Research Article

Emerging concerns: Prevalence of antimicrobial resistance in *Aeromonas* and *Escherichia coli* in retail fish from Ganges delta's Diamond harbour region

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ABSTRACT

The study investigated the prevalence of *Aeromonas* spp. and *Escherichia coli*, pathogens associated with diseases in both fish and humans, within retail market *Labeo rohita* and *L. catla* samples obtained from the Diamond Harbour region of the Ganges delta. The primary focus was to assess the potential risks posed by these bacteria in terms of antibiotic resistance. A total of 46 presumptive *Aeromonas* and 29 nos of *E. coli* strains were successfully isolated from the sampled retail market carps. Among the isolated aeromonads, *A. sobriae* (15) and *A. hydrophila* (13) were dominant. The study revealed that 70 strains, comprising 42 aeromonads and 28 *Escherichia coli*, exhibited multiple antibiotic resistance (MAR). Notably, a significantly higher percentage of MAR was observed in bacterial strains isolated during the monsoon season. Analysis of the antibiotic resistance profiles demonstrated a total of 43 distinct profiles among the isolated strains. The contamination of farmed carps, particularly with enteric bacteria such as *Escherichia coli*, emerged as a significant concern for consumers in retail markets. The high frequency of multiple antibiotic-resistant aeromonads and *Escherichia coli* in retail carps, coupled with their potential dissemination through the food chain, poses serious threats to consumer health. Importantly, this report is likely the inaugural documentation of antimicrobial resistance (AMR) in aquacultured fish in the Diamond Harbour region of West Bengal. Given its proximity to the Ganges delta, there is a pressing need for ongoing and systematic documentation to address and mitigate these emerging health risks.

Keywords: *Aeromonas*, Antibiotic profiling, Antibiotic-resistance, *Escherichia coli*, Retail markets

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INTRODUCTION

Aquaculture is experiencing exponential growth, surpassing all other sectors of animal production worldwide. In this regard, India holds the second position in aquaculture production after China (FAO, 2022). The annual growth rate of India's fisheries sector stands at an impressive 7%. The primary contributors to freshwater aquaculture production in India are the Indian major carps (IMCs), which include *Labeo rohita*, *L. catla*, and *Cirrhinus mrigala*, accounting for over 75% of the production. West Bengal, as the second-largest fish-producing state in India, achieved a production of 18.56 lakh tonnes in 2022-23 (Department of Fisheries, 2022). However, the rapid expansion of aquaculture has led to the extensive use of antibiotics to combat bacterial fish diseases. This has resulted in the emergence of antimicrobial resistance (AMR) in the bacterial flora associated with fish, posing a substantial risk of horizontal gene transfer (HGT) from fish to humans (ECDC/EMEA 2009; Limbu et al., 2020; Schar et al., 2021) thus evolving into a global concern (WHO/FAO/OIE, 2020; Schar et al., 2021; FAO, 2022). The regulatory framework governing antibiotic use in aquaculture remains limited. Consequently, there is a pressing need for information on AMR occurrence at local, national, and international levels to guide policymakers and regulatory authorities toward the responsible use of antibiotics (Watts et al., 2017; Silva et al., 2019; Schar et al., 2021; FAO, 2022; Hossain et al., 2022). Despite some regions implementing strict regulations in leading aquaculture production countries, the regulatory framework remains scarce. The Codex Alimentarius Commission (CAC), operating under the WHO and FAO, has issued recommendations for all countries to follow as a code of practice to minimize and contain AMR (Codex Alimentarius, 2005; WHO/FAO/OIE, 2020). In line with this, the Food Safety and Standards Authority of India (FSSAI) has enacted a comprehensive ban on the use of antibiotics and various pharmacologically active substances in Indian fisheries (FSSAI, 2022). The genus *Aeromonas* is ubiquitous in aquatic environments and has been recognized as an ideal subject for studying AMR in such settings (Parker and Shaw, 2011; Patil et al., 2016). Additionally, *Aeromonas* is not only a native microorganism of aquatic environments but also a causative agent of food poisoning and gastrointestinal diseases in humans (Graf, 2015). Motile *Aeromonas* spp. are responsible for several diseases and pathological conditions in carps, often necessitating antibiotic use (Janda and Abbott, 2010). *Escherichia coli* has been employed as an indicator of fecal contamination and water and seafood's sanitary status. Fish displaying signs of fecal contamination pose a greater health risk to humans as they are more likely to harbor human-specific enteric pathogens (Croxen et al., 2013; Dutta and Sengupta, 2016). The unsanitary conditions prevalent in landing centers, storage facilities, and domestic retail markets exacerbate hygiene and consumer safety issues (Bardhan and Abraham, 2021). While most *E. coli* or faecal coliform strains are harmless, some can pose health hazards. Foodborne illnesses often result from the consumption of raw or inadequately cooked food, including fish, which may be contaminated with bacteria from water environments (*E. coli*) or terrestrial sources (coliforms) (Croxen et al., 2013; Silva et al., 2019; Limbu et al., 2020). Fish and related products can pose a significant health risk as they may harbor important pathogenic

bacteria on or within them, potentially leading to bacterial infections through improper handling or consumption of undercooked fish meat (Gufe et al., 2019). Therefore, the present study aimed to isolate *Aeromonas* spp. and *E. coli* from carp sold in Diamond Harbour's retail markets and evaluate their susceptibility to 8 broad-spectrum antibiotics. The study also shed light on the potential threats posed by these motile *Aeromonas* strains to consumers in terms of AMR. Importantly, there have been no previous reports of AMR or MAR strains in the Diamond Harbour region. Given its status as the largest wholesale market in South 24 Parganas, a tourist spot, its proximity to the Hooghly River and the rich diversity of fish species, this area warrants regular surveillance of AMR in fish sold in its markets. Thus, this study provided valuable insights into the current status of AMR in the region.

MATERIALS AND METHODS

Sampling and experimental fish

The experimental study was conducted over two seasons, specifically the pre-monsoon period (April-June) and the monsoon season (July-September). Sampling activities were carried out in four distinct fish markets: Sarisha fish market (22°25'06" N, 88°18'61" E), Amtala fish market (23°92'85" N, 88°44'92" E), Sirakol fish market (22°31'70" N, 88°26'79" E), and Diamond Harbour fish market (22°19'30" N, 88°19'12" E). The study focused on the targeted experimental biota, which included *Labeo rohita* and *L. catla*. Sampling was conducted on a weekly basis, resulting in a total of four sampling events per month for each fish market. During each sampling event, three live fish for each species, ranging in weight from 18.20±2.23 g to 25.64±3.41 g, were procured from each fish market. These live fish were promptly euthanized through a precise cranial impact, then carefully placed in sterile polythene zipper bags and transported through cold chain for further analysis.

Bacterial isolation and phenotyping

For each fish species (n=3), aseptic degutting and filleting was performed to obtain approximately 25 g of edible meat, including the skin. The pooled meat and skin (25 g) from each fish species, from one particular market location, were individually mixed with 225 ml of sterile saline and homogenized under aseptic conditions using sterilized pestle and mortar. A loopful of the homogenized fish meat samples was carefully streaked onto Rimler-Shotts agar (RSA) plates that were supplemented with novobiocin (10 µg/ml). These plates were appropriately labeled and then placed in an incubator at 35±2 °C (24 hours). The RSA agar, chosen for its selective properties, was used to isolate and identify *Aeromonas* spp. This selection was based on specific characteristics such as lysine and ornithine decarboxylation, maltose fermentation, and hydrogen sulfide production (Collins et al., 2004). Distinct colonies, displaying a yellow color, convex shape, smooth texture, and a round form, were singled out and provisionally categorized as *Aeromonas* spp. These representative colonies were then purified through successive streaking on nutrient agar (NA) plates. The colonies on NA plates, which exhibited the characteristic

circular shape with a diameter of 2-3 mm, were subsequently preserved on NA slants for phenotypic characterization. The presumptive *Aeromonas* spp. (n = 46) underwent further characterization through a series of biochemical tests (Collins et al., 2004) ultimately leading to identification at the species level. For *Escherichia coli* identification, enrichment process is mandatory. To initiate the enrichment process, 1 g of homogenized fish meat was introduced separately into two test tubes containing 10 ml of MacConkey broth. One of these tubes was then incubated at 37°C for a period of 18–24 hours, while the second tube was incubated at 44°C for the same duration. After the completion of the incubation period, both tubes were examined for a color change from purple to yellow, indicating successful enrichment. Subsequently, a loopful of inoculum from the enriched MacConkey broth and the homogenized meat was streaked onto MacConkey agar. This agar was incubated at 30°C for 18–72 h, following outlined protocols (Collins et al., 2004). Colonies meeting the criteria of being pink to dark pink, dry and donut shaped surrounded by a halo of dark pink area, were identified as *E. coli* colonies. These *E. coli* colonies were aseptically collected, subjected to purification through repeated streaking on nutrient agar (NA) plates, and preserved in NA slants for subsequent biochemical characterization. Confirmation of the isolates as *E. coli* (n=29) was achieved through a series of biochemical tests, as detailed in Table 1 and following outlined procedures (Collins et al., 2004).

Antibiotic sensitivity assay and determination of antibiotic resistant profiles

The study assessed the antibiotic sensitivity of 46 *Aeromonas* spp. and 29 *E. coli* strains isolated from retail market carps. Eight broad-spectrum antibiotics, namely amoxyclav (30 µg), azithromycin (15 µg), cefalexin (30 µg), chloramphenicol (30 µg), enrofloxacin (10 µg), oxytetracycline (30 µg), sulfafurazole (300 µg), and erythromycin (15 µg), were used for testing. The agar-disc diffusion technique (CLSI, 2012) was employed on Mueller Hinton agar (MHA) at a controlled temperature of 35±2°C. The interpretation of sensitivity was based on a zone size interpretation chart (CLSI, 2012). To ensure accuracy, reference strains *Aeromonas hydrophila* ATCC 7966 and *Escherichia coli* ATCC 25922 were included in the testing. The resistance pattern and index were determined using the antibiogram data. Isolates that exhibited resistance to three or more antibiotic groups (ECDC/EMEA, 2009) were classified as multiple antibiotic resistant (MAR).

RESULTS

Prevalence of Aeromonas spp. and Escherichia coli

A total of 46 and 29 presumptive *Aeromonas* and *E. coli* were isolated respectively. Following biochemical characterization (Table 1) among the 46 *Aeromonas* isolates, *A. sobriae* (32.61%; n=15), *A. tecta* (19.56%; n=9), *A. hydrophila* (28.26%; n=13) and *A. veronii* (19.56%; n=9) were segregated. Similarly, all the 29 presumptive *E. coli* isolates were also biochemically characterized (Table 1).

Table 1: Percentage (%) of isolates depicting positive results for various biochemical characterization

Biochemical tests	*Aeromonas sobriae* (n=15)	*A. hydrophila* (n=13)	*A. tecta* (n=9)	*A. veronii* (n=9)	*Escherichia coli* (n=29)
Gram reaction, Negative	100	100	100	100	100
Morphology, Rod	100	100	100	100	100
Oxidase	100	100	100	100	0
O/F reaction	88	100	95	95	98
Catalase	0	100	100	100	98
Motility	20	100	80	88	100
CF reaction	100	100	100	100	100
Indole production	80	100	0	100	100
MR production	88	100	100	100	100
VP reaction	20	100	20	80	0
Citrate utilization	100	100	100	100	0
Starch hydrolysis	100	100	100	100	100
Esculin hydrolysis	100	100	100	20	0
Arabinose utilization	20	90	20	90	100
Cellobiose utilization	100	100	15	100	100
Sorbitol utilization	0	0	100	0	100
LDC	88	10	98	98	96
ODC	0	0	0	0	0
Arginine dihydrolase	89	0	0	86	92
Saccharose/Sucrose	100	100	100	100	100
Urease	100	0	100	0	0
H_2S production	100	100	0	100	0

O/F: Oxidation and fermentation; CF: Carbohydrate fermentation; MR: Methyl red: VP: Voges Proskaeur; LDC: Lysice decarboxylase utilization; ODC: Ornithine decarboxylase utilization; H_2S: Hydrogen sulphide

Antibiogram

Figure 1 illustrates the antibiotic sensitivity of a total of 75 strains of *Aeromonas* sp. and *E. coli* isolated from carp samples. These strains were subjected to testing with 8 broad-spectrum antibiotics sourced from HiMedia, India. In the case of *Labeo rohita* samples, the highest level of resistance, reaching 100%, was observed against amoxyclav and azithromycin (notably in *A. tecta* and *A. veronii* strains), as well as cephalexin (predominantly in *A. hydrophila* strains). Conversely, the lowest resistance rate, at 22%, was recorded against sulfafurazole and erythromycin, particularly in *A. sobriae* strains. For *E. coli* strains, a resistance rate of 94.44% was observed against chloramphenicol, while oxytetracycline exhibited a notably lower resistance rate of 5.56%. In samples from *Labeo catla*, *A. tecta* demonstrated complete resistance (100%) against amoxyclav, azithromycin, and cephalexin. Similarly, both *A. sobria* and *A. hydrophila* strains exhibited 100% resistance against chloramphenicol. On the other hand, *A. veronii* displayed the lowest resistance rate (20%) to erythromycin, oxytetracycline, and sulfafurazole, which was consistent with *A. tecta*'s relatively lower resistance to oxytetracycline and sulfafurazole. In contrast, *A. hydrophila* strains showed only a 20% resistance rate against erythromycin. Among *Escherichia coli* strains, a high resistance rate of 90.91% was observed against chloramphenicol and enrofloxacin, while there was a notably lower resistance rate of 9.09% against oxytetracycline and sulfafurazole.

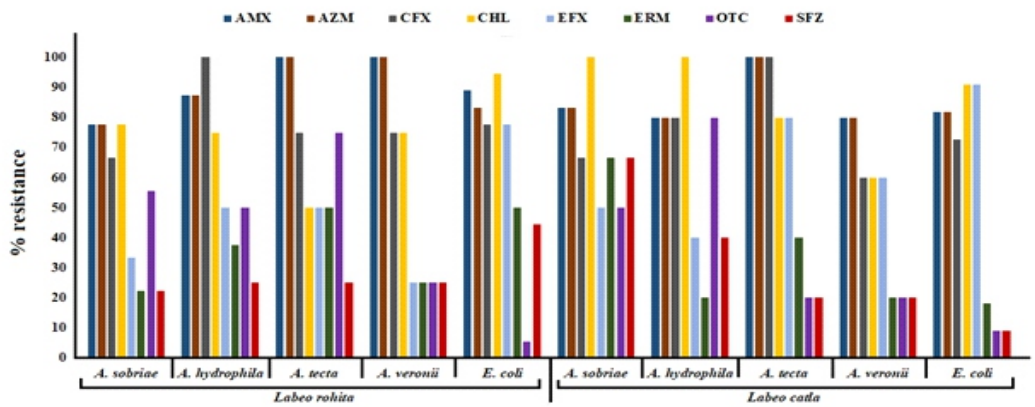

Figure 1: Prevalence of antimicrobial resistance in isolated strains from retail market *Labeo rohita* and *L. catla* samples against 8 broad spectrum antibiotics

A total of 70 strains exhibited resistance to three or more antibiotic groups, categorizing them as multiple antibiotic resistant (MAR). Within this MAR group, approximately 30% of strains demonstrated resistance to six or more antibiotic groups, while 21.43% exhibited resistance to seven or more antibiotic groups. The comprehensive breakdown of MAR groups and the corresponding number of strains in each group can be found in Table 2. Among these 70 strains displaying resistance to three or more antibiotic groups, a noteworthy 96.55% of the *E. coli* strains (n=28) demonstrated MAR.

Interestingly, bacterial strains isolated during the monsoon season exhibited a significantly higher MAR percentage (98%) compared to those isolated during the pre-monsoon season (84%). Additionally, strains isolated from samples obtained at the Amtala fish market displayed the highest MAR rate, while those from the Sarisha fish market exhibited the lowest MAR rate.

Table 2: Multiple antibiotic resistance (MAR) among the motile aeromonads of the retail markets

Particulars	Number of antibiotic groups to which the isolated strains demonstrated resistance					
	3 groups	4 groups	5 groups	6 groups	7 groups	8 groups
	Per cent resistance					
Labeo rohita (n=43)	93.02	88.37	34.88	25.58	16.28	0.00
Labeo catla (n=32)	93.75	93.75	68.75	31.25	25.00	0.00
Pre-monsoon (n=25)	84.00	80.00	72.00	32.00	8.00	0.00
Monsoon (n=50)	98.00	96.00	38.00	26.00	26.00	0.00
Sarisha fish market (n=21)	85.71	90.48	52.38	23.81	23.81	0.00
Amtala fish market (n=12)	100.00	91.67	33.33	33.33	25.00	0.00
Sirakol fish market (n=20)	95.00	90.00	50.00	25.00	10.00	0.00
DH fish market (n=22)	95.45	90.91	54.55	31.82	22.73	0.00
A. sobriae (n=15)	93.33	86.67	33.33	26.67	20.00	0.00
A. hydrophila (n=13)	92.31	92.31	76.92	46.15	38.46	0.00
A. tecta (n=9)	77.78	77.78	33.33	22.22	22.22	0.00
A. veronii (n=9)	100.00	88.89	22.22	11.11	11.11	0.00
E. coli (n=29)	96.55	96.55	58.62	27.59	13.79	0.00

Antibiotic resistance profiling

The antibiotic-resistance profiling revealed the existence of 43 distinct profiles, as detailed in Table 3, across both *L. rohita* and *L. catla* samples. Specifically, *L. rohita* samples from the Sirakol fish market displayed 19 different profiles, while *L. catla* samples from the same market exhibited 16 unique profiles. One of the most notable profiles, labeled 'Azithromycin, oxytetracycline, sulfafurazole,' stood out as it was present in nearly all the retail markets. The most frequently documented resistance profile was 'Amoxyclav, chloramphenicol, cefalexin, oxytetracycline' (n=9). However, it's worth noting that a greater variety of antibiotic-resistance profiles were observed within the '3 antibiotic groups' category. Many of these profiles exhibited resistance to amoxyclav and azithromycin, with minimal instances of resistance to enrofloxacin, erythromycin, oxytetracycline, and sulfafurazole. Profiles combining resistance to amoxyclav and azithromycin (n=22), as well as profiles encompassing resistance to amoxyclav, azithromycin, and cephalexin (n=11), were also frequently encountered.

Similarly, antibiotic-resistance profiling was done among the isolated strains, which are displayed as a heat map (Fig 2). Among the *Aeromonas* strains isolated, the frequency in variability of antibiotic-resistance profiles followed the order *A. sobriae* > *A. hydrophila* > *A. veronii* > *A. tecta,* wherein the most documented profile was 'Amoxyclav, azithromycin, cefalexin' (15.40%) and 'Amoxyclav, azithromycin, enrofloxacin' (15.40%). *A. sobriae* and *A. hydrophila* depicted huge versatility in resistance-profiling. However, *A. tecta* showed a high number of strains showing resistance to more than 6 antibiotic groups. *E. coli* strains demonstrated nearly 30 different resistance-profiles.

Table 3: Antibiotic* resistance profiles of isolated strains from *Labeo rohita* and *L. catla* samples isolated from retail fish markets of Sarisha, Amtala, Sirakol and Diamond Harbour (DH)

Resistance profile	*Labeo rohita*				*Labeo catla*			
	Sarisha	Amtala	Sirakol	DH	Sarisha	Amtala	Sirakol	DH
3 antibiotic groups								
Am,Az,Cf	1		1	2	1			
Am,Az,Ch		1			1			3
Am.Az,Ex	2	1	1		1			
Am,Az,O		1					2	
Am,Az,Sf	1			2		1		
Am,Ch,O	2					1		
Am,Ex,O	1			1	3	1		
Am,Er,Ex			1	1			2	2
Am,O,Sf	1						2	
Az,Ch,Ex	2						1	
Az,Ch,O		1		3	1			
Az,Er,Sf			1				1	
Az,O,Sf		1	1		1	2		1
Er,O,Sf	1						1	
Ex,Er,O		1					1	1
4 antibiotic groups								
Am,Az,Cf,Ch			1		1			1
Am,Az,Ch,Ex	1		1				1	1
Am,Az,Er,O		1				1		1
Am,Az,Ch,Sf			1		1			1
Am,Az,Ch,O				2				
Am,Az,O,Sf		1					1	
Am,Ch,Cf,O			2	1	3		3	
Am,Ex,Er,Sf		1		1		1	1	
Cf,Ch,Ex,Er	1	1					1	
Cf,Ch,Ex,O	2				2	1		3
5 antibiotic groups								
Am,Az,Cf,Ch,Ex	1	1	1			1		
Am,Az,Cf,Ch,Er			1			1		
Am,Az,Cf,Ch,O		1					1	
Am,Az,Cf,Ch,Sf			1	1	1			
Am,Az,Ch,Er,O			1	1			1	1
Am,Az,Ex,O,Sf			1	1		1		
Am,Cf,Ch,Er,O			1					1
Am,Ch,Er,O,Sf	1		1		1			1

6 antibiotic groups										
Am,Az,Cf,Ch,Ex,Er	1			1			1			
Am,Az,Cf,Ch,Ex,O	1						1			
Am,Az,Cf,Ch,Ex,Sf			1				1			
Am,Cf,Ch,Ex,Er,O			1		1					
Am,Cf,Ch,Ex,Er,Sf				1					1	
Am,Ch,Ex,Er,O,Sf			1		1	1				
7 antibiotic groups										
Am,Az,Cf,Ch,Ex,Er,O	1			2					1	
Az,Cf,Ch,Ex,Er,O,Sf				1	1				1	
Am,Az,Cf,Ch,Ex,Er,Sf	1			1					1	

*Amoxyclav (Am), azithromycin (Az), cefalexin (Cf), chloramphenicol (Ch), enrofloxacin (Ex), oxytetracycline (O), sulfafurazole (Sf), and erythromycin (Er) were the antibiotics tested

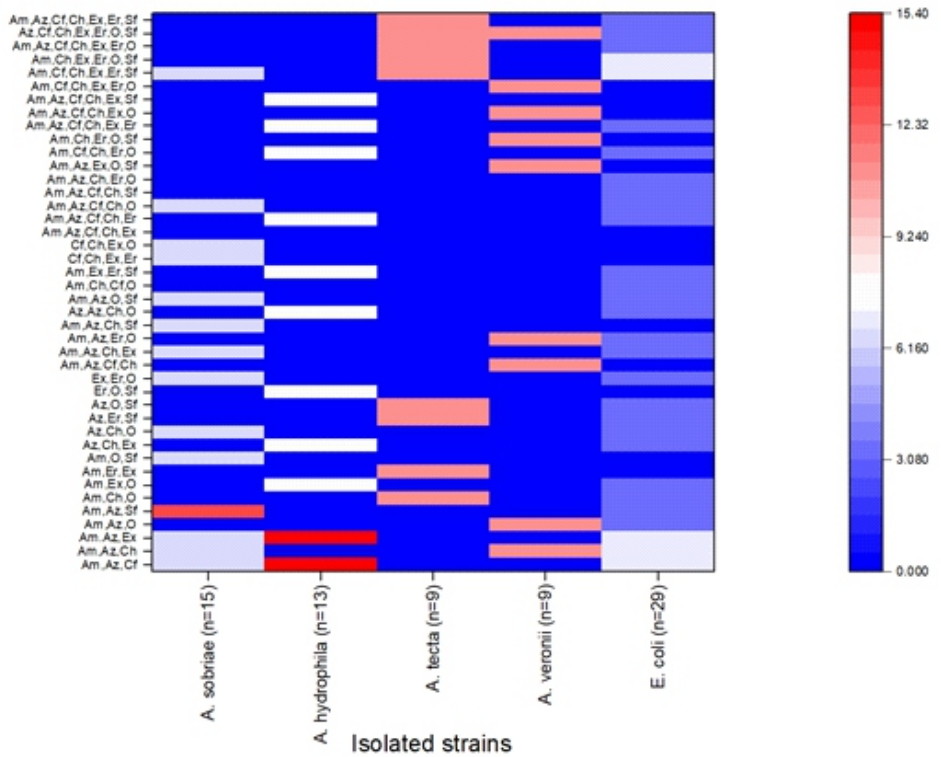

Figure 2: Heat map exhibiting total numbers of diversified antibiotic-resistance profiles observed in isolated strains from retial market *Labeo rohita* and *L. catla* samples

DISCUSSION

The evaluation of AMR has become a pivotal focus within aquaculture research, particularly due to the escalating instances of disease resistance and the excessive use of antimicrobials. Retail markets, including fish wet markets, are recognized sources of AMR dissemination, primarily attributed to contamination during mishandling, inadequate storage conditions, and exposure to mucus secretions or ice (Bardhan and Abraham, 2021). While there is a growing emphasis on implementing biosecurity measures in fish farms, the same level of attention to safety procedures is often lacking in retail markets, raising significant concerns about consumer safety (Biswas et al., 2023). This study specifically addresses two major groups of zoonotic pathogens that induce gastrointestinal anomalies in both fish and humans (Abraham et al., 2022). The Diamond Harbour region, characterized by its rural nature, lacks the necessary amenities and privileges for enforcing proper safety protocols. The proximity to the Ganges introduces environmental safety challenges due to the disposal of market wastes, including viscera and enterals. Additionally, as a tourist attraction, ensuring public safety, particularly that of consumers, is imperative. The research focuses on providing insights into the current scenario of AMR in two widely consumed carp species, *Labeo rohita* and *Labeo catla*, prevalent in the Diamond Harbour area. The selection of these species is strategic, given their popularity in the region. In summary, this research contributes to a comprehensive understanding of the prevailing situation of AMR, emphasizing the need for enhanced safety measures, particularly in the context of retail markets and the consumption of carp species in the Diamond Harbour region.

Aeromonas strains, commonly found in freshwater environments, serve as indicators in this study for assessing antimicrobial resistance (AMR) in cultured carps sold in retail markets (Bardhan and Abraham, 2021). These strains are known to cause various human health issues. AMR bacteria can be transmitted to humans through fish consumption or contact. Investigating different aquatic sources for *Aeromonas* presence and resistance to antibiotics is crucial. Antibiotics like oxytetracycline and sulphonamides are widely used in aquaculture to combat bacterial infections (Patil et al., 2016). However, some of the antibiotics to which aeromonad and *E. coli* strains in this study demonstrated resistance are also prescribed for human infections (Bardhan and Abraham, 2021). Despite the peri-urban location of these markets, the high prevalence of MAR strains suggests contamination risks along the production chain. Resistance to antibiotics like cefalexin is concerning, given the popularity of carps as a food source. The aeromonads from all the market carps showed a higher MAR rate possibly due to culture system contamination or the use of poor-quality water (Bardhan and Abraham, 2021). These findings emphasize the potential role of Indian major carps as a source of MAR aeromonads, with contamination occurring through mucus contact or water use. India ranks among the major global producers of veterinary-based drugs, yet the regulation of antibiotic sales and usage in the country faces challenges (Done et al., 2015). Prolonged antibiotic use in aquaculture exerts selective pressure on bacterial populations, even at antibiotic concentrations below the minimum inhibitory concentration for susceptible wild-type populations. This practice

also elevates horizontal gene transfer rates, involving both human and fish pathogens (Watts et al., 2017; Limbu et al., 2020; Hossain *et al.,* 2022). *Escherichia coli* is frequently employed as an indicator organism to monitor emerging resistance patterns and specific resistance genes that could potentially transfer to other pathogenic Gram-negative bacteria (Ryu et al., 2012). Given their widespread presence in aquatic environments and their capacity to develop antimicrobial resistance (AMR) under selective conditions, *E. coli* organisms serve as suitable indicators for assessing AMR in such settings. Previous studies have reported varying degrees of amoxyclav resistance in *E. coli* from different sources, with rates of 97.5%, 82.40%, and 80% in *O. niloticus* (Saqr et al., 2016), catfish (Efuntoye et al., 2012), and an integrated fish farm (Su et al., 2011), respectively. An earlier study by Akinbowale et al., (2006) documented a 41.4% cephalexin resistance in *E. coli* strains from aquaculture sources in Australia. Furthermore, anthropogenic influences, such as hospital waste and household effluents, have been identified as factors promoting antibiotic resistance through the contamination of water and soil (Diwan et al., 2012).

Despite the limited scope of seasonal investigation, specifically focusing on pre-monsoon and monsoon periods, noteworthy trends emerged. Notably, a higher prevalence of isolates was evident during the monsoon season. Correspondingly, the incidence of MAR isolates was also notably elevated during the monsoon, suggesting a potential association with surface runoff as a significant factor in the contamination and horizontal spread of AMR. This observation aligns with findings from Mohanta and Goel (2014), who similarly noted an increased percentage of MAR and antibiotic resistance indices in cultured fish during the monsoon and post-monsoon seasons, relative to other periods. This supports the inference that additional terrestrial remnants of antibiotic residues, conveyed through surface runoff, may contribute to the heightened incidence of AMR in retail markets. The amplification of AMR in these settings is primarily attributed to contamination resulting from the transportation of antibiotic residues via surface runoff (Pereira et al., 2011), emphasizing the need for comprehensive strategies to mitigate AMR dissemination in aquaculture and retail markets. The significant presence of *A. hydrophila* and *A. sobriae*, along with the concerning levels of AMR, is a matter of concern. These two species are known to play a prominent role in gastrointestinal infections among the human population (Igbinosa et al., 2012). The high levels of AMR pose a dual threat, impacting not only cultured carps but also public health by potentially facilitating the transmission of AMR to humans (Borella et al., 2020). Antibiotics such as azithromycin and cefalexin are commonly used to treat various aeromonad infections in humans, including gastroenteritis and urinary tract infections (Igbinosa et al., 2012; Jover-Garcia et al.,2016). Furthermore, MAR strains from market carps could lead to infections where antibiotic therapy may prove ineffective. The findings also raise questions about market cleanliness, hygiene practices, and transportation methods, necessitating strict adherence to government guidelines. The logical explanation for the high MAR index is that the isolated strains likely originate from environments with substantial antibiotic exposure. Since these markets are situated in densely populated areas, comprehensive studies on source tracing should be conducted to identify potential causes of antibiotic resistance beyond the farms. Given the substantial

prevalence of antibiotic resistance observed, the effectiveness of these therapeutic agents in treating future *Aeromonas* infections is likely to diminish. Our observations regarding the prevalence of elevated levels of MAR *E. coli*, particularly those resistant to 5 to 7 antibiotic groups, highlight unsanitary conditions in fish farming and retail market areas. These conditions are susceptible to contamination with enteric pathogenic bacteria and MAR fecal bacteria from high-risk sources, which is a matter of serious concern. These resistant strains have the potential to serve as a source for the transmission of AMR to human pathogens through the consumption of contaminated food or direct contact with infected animals. Given that MAR *E. coli* pathotypes can be pathogenic to humans, it is strongly recommended that individuals involved in carp trading adhere to rigorous hygiene practices and maintain sanitary conditions.

CONCLUSION

The notable presence of antibiotic-resistant motile aeromonads and *E. coli* in carps sold at retail markets, along with the potential for their dissemination through the food chain, presents significant and pressing public health concerns. The wide range and diversity of MAR underscore the ongoing need for vigilant and effective monitoring of AMR patterns in cultured fish. It is paramount to ensure that carps are thoroughly cooked to mitigate the risk of foodborne infections. To reduce the prevalence of such foodborne infectious strains, continuous surveillance of AMR in farmed carps and the implementation of appropriate control measures are imperative. Additionally, there is a critical need to educate fish farmers and retailers about the far-reaching implications of antibiotic resistance on both public and environmental health. Further comprehensive studies are essential to delve into the genetic determinants responsible for AMR among motile aeromonads in carps and to investigate their potential transmission to the wider community.

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ANIMAL ETHICS

The following work was performed in compliance with the guidelines of the Committee for the Purpose of Supervision of Experiments of Animals (CPCSEA), Government of India. The experimental protocols were approved by the The Neotia University (TNU) under the minor research grant (TNU/R&D/F/M/001) dated 14.02.2023.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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AUTHORSHIP CONTRIBUTION STATEMENT

AB: Execution of experiments, laboratory investigation, formal analysis, generation of data, statistical analysis and writing of the original draft of the manuscript; SHM: Review and editing of the manuscript.

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