reared fish from Kerala

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Abstract

Diarrheagenic *Escherichia coli* strains can potentially cause gastrointestinal illnesses in humans upon consuming contaminated seafood. The present study investigated the prevalence of diarrheagenic *Escherichia coli* (DEC) in fish reared through aquaculture practices in districts D1 and D2 of Kerala, India. The samples comprised fish fed with both raw (115) and cooked (123) chicken slaughter waste (CSW) as well as pellet-fed fish (120) and water samples (100) from the fish rearing ponds. The confirmation of *Escherichia coli* (*E. coli*) was done through PCR amplification of the *uidA* gene. The results indicated that a significant proportion of fish reared with raw and cooked CSW carried *E. coli*, with no statistically significant difference between the districts. In contrast, pellet-fed fish exhibited a lower occurrence of *E. coli*, and a significant difference was observed when compared to CSW-fed fish. Further analysis revealed the prevalence of specific *E. coli* pathotypes in the samples. In raw CSW-fed fish, the *astA* gene, indicative of Enteroaggregative *E. coli* (EAEC), was detected in 7 isolates, while no other pathotypes were detected. In cooked CSW-fed fish, EPEC and EAEC pathotypes were identified in both districts, along with a few EHEC and ETEC isolates (in district 2). Pellet-fed fish predominantly harbored EHEC and EAEC pathotypes in district 1. These findings emphasized the potential health risks associated with specific aquaculture feeding practices and highlighted the need for targeted interventions to ensure seafood safety.

Keywords: DEC pathotype, EAEC, *E. coli*, Fish, Pond water

Highlights

- The study revealed a high level of contamination of farm-reared fish with pathogenic *E. coli*.
- Feeding chicken slaughter waste as feed to fish enhances the chances of entry of pathogenic *E. coli* into human food chain.
- The presence of different DEC pathotypes of *E. coli* is of public health importance as it has the potential to cause potential diarrheal diseases in humans.

INTRODUCTION

Fisheries and aquaculture are considered as a sunrise sector in the Indian economy. India is the third largest fish-producing country with an annual turnover of 16.25 million metric tonnes in 2022-23, which accounts for 7.96 per cent of global fish production. The fish production statistics in India revealed that around 74.58 per cent of total fish production is contributed by inland aquaculture and 25.42 per cent by the marine sector (DADF, 2022). The aquaculture industry in Kerala has witnessed substantial growth in recent years, becoming a vital source of seafood production. However, with this rapid expansion comes the need for increased vigilance in ensuring the safety and quality of aquaculture products. One of the key concerns in this regard is the presence of *Escherichia coli* (*E. coli*) in fish reared through aquaculture practices.

In developing countries, the second major cause of illness and death in children below five years of age is reported to be diarrhea of various etiology, of which

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bacterial diarrheal disease is very common. The *E. coli* organisms have evolved by the production of various enterotoxins and adherence factors, which help them to easily invade the host system and produce disease effortlessly (Gomes *et al.*, 2016). Diarrheagenic *E. coli* (DEC) strains can potentially cause gastrointestinal illnesses in humans upon consuming contaminated seafood. Hence, understanding the occurrence and prevalence of these pathogenic DEC strains in aquaculture-reared fish from Kerala is of paramount importance. The present study embarks on a comprehensive exploration of the presence of DEC in fish cultivated in Kerala's aquaculture systems, shedding light on the potential risks to public health and offering insights into strategies for ensuring the safety of seafood products originating from this region. The findings of this research hold significant implications for the aquaculture industry, regulatory bodies, and consumers alike, as they contribute to the ongoing discourse surrounding food safety and sustainable aquaculture practices in Kerala.

MATERIALS AND METHODS

Study location: The study was designed to assess the occurrence of *E. coli* and diarrheagenic *E. coli* (DEC) pathotypes in aquaculture-reared fish samples and fish pond water samples collected from two selected districts in Kerala. The study area was divided into two: a high-altitude zone (D1 district, 11.6854° N, 76.1320° E) and a low-altitude zone (D2 district, 9.9816° N, 76.2999° E).

Sampling: Samples of fish fed with raw chicken slaughter waste (CSW) and/or cooked chicken slaughter waste and fish fed with pellet feed along with fish pond water samples were collected from both districts. Two fish farms following the practice of CSW feeding and two fish farms following the pellet feeding practice were selected from each district. Hence, a total of eight farms were selected for the study purpose, four each from D1 and D2 districts. A total of 165 samples consisting of 55, 60 and 50 samples of fish fed with raw CSW, cooked CSW and pellet feed, respectively were collected from D1 district. In a similar manner, 60, 63 and 70 samples of fish fed with raw CSW, cooked CSW and pellet feed, respectively were collected from D2 district also. The study also assessed the presence of *E. coli* and DEC in 25 water samples, each from CSW-feeding fish ponds and pellet-feeding fish ponds from both districts. The samples collected in the Cary-Blair transport medium and transported under insulated chilled conditions were processed in the laboratory for isolation and molecular confirmation of *E. coli.*

Isolation of *E. coli*: The samples were enriched at a rate of 1:10 dilution in buffered peptone water (BPW), and the enriched samples were streaked onto MacConkey agar and incubated at 37ºC for 18 h for the isolation of *E. coli*. At the end of incubation, the typical pink-coloured colonies were presumptively identified as *E. coli*. Further, selective plating of *E. coli* was done on eosin methylene blue (EMB) agar and the colony characteristics were noted. Biochemical characterisation of bacterial isolates obtained from the cultural isolation was done as per the procedure described by Barrow and Feltham (2003).

Molecular confirmation of *E. coli*: Presumptive colonies of *E. coli* were confirmed using *uid*A gene targeted PCR. The PCR mixture was prepared in a 0.2 mL PCR tube and vortexed thoroughly to mix the reagents properly. The PCR was performed in an automated thermal cycler (Bio-Rad, USA) with a preheated lid. The cycling conditions for the selected gene under study included an initial denaturation of DNA at 94ºC, 5min. followed by 35 cycles each of 40 sec denaturation at 94ºC, 40 sec annealing at 55ºC and 50 sec extension at 72ºC, followed by a final extension of 5 min at 72ºC and hold at 4ºC (Bej *et al*., 1991). The PCR products were separated and visualised by gel electrophoresis in 1.50 per cent agarose (Origin, India) containing ethidium bromide, in TAE buffer. The resolution of the amplified fragment in the gel was visualised and recorded by the gel documentation system (Bio-Rad, USA).

Identification of Diarrheagenic *E. coli* (DEC) pathotypes: All the confirmed *E. coli* isolates were subjected to standardised PCR assays for identification of DEC pathotypes *viz*., entero-pathogenic *E. coli* (EPEC), entero-haemorrhagic *E. coli* (EHEC), entero-aggregative *E. coli* (EAEC) and entero-toxigenic *E. coli* (ETEC) (Vergis, 2013). The genes selected for the present study were *eae* (917 bp) and *bfp*A (385 bp) genes for EPEC, stx_1 (470 bp) and stx_2 (255 bp) genes for EHEC, and *ST* (171 bp) genes for ETEC and *ast*A (102 bp) gene for EAEC. In brief, the standardized PCR protocol for 25 µL reaction mixture included 10 µL of PCR master mix, 20 µM of a primer set containing forward and reverse primers (a final volume of 0.8 µL of each primer), 3 µL of DNA template and sterilized Milli-Q water to make up the reaction volume. The cycling conditions for PCR is as given in Table 1. The PCR products were visualized by gel electrophoresis in 1.50 per cent agarose containing ethidium bromide in TAE buffer.

Table 1. Standardisation of the PCR for DEC pathotypes

PCR for	PCR conditions									
	Initial denaturation		Denaturation		Annealing		Extension		Repeat denaturation to extension 34 X and 39 X	Final	Extension
eae gene	94	5.00	94	0.30	59	1.00	72	1.30		72	10.00
*bfp*A gene	94	5.00	94	0.30	59	1.00	72	1.30		72	10.00
*stx*1 gene	94	5.00	94	0.30	59	1.00	72	1.30		72	10.00
*stx*2 gene	94	5.00	94	0.30	59	1.00	72	1.30		72	10.00
ST gene	95	5.00	95	0.45	60	1.00	72	2.00		72	10.00
*ast*A gene	95	5.00	95	0.45	60	1.00	72	2.00		72	10.00

RESULTS

Occurrence of *E. coli*: Aquaculture-reared fish fed with raw and cooked CSW and pellet-fed fish samples along with water samples from respective fish ponds were collected for the study from D1 and D2 districts and subjected to the isolation and identification of *E. coli*. Out of the total 55 and 60 samples of raw CSW-fed fish taken from D1 and D2 districts, respectively, 90.91 and 90.00 per cent samples were found to be carrying *E. coli*, confirmed using the PCR amplification of *uid*A gene.

A total of 60 and 63 samples of cooked CSW-fed-fish samples were collected and analyzed from D1 and D2 districts, out of which 85.00 and 87.30 per cent isolates, respectively, were confirmed as *E. coli* using the PCR technique. The data regarding the occurrence of *E. coli* in raw and cooked CSW-fed fishes from D1 and D2 districts were statistically analyzed using a z-test for two independent proportions, and was found that there was no significant difference (P>0.05) between the two sets of data (Table 2).

Samples were also collected from intensively reared fish fed with pellet feed from both districts, and out of the 50 and 70 samples of fish collected from pellet feeding farms of D1 and D2 districts, on confirmation using the molecular method, only 40.00 and 50.00 per cent samples, respectively, were found to carry *E. coli*. There was no significant difference (P>0.05) between the two sets of data regarding the occurrence of *E. coli* in pellet-fed fishes collected from the two districts as per the z-test for two independent proportions (Table 2).

A comparison of the *E. coli* isolates obtained from fish fed with CSW with that of pellet-fed fish was done by using the Chi-square test for multiple proportions (Table 3). As chi-square was found to be significant, pairwise comparison was done by using a z-test for two independent proportions. The analysis revealed that there was a highly significant difference (P<0.01) between the occurrences of *E. coli* in samples of fish fed with pellet feed and CSW with the lowest occurrence noticed in pellet-fed fish samples.

Samples of pond water in which fishes are reared were also taken for analysis from both districts. As mentioned earlier, two ponds following CSW feeding practice and two ponds following pellet feeding practice were selected from each district for water analysis. Twenty five samples of pond water were collected, each from the two farms following CSW-feeding practice and 25 samples each were taken similarly, from the two farms in which pellet-feeding practice was followed. From the samples of CSW-feeding farms, 88.00 per cent of samples

Table 2. Details of *E. coli* isolates obtained from different sources of D1 and D2 districts

Sl No.	Sources	D1 district			D2 district			Z-value (P-value)
		Total no. of samples	Confirmed *E. coli* isolates		Total no. of samples	Confirmed *E. coli* isolates		
			No	Per cent		No	Per cent	
1	Fish fed with raw CSW	55	50	90.91	60	54	90.00	0.166^{ns} (0.868)
2	Fish fed with cooked CSW	60	51	85.00	63	55	87.30	0.369^{ns} (0.712)
3	Fish fed with pellet feed	50	20	40.00	70	35	50.00	1.093^{ns} (0.274)

Table 3. Comparison of *E. coli* isolates obtained from CSW-fed fish with that of pellet-fed fish in D1 and D2 districts

Sl No.	Sources	D1 district			D2 district		
		Total no. of samples	*E. coli* isolates recovered		Total no. of samples	*E. coli* isolates recovered	
			No	Per cent		No	Per cent
1	Fish fed with raw CSW	55	50	90.91[a]	60	54	90.00[a]
2	Fish fed with cooked CSW	60	51	85.00[a]	63	55	87.30[a]
3	Fish fed with pellet feed	50	20	40.00[b]	70	35	50.00[b]
	X^2 Value		41.273**			35.240**	
	(P-value)		(<0.001)			(<0.001)	

** Significant at 0.01 level; Percentage having different letter as superscript differ significantly within a column

of both districts carried the organism and in the case of water samples collected from pellet-feeding farms from D1 and D2 districts, 52.00 and 40.00 per cent samples, respectively harboured *E. coli*.

Occurrence of Diarrheagenic *E. coli* (DEC) pathotypes: All the confirmed *E. coli* isolates were subjected to standardized PCR for identification of DEC pathotypes *viz*., *eae* (917 bp) and *bfp*A (385 bp) genes for EPEC, stx_1 (470 bp) and stx_2 (255 bp) genes for EHEC, *ST* (171 bp) gene for ETEC and *ast*A (102 bp) gene for EAEC as shown in Fig. 1-6.

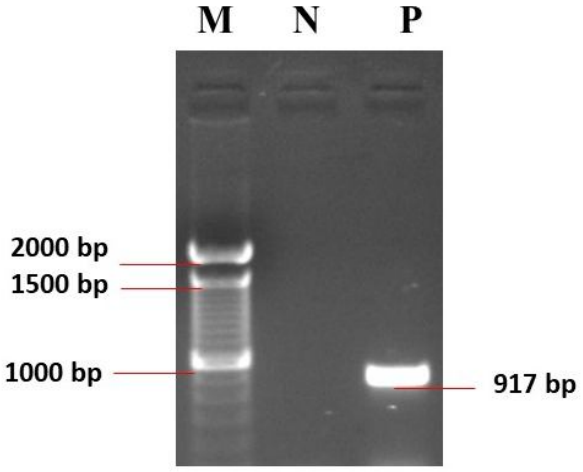

Fig. 1. PCR standardization of *eae* gene. Lane M: Marker; Lane N: Negative Control; Lane P: Positive Control

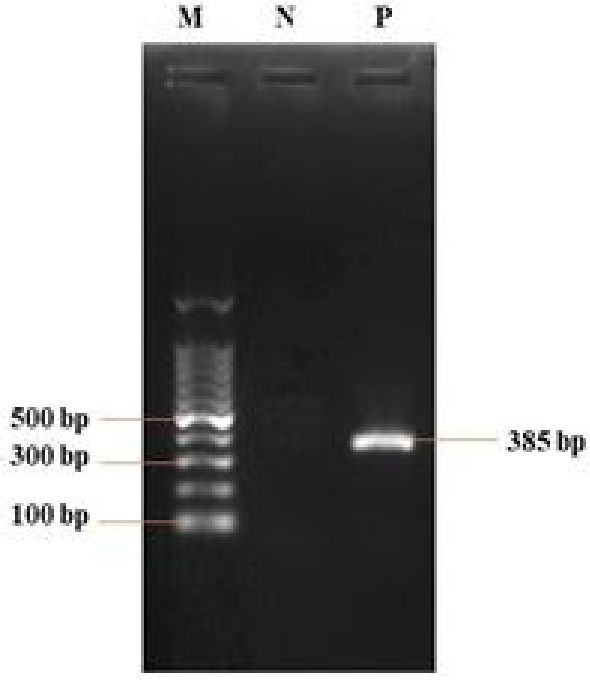

Fig. 2. Standardization of *bfp*A gene amplification. Lane M: Marker; Lane N: Negative Control; Lane P: Positive Control

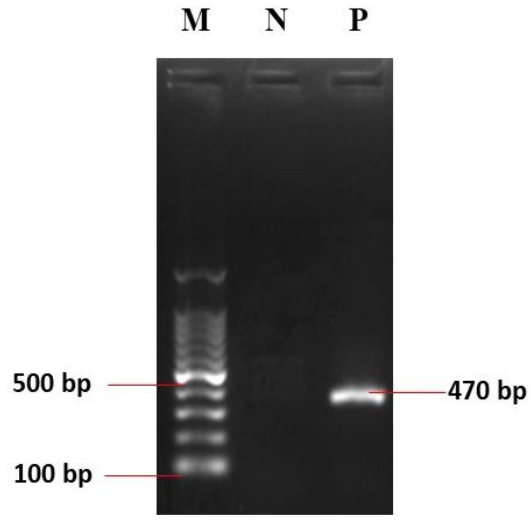

Fig. 3. Standardization of *stx*1 gene amplification. Lane M: Marker; Lane N: Negative Control; Lane P: Positive Control

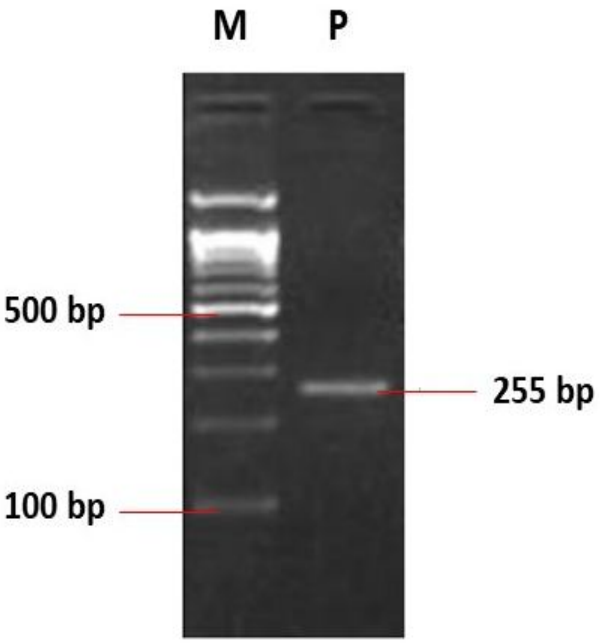

Fig. 4. Standardization of *stx*2 gene amplification. Lane M: Marker; Lane P: Positive Control

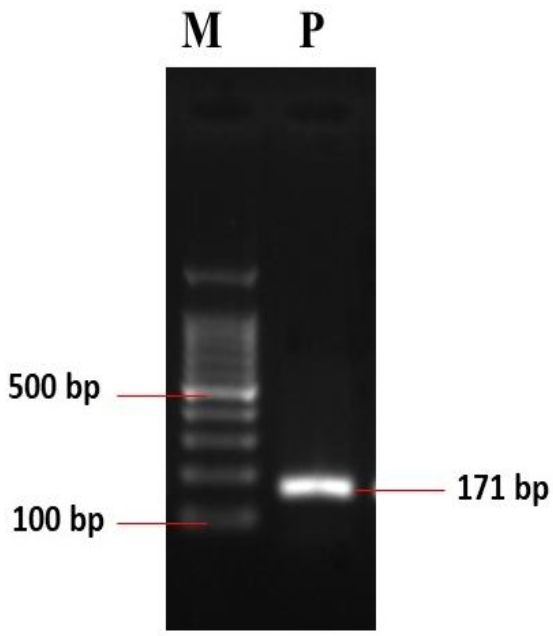

Fig. 5. Standardization of ST gene amplification. Lane M: Marker; Lane P: Positive Control

From the D1 district, 50 *E. coli* isolates obtained from raw CSW-fed fish samples were screened for the detection of DEC pathotypes. The *ast*A gene suggestive of EAEC was detected in seven isolates. None of the isolates were found to carry genes specific to EPEC, EHEC and ETEC pathotypes. From the 54 isolates recovered from raw CSW-fed fish samples collected from D2 district, one isolate carried gene specific for EPEC, and three isolates harboured genes specific for aEPEC type. Two isolates were found to carry stx_1 gene indicative of the EHEC, and seven isolates were found to carry *ast*A gene indicative of the EAEC type. None of the isolates carried *ST* gene, and hence the ETEC was not detected among the isolates.

A total of 51 *E. coli* isolates were obtained from cooked CSW-fed fish samples collected from D1 district, and the pathotype screening of the same revealed that one isolate carried both *eae* and *bfp*A genes suggestive of the EPEC type and sixteen isolates harboured *ast*A gene indicative of the EAEC type. None of the isolates were found to belong to the EHEC or ETEC groups as the specific genes for these types were not detected. Of the 55 *E. coli* isolates recovered from cooked CSW-fed fish samples collected from D2 district, one isolate showed the presence of both *eae* and *bfp*A genes indicative of the EPEC group, and 12 isolates belonged to aEPEC as they carried only *eae* gene in the absence of *bfp*A gene. Three of the isolates were found to carry stx_1 gene; hence, categorised as the EHEC and another three

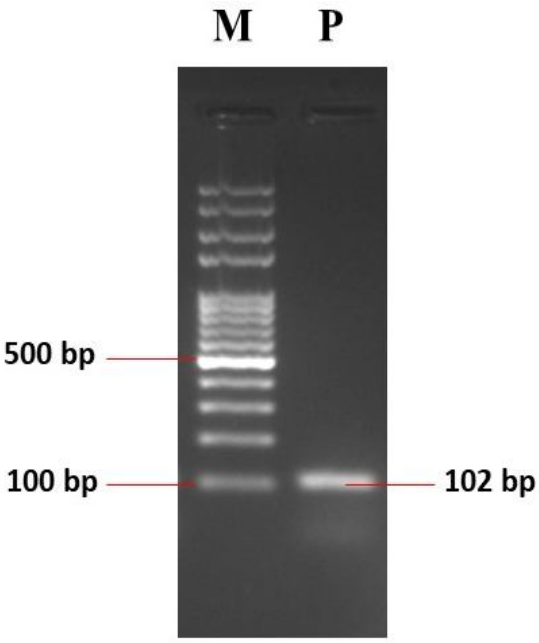

Fig. 6. Standardization of *ast*A gene amplification. Lane M: Marker; Lane P: Positive Control

isolates were found to carry *ST* gene indicative of the ETEC group. Out of the 55 isolates subjected to the pathotype characterization, 11 isolates were found to harbour *ast*A gene, suggestive of EAEC (Table 4).

Table 4. Details of DEC pathotypes detected from fish samples from different sources in the two districts

Sl No.	Type of sample	Sample No.	No. of PCR confirmed *E. coli* isolates	Pathotypes detected					
				EPEC		EHEC		ETEC	EAEC
	D1			*eae*	*bfp*A	stx_1	stx_2	*ST*	*ast*A
1	CSW-fed fish (raw)	55	50						7
2	CSW-fed fish (cooked)	60	51	1	1				16
3	Pellet-fed fish	50	20			7	5	1	6
4	Water	Pellet	25	13				1	
		CSW	25	22	3	2	2		
	Total	**215**	**156**	**4**	**3**	**9**	**6**	**1**	**29**
	D2								
5	CSW-fed fish (raw)	60	54	4	1	2			7
6	CSW-fed fish (cooked)	63	55	13	1	3		3	11
7	Pellet-fed fish	70	35					1	22
8	Water	Pellet	25	10	4	2			4
		CSW	25	22	2	1			8
	Total	**243**	**176**	**23**	**5**	**5**		**4**	**52**

A total of 20 *E. coli* isolates were recovered from pellet-fed fish samples from D1 district, and the PCR screening for the pathotypes revealed that seven isolates harboured the stx_1 gene out of which two isolates co-harboured the stx_2 gene and three other isolates harboured the stx_2 gene alone. So, a total of 10 isolates were found to belong to the EHEC type. One isolate was found to carry *ST* gene and belonged to the ETEC type and six isolates were found to carry *ast*A gene, suggestive of EAEC type. Out of the 35 *E. coli* isolates recovered from pellet-fed fish samples from D2 district, none of the isolates were found to belong to the EPEC and EHEC types, as the genes specific to these types were not detected. However, one isolate was found harbouring *ST* gene specific to ETEC type, and 22 isolates harboured *ast*A gene, indicative of the EAEC type.

From the water samples from pellet-fed fish ponds selected from D1 district, 13 *E. coli* isolates were recovered. The screening of these isolates for detection of pathotypes revealed that only one of the isolates harboured a pathotype gene selected under the current study (stx_2) and belonged to the EHEC while the rest of the isolates were negative for all the genes studied. However, from D2 district, of the 10 *E. coli* isolates recovered from the water samples, two isolates carried both *eae* and *bfp*A genes and belonged to the EPEC type, while another two isolates carried only *eae* gene and were typed as aEPEC. Also, four of the isolates were found to harbour *ast*A gene and were categorised as EAEC.

From the water samples collected from CSW-fed fish ponds in D1 district, 22 *E. coli* isolates were obtained, of which two isolates carried genes specific to the EPEC and one isolate carried only *eae* gene suggestive of aEPEC. Also, another two isolates harboured stx_2 gene and were categorised as the EHEC. None of the isolates belonged to the ETEC and EAEC types. In D2 district, 22 *E. coli* isolates were recovered from the water samples and the PCR based pathotype screening revealed that one isolate harboured *eae* and *bfp*A genes and belonged to the EPEC, while another isolate carried only *eae* gene indicative of aEPEC. Another eight isolates carried *ast*A gene and were typed as EAEC (Table 4).

DISCUSSION

Occurrence of *E. coli* in fish and environment: Among the 55 samples of farm-reared fish fed with raw CSW from D1 district, 90.90 per cent of samples were found to be positive for *E. coli,* whereas out of the 60 samples of farm-reared fish fed with cooked CSW from the same district, 85.00 per cent of samples were found to carry *E. coli*. A similar study conducted by Dang and Dalsgaard (2012) in Vietnam reported that the *E. coli* count in the intestinal content of fish fed with pig waste was 100 times higher than that of fish not fed with pig waste. The study by Jiang *et al*. (2012) found that 72.67 per cent of fish samples carried *E. coli*. A study by Saharan *et al*. (2020) showed that fish samples collected from aquaculture farms in India had a 51.00 per cent isolation rate.

A total of 60 samples of fish fed with raw CSW and 63 samples of fish fed with cooked CSW were collected from D2 district and analyzed for the presence of *E. coli*, and it was found that 90.00 and 87.30 per cent of samples were harbouring the organism, respectively. The occurrence of *E. coli* in raw CSW-fed and cooked CSW-fed fish samples did not differ significantly in either of the two districts, indicating that the heat treatment of the CSW is not an effective way to reduce the microbial load in the CSW feed.

Ava *et al*. (2020) conducted a study in Bangladesh and reported that 75.63 per cent of samples of fish collected from selected fish farms were positive for *E. coli*. Bollache *et al.* (2019) analyzed the prevalence of *E. coli* in omnivorous fishes collected from a French water shed in Burgundy and reported that the prevalence rates of *E. coli* in different types of fishes ranged from 25.00 to 92.30 per cent. The authors stated that the high isolation rates may be due to the water pollution from sewage and animal waste entering the watershed at various points.

In the case of farmed fish fed with pellet feed, a total of 50 samples were collected from D1 district, of which 40.00 per cent samples were found to carry *E. coli*. Similar findings were obtained in the study by Dewi *et al*. (2022), who discovered that 44.50 per cent of fish samples from aquaculture ponds contained *E. coli*. Contrary to the findings of the present study, an investigation conducted by Kikomeko *et al*. (2016) in Uganda reported a very high prevalence rate (83.33 per cent) of *E. coli* in farmed fish, whereas Silva *et al*. (2019) reported a lower prevalence rate of 30.0 per cent in farmed fish samples collected from Portugal.

Compared to fish samples from farms following CSW feeding practice, the isolation of *E. coli* was much less from the pellet-fed fish samples. The statistical comparison also revealed a significant difference in the number of *E. coli* recovered from fish fed with CSW and pellet feed samples. However, no significant difference was noted in the number of positive samples between the samples of fish collected from raw and cooked CSW feeding farms.

The samples of fish collected from farms using pellet feed in D2 district were also analyzed, and out of the 70 fish samples, only 50.00 per cent were found to carry *E. coli*. A study conducted by Hassan *et al*. (2022) on farmed fish collected from Egypt reported a finding (44.33 per cent) similar to the current study. However, *E. coli* isolation rates as high as 90.00 per cent and as low as 20.00 per cent were also reported by contemporary workers like Akter *et al*. (2022) and Yohans *et al*. (2022), respectively.

The rate of isolation of *E. coli* from pellet-fed fish collected from D2 district was higher than that from D1 district. The hygienic condition of farm water and surroundings was poor in the former district, which might have added to the increased isolation rate of the organism. This finding indicated that farm hygiene and management practices have a major impact on the microbial quality of the fish farmed there. The rate of isolation of *E. coli* from different fish samples collected from D2 district revealed that the highest (90.00 per cent) rate of recovery was found in samples of fish fed with raw CSW, and the lowest (50.00 per cent) was seen in samples of fish fed with pellet feed. The contamination of fish with the CSW used as feed was highly evident from the results.

The water samples were collected from both CSW-feeding fish farms and pellet-feeding fish farms from both districts. Analysis of samples revealed that 88.00 per cent of samples were positive for *E. coli* in CSW feeding farms of both districts, whereas only 52.00 and 40.00 per cent of samples from pellet feeding farms of D1 and D2 districts, respectively were positive for the organism. This result clearly indicated the extent of contamination caused by the addition of CSW to the pond water as feed for fish. Similar studies conducted elsewhere had reported a prevalence of *E. coli* as high as 100 per cent (Liao *et al*., 2021) and as low as 13.84 per cent (Valenzuela-Armenta *et al*., 2018) in aquaculture water samples. A study by Ava *et al*. (2020) reported that 81.40 per cent of fish pond water samples were positive for *E. coli*, and the findings agree with the current study's results. Similar to the results of the current investigation, a previous study by Hamza *et al*. (2020) reported that the water samples collected from three different sources in integrated agriculture-aquaculture farms revealed the presence of *E. coli* in 42.00 per cent of samples.

Occurrence of Diarrheagenic *E. coli* (DEC) pathotypes

Out of the 50 and 51 isolates of *E. coli* recovered from fish samples fed with raw and cooked CSW from D1 district, only the EAEC type was detected except for one isolate that harboured genes suggestive of EPEC type. In the case of 54 and 55 isolates obtained from samples of fish fed with raw and cooked CSW from D2 district, a major percentage of EAEC, aEPEC and a minor percentage of EHEC and ETEC were detected. In the case of the detection of DEC from aquaculture fish samples, it was observed that most isolates belonged to either EPEC or ETEC, as reported by many authors (Nayani *et al*., 2013; Barbosa *et al*., 2014; Kambire *et al*., 2017), while in the current study, the most common DEC type detected was EAEC followed by aEPEC. The typical EPEC is defined as *E. coli* isolates harbouring both *eae* and *bfp*A genes, whereas atypical EPEC carries only *eae* and not *bfp*A gene (Amir *et al*., 2021). The EPEC was the first recognized *E. coli* pathotype and is known to cause infantile diarrhea, mostly in developing

countries. Nevertheless, nowadays, it is reported that the aEPEC isolates are recovered from the majority of diarrhea cases in developing as well as developed countries (Mare *et al*., 2021), and it was also reported that the aEPEC had a more natural tendency to cause chronic infection compared to other DEC pathotypes (Ekici and Dumen, 2019).

Among the isolates recovered from samples of fish fed with pellet feed from D1 district, most of the isolates were found to carry genes suggestive of EHEC and EAEC. In the case of 35 isolates obtained from similar samples from D2 district, the larger share of isolates was found to carry *ast*A gene indicative of EAEC, and only one isolate was found to harbour gene specific for ETEC type. Prakasan *et al*. (2022) reported a similar result and found that the most common DEC pathotype detected from the fish samples analyzed was EHEC. Also, another study conducted in freshwater fish samples revealed that all the *E. coli* isolates from fish samples harboured *stx* genes suggestive of EHEC type (Taha and Yassin, 2019). The EHEC pathotype is one of the most studied pathotypes of *E. coli* and is known to cause diseases ranging from simple diarrhea to severe ones like haemorrhagic colitis and haemolytic uremic syndrome (HUS). This pathotype is highly zoonotic and is mostly reported from food animals like cattle and swine. The pathotype ETEC is mostly reported from cases of traveller's diarrhea occurring in developing countries and is mostly isolated from areas with poor hygiene and lack of clean drinking water (Jafari *et al*., 2012).

The screening of the 13 isolates recovered from the water samples from the pellet-feeding fish ponds in D1 district revealed that only one isolate harboured the *stx* gene suggestive of EHEC type, and the rest of the isolates were negative for all other genes screened under the current study. Out of the 10 isolates obtained from similar samples from D2 district, two isolates each were categorised as EPEC and aEPEC, as they were observed to carry the specific genes for the pathotype. Among the 22 isolates recovered from water samples collected from fish ponds using CSW as feed from D1 district, the EPEC, aEPEC and EHEC pathotypes were detected. Similarly, from the 22 isolates recovered from the water samples from D2 district, EAEC, EPEC and aEPEC types were detected, and the result was in agreement with the findings of Canizalez-Roman *et al*. (2019), who reported that out of the total *E. coli* isolates recovered from water samples, most commonly detected DEC types were EAEC followed by EPEC and the least detected one was ETEC. The number and types of DEC detected from CSW-fed fish pond water samples were higher than those detected from pellet-fed fish pond water samples. The contamination of waterbodies and thereby facilitating the spread of such DEC pathotypes resulting from the use of CSW is evident from the data. The pathotype EPEC was seen commonly from the water samples of both districts in the current study, and similar findings were reported by different authors (Roy *et al*., 2013; Huang *et al*., 2016).

The study has provided a comprehensive assessment of the occurrence and distribution of DEC pathotypes in aquaculture-reared fish from districts D1 and D2 in Kerala, India. The investigation encompassed fish fed with both raw and cooked CSW and pellet-fed fish, shedding light on the potential risks associated with various feeding practices. The result indicated a notably high prevalence of *E. coli* in fish reared with both raw and cooked CSW. These findings underscore the importance of monitoring and improving the safety of fish produced under such feeding conditions. Conversely, pellet-fed fish displayed a lower occurrence of *E. coli,* suggesting that this feeding practice may be associated with reduced pathogen presence in aquaculture-reared fish. This distinction is statistically significant and emphasizes the potential benefits of alternative feeding strategies in enhancing food safety. Furthermore, the study investigated the characterization of *E. coli* pathotypes among the isolates. The presence of Enteroaggregative *E. coli* (EAEC) was prominently identified in fish fed with CSW. In contrast, pellet-fed fish exhibited a higher prevalence of Enterohemorrhagic *E. coli* (EHEC) pathotype, emphasizing the need for targeted interventions and rigorous food safety measures. Overall, these findings highlighted the critical importance of feeding practices in aquaculture and their impact on the prevalence of *E. coli* pathotypes in fish. Implementing strategies to reduce the presence of these pathogens in aquaculture systems is crucial for ensuring food safety and safeguarding public health.

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