Journal of Aquatic Biology & Fisheries | 2024 | 12(1) | pp. 96-103
ISSN 2321–340X
OPEN ACCESS
http://www.jabf.in/
University of Kerala, INDIA

Antimicrobial resistance in *Aeromonas* and *Escherichia coli* from retail fish on Ganges Delta

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ABSTRACT

The study examined the presence of Aeromonas spp. and Escherichia coli—pathogens linked to diseases in fish and humans—in retail *Labeo rohita* and *L. catla* samples from the Diamond Harbour region of the Ganges delta.The primary focus was to assess the potential risks these bacteria posed regarding antibiotic resistance. 46 presumptive *Aeromonas* and 29 *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 43 distinct profiles among the isolated strains. The contamination of aquacultured carp, 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 and their potential dissemination through the food chain pose severe threats to consumer health. This report is likely the first to document antimicrobial resistance (AMR) in aquacultured fish from the Diamond Harbour region of West Bengal. Due to the region's proximity to the Ganges delta, it is crucial to continue systematic monitoring to address and mitigate these emerging health risks.

ARTICLE HISTORY

Received on: 29-01-2024
Revised on: 27-07-2024
Accepted on: 09-08-2024

KEYWORDS

Indian major carps;
Retail markets;
Antibiotic-resistance;
Antibiotic profiling; *Aeromonas; Escherichia coli*

1. Introduction

Aquaculture is experiencing exponential growth, surpassing all other animal production sectors worldwide. In this regard, India is second in aquaculture production after China (FAO, 2022). India's fisheries sector boasts an impressive annual growth rate of 7%. Indian major carps (IMCs)—*Labeo rohita*, *L. catla*, and *Cirrhinus mrigala*—are the primary contributors to freshwater aquaculture, making up more than 75% of the country's production. West Bengal, the second-largest fish-producing state in India, achieved a production of 14.16 million metric tons in 2019–20, with inland production contributing 10.53 million metric tons (Department of Fisheries, 2020). However, the rapid expansion of aquaculture has led to the extensive use of antibiotics to combat bacterial fish diseases. This has led to the rise of antimicrobial resistance (AMR) in the bacterial flora associated with fish, creating a significant 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 for antibiotic use in aquaculture is still limited. As a result, there is an urgent need for data on AMR prevalence at local, national, and international levels to help policymakers and regulatory authorities promote responsible antibiotic usage (Watts *et al.,* 2017; Silva *et al.,* 2019; Schar *et al.,* 2021; FAO, 2022; Hossain *et al.,* 2022). The regulatory framework remains scarce despite some regions implementing strict regulations in leading aquaculture production countries. 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). The Food Safety and Standards Authority of India (FSSAI) has enacted a comprehensive ban on antibiotics and various pharmacologically active substances in Indian fisheries (FSSAI, 2022).

Aeromonas, a genus commonly found in aquatic environments, is widely considered an ideal candidate for studying antimicrobial resistance in these settings (Patil et al., 2016). Beyond its role as an aquatic microorganism, Aeromonas is also known to cause food poisoning and gastrointestinal illnesses 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 to indicate faecal contamination and water and seafood's sanitary status. Fish displaying signs of faecal contamination pose a greater health risk to humans as they are more likely to harbour 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 harbour 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).

This study focused on isolating Aeromonas species and E. coli from carp available in retail markets in Diamond Harbour, with the objective of assessing their susceptibility to eight broad-spectrum antibiotics.The study also shed light on the potential threats these motile *Aeromonas* strains pose to consumers regarding AMR. Importantly, no previous reports of AMR or MAR strains in the Diamond Harbour region have been reported. 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.

2. Materials and Methods

2.1. 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 *Labeo 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, two 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 to the laboratory for further analysis.

2.2. Isolation and biochemical characterization

For each fish species (n = 3), aseptic degutting and filleting were performed to obtain approximately 25 g of edible meat, including the skin. The pooled meat and skin (25 g) from each fish species were mixed with 225 ml of sterile saline and homogenized under aseptic conditions. 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 labelled 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 colour, 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 2–3 mm diameter, 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), which ultimately led to identification at the species level.

For *Escherichia coli* identification, the 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 18–24 hours, while the second tube was incubated at 44°C for the same duration. After incubation, both tubes were examined for a colour change from red to yellow, indicating successful enrichment. Subsequently, a loopful of inoculum from the enriched MacConkey broth and the homogenized meat was streaked onto MacConkey agar. Following outlined protocols, this agar was initially incubated at 30°C forfour4 h and then at 44°C for 18–2ols (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 biochemical tests, as detailed in Table 1 and following outlined procedures (Collins *et al.,* 2004).

2.3. Antibiotic sensitivity assay

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, 2023) was employed on Mueller Hinton agar (MHA) at a controlled temperature of 35±2°C.The interpr etation of sensitivity was based on a zone size interpretation chart (CLSI, 2023). The testing included reference strains *Aeromonas hydrophila* ATCC 7966 and *Escherichia coli* ATCC 25922 to ensure accuracy. 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).

2.4. Software involvement

The graphical markings were carried out using Origin Version: 2023b. The graphical abstract was constructed using BioRender.

3. Results

3.1. Prevalence of *Aeromonas* spp. and *Escherichia coli*

A total of 46 and 29 presumptive *Aeromonas and E. coli* were isolated. 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 prseumptive *E. coli* isolates were also biochemically characterized (Table 1).

3.2. Antibiotic sensitivity

Fig. 1 illustrates the antibiotic sensitivity of 75 strains of *Aeromonas* and *E. coli* isolated from carp samples. The strains underwent testing with eight broad-spectrum antibiotics from HiMedia, India. Among the *L. rohita* samples, the highest resistance, reaching 100%, was noted against amoxyclav and azithromycin, especially in *A. tecta* and *A. veronii* strains, as well as against cephalexin, primarily in *A. hydrophila* strains. In contrast, the lowest resistance rate was 22%, observed against sulfafurazole and erythromycin, particularly in *A. sobriae* strains. For *E. coli* strains, resistance was highest at 94.44% against chloramphenicol, whereas oxytetracycline showed a significantly 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.

A total of 70 strains exhibited resistance to three or more antibiotic groups, categorizing them as multiple antibiotic resistant (MAR). Approximately 30% of strains in this MAR group demonstrated resistance to six or more antibiotic groups, while 21.43% exhibited resistance to seven or more antibiotic groups. Table 2 provides a detailed breakdown of the MAR groups and the number of strains in each group. Of the 70 strains that showed resistance to three or more antibiotic groups, a remarkable 96.55% of the E. coli strains (n=28) exhibited MAR. Bacterial strains collected during the monsoon season had a higher MAR percentage (98%) compared to those collected during the pre-monsoon season (84%). Moreover, strains from the Amtala fish market had the highest MAR rate, while those from the Sarisha fish market had the lowest.

3.3. Antibiotic resistance profiling

The antibiotic resistance profiling identified 43 unique profiles across *L. rohita* and *L. catla* samples, as shown in Table 3. Specifically, *L. rohita* samples from the Sirakol fish market revealed 19 different profiles, while *L. catla* samples from the same location showed 16 distinct profiles. A prominent profile, termed 'Azithromycin, oxytetracycline, sulfafurazole,' was notable for its widespread presence across nearly all retail markets. The most commonly observed resistance profile was 'Amoxyclav, chloramphenicol, cephalexin, oxytetracycline' (n = 9). Additionally, a broader range of resistance profiles was found within the category of '3 antibiotic groups.'Many of these profiles exhibited resistance to amoxyclav and azithromycin, with minimal resistance to enrofloxacin, erythromycin, oxytetracycline, and sulfafurazole. Profiles combining resistance to amoxyclav and azithromycin (n = 22) and profiles encompassing resistance to amoxyclav,

Table 1. Percentage of isolates showing positive results for different biochemical characterizations

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	100
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: VogesProskaeur; LDC: Lysice decarboxylase utilization; ODC: Ornithine decarboxylase utilization; H_2S: Hydrogen sulphide

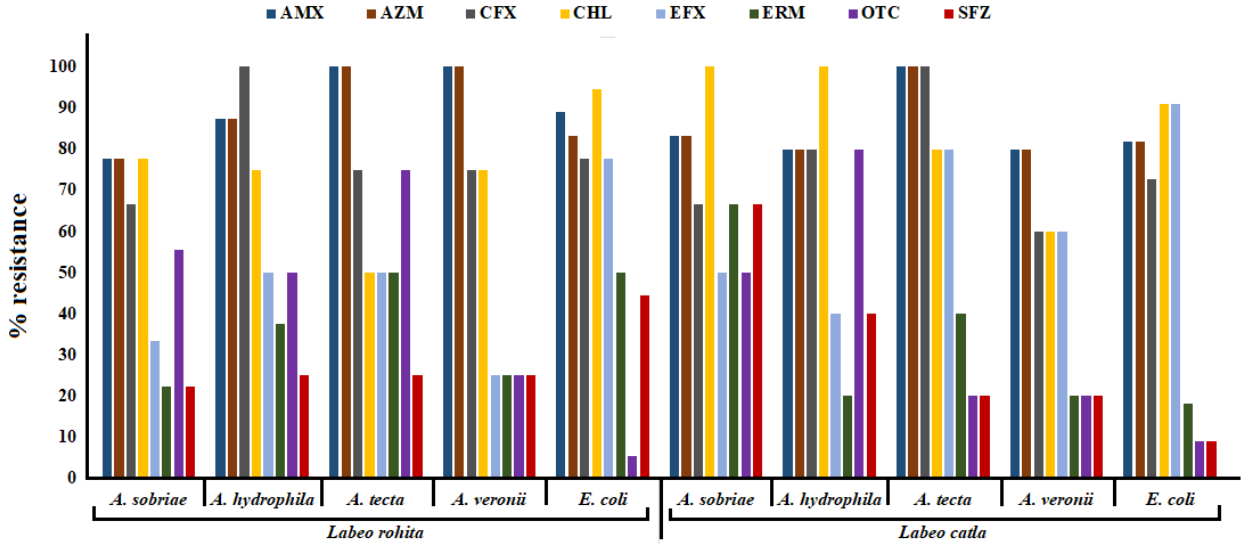

Fig. 1. Prevalence of antimicrobial resistance in isolated strains from retail market *Labeo rohita* and *L. catla* samples against 8 broad spectrum antibiotics

azithromycin, and cephalexin (n = 11) were also frequently encountered.

Similarly, antibiotic-resistance profiling was done among the isolated strains, displayed as a heat map (Fig. 3). 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.

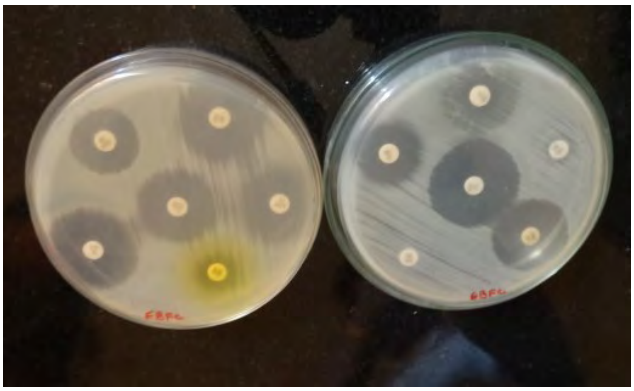

Fig. 2. Antibiogram of *A. hydrophila* selected strain observed after agar-disc diffusion assay of antibiotic-susceptibility testing assay

Table 2. Antibiotic resistance patterns in motile aeromonads from 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					
Labeorohita (n=43)	93.02	88.37	34.88*	25.58*	16.28*	0.00
Labeocatla (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

DH: Diamond Harbour; MAR index range (0.33-0.875) was same for all the obtained isolates. For a particular fish species, season, retail market or bacterial species, data showing asterisks as superscripts were found to be significant ($P < 0.05$). For a particular season, retail market or bacterial species data showing octothorpe as superscripts were found to be insignificant ($P > 0.05$).

Table 3. Antibiotic resistance patterns of strains isolated from Labeorohita and L. catla samples collected from retail fish markets in Sarisha, Amtala, Sirakol, and Diamond Harbour (DH)

Sl. no	Resistance profile	*Labeo rohita*				*Labeo catla*			
		Sarisha	Amtala	Sirakol	DH	Sarisha	Amtala	Sirakol	DH
	3 antibiotic groups								
1	Am, Az,Cf	1		1	2	1			
2	Am,Az,Ch		1			1			3
3	Am.Az,Ex	2	1	1		1			
4	Am,Az,O		1					2	
5	Am,Az,Sf	1			2		1		
6	Am,Ch,O	2					1		
7	Am,Ex,O	1			1	3	1		
8	Am,Er,Ex			1	1			2	2
9	Am,O,Sf	1						2	
10	Az,Ch,Ex	2						1	
11	Az,Ch,O		1		3	1			
12	Az,Er,Sf			1				1	
13	Az,O,Sf		1	1		1	2		1
14	Er,O,Sf	1						1	
15	Ex,Er,O		1					1	1
	4 antibiotic groups								
16	Am,Az,Cf,Ch			1		1			1
17	Am,Az,Ch,Ex	1		1				1	1
18	Am,Az,Er,O		1				1		1
19	Am,Az,Ch,Sf			1		1			1
20	Am,Az,Ch,O				2				
21	Am,Az,O,Sf		1					1	
22	Am,Ch,Cf,O			2	1	3		3	
23	Am,Ex,Er,Sf		1		1		1	1	
24	Cf,Ch,Ex,Er	1	1					1	
25	Cf,Ch,Ex,O	2				2	1		3
	5 antibiotic groups								
26	Am,Az,Cf,Ch,Ex	1	1	1			1		
27	Am,Az,Cf,Ch,Er			1			1		
28	Am,Az,Cf,Ch,O		1					1	
29	Am,Az,Cf,Ch,Sf			1	1	1			
30	Am,Az,Ch,Er,O			1	1			1	1
31	Am,Az,Ex,O,Sf			1	1		1		
32	Am,Cf,Ch,Er,O			1					1
33	Am,Ch,Er,O,Sf	1		1		1			1
	6 antibiotic groups								
34	Am,Az,Cf,Ch,Ex,Er	1			1			1	
35	Am,Az,Cf,Ch,Ex,O	1						1	
36	Am,Az,Cf,Ch,Ex,Sf			1				1	
37	Am,Cf,Ch,Ex,Er,O			1		1			
38	Am,Cf,Ch,Ex,Er,Sf				1				1
39	Am,Ch,Ex,Er,O,Sf			1		1	1		
	7 antibiotic groups								
41	Am,Az,Cf,Ch,Ex,Er,O	1			2				1
42	Az,Cf,Ch,Ex,Er,O,Sf				1	1			1
43	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.

4. Discussion

The evaluation of AMR has become a pivotal focus within aquacultural research, mainly due to the escalating disease resistance and the excessive use of antimicrobials. Retail markets, includingwet fish 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). Despite the increasing focus on biosecurity measures in fish farms, similar attention is often missing in retail markets, raising serious concerns about consumer safety (Biswas et al., 2023). This study specifically addresses two major groups of zoonotic pathogens that cause gastrointestinal issues in both fish and humans (Abraham et al., 2022). The Diamond Harbour area, being rural, lacks the necessary infrastructure to enforce effective safety protocols. The proximity to the Ganges adds environmental safety challenges due to the disposal of market waste, including viscera and entrails. Furthermore, as a tourist destination, maintaining public safety, especially for consumers, is crucial. The research provides insights into the current scenario of AMR in two widely consumed carp species, *Labeorohita* and *Labeocatla*, 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

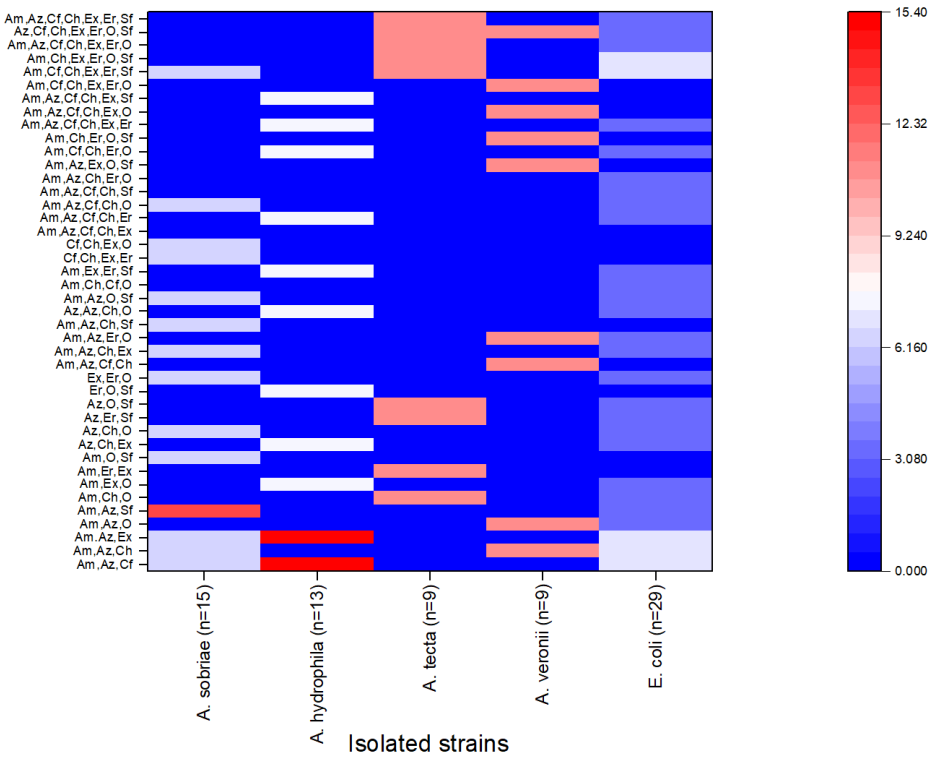

Fig. 3. Heat map exhibiting total numbers of diversified antibiotic-resistance profiles observed in isolated strains from retial market *Labeo rohita* and *L. catla* samples

retail markets and the consumption of carp species in the Diamond Harbour region.

Aeromonas strains, frequently found in freshwater habitats, are utilized in this study to evaluate antimicrobial resistance (AMR) in cultured carps sold at retail markets (Bardhan and Abraham, 2021). These bacteria are known to cause various health problems in humans. AMR bacteria can be transferred to humans through the consumption of fish or direct contact. Examining different aquatic sources for the presence of Aeromonas and their antibiotic resistance is essential. In aquaculture, antibiotics such as oxytetracycline and sulphonamides are commonly used to treat bacterial infections (Patil et al., 2016). However, some antibiotics to which Aeromonas and E. coli strains in this study have shown resistance are also used to treat 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 country faces challenges in the regulation of antibiotic sales and usage (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 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). Due to their widespread occurrence in aquatic environments and their ability to develop antimicrobial resistance (AMR) under selective pressures, *E. coli* strains are effective indicators for evaluating AMR in these settings. Previous research has shown variable levels of amoxyclav resistance in *E. coli* from different sources, including 97.5% in *O. niloticus* (Saqr et al., 2016), 82.40% in catfish (Efuntoye et al., 2012), and 80% in an integrated fish farm (Su et al., 2011). A study by Akinbowale et al. (2006) reported a 41.4% resistance rate to cephalexin in E. coli strains from aquaculture sources in Australia. Additionally, human activities such as hospital waste disposal and household effluents have been identified as contributors to antibiotic resistance by contaminating water and soil (Diwan et al., 2012).

Although the study was limited to seasonal analysis during the pre-monsoon and monsoon periods, significant trends were observed. There was a notable increase in the prevalence of isolates during the monsoon season. This was accompanied by a higher incidence of multiple antibiotic-resistant (MAR) isolates during this period, suggesting that surface runoff might be a key factor in the spread and contamination of AMR. This observation is consistent with findings from Mohanta and Goel (2014), who also reported

higher levels of MAR and antibiotic resistance indices in aquacultured fish during the monsoon and post-monsoon seasons compared to other times of the year. This supports the hypothesis that additional terrestrial antibiotic residues, transported through surface runoff, may contribute to the increased 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.,* 2017). 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 faecal bacteria from high-risk sources, which is a matter ofgraves concern. These resistant strains can potentially 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.

5. Conclusion

The significant presence of antibiotic-resistant motile aeromonads and E. coli in carps sold at retail markets, combined with their potential spread through the food chain, raises serious public health concerns. The broad spectrum and variety of multiple antibiotic resistance (MAR) highlight the critical need for ongoing and effective monitoring of antimicrobial resistance patterns in cultured fish. It is crucial to ensure that carps are thoroughly cooked to reduce the risk of foodborne infections. To decrease the occurrence of these infectious strains, continuous surveillance of AMR in farmed carps and the implementation of appropriate control measures are essential. Moreover, educating fish farmers and retailers about the widespread impact of antibiotic resistance on public and environmental health is vital. Further detailed studies are needed to explore the genetic factors driving AMR in motile aeromonads in carps and to assess their potential transmission to the broader community.

Acknowledgement

The work was supported by The Neotia University. The authors thank the Department of Biotechnology, The Neotia University for providing additional facilities to carry out the work.

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