Journal of Advances in Microbiology

Volume 24, Issue 11, Page 51-61, 2024; Article no.JAMB.125687
ISSN: 2456-7116

Microbial Profile and Antimicrobial Resistance in Enteric Pathogens of Farmed Catfish (*Pangasianodon hypophthalmus*) in Tropical Freshwater Farms

Kuttanapilly Velayuthanelayadom Lalitha [a++*], Murugadas Vaiyapuri [a] and Toms Cheriath Joseph [a]

[a] *Microbiology Fermentation and Biotechnology Division, ICAR- Central Institute of Fisheries Technology, Cochin- 682 029, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jamb/2024/v24i11864

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125687

Original Research Article

Received: 03/09/2024
Accepted: 05/11/2024
Published: 13/11/2024

ABSTRACT

Background: Recently, *Pangasianodon hypophthalmus* has emerged as an important fish species farmed in India, contributing to food security in the region. Understanding the bacterial flora associated with this fish is essential to ensure food safety, particularly in the Indian context where such studies have been limited.

[++] *Principal Scientist & Head (Retd);*
**Corresponding author: E-mail: kvlalithaa@gmail.com;*

Cite as: *Lalitha, Kuttanapilly Velayuthanelayadom, Murugadas Vaiyapuri, and Toms Cheriath Joseph. 2024. "Microbial Profile and Antimicrobial Resistance in Enteric Pathogens of Farmed Catfish (Pangasianodon Hypophthalmus) in Tropical Freshwater Farms". Journal of Advances in Microbiology 24 (11):51-61. https://doi.org/10.9734/jamb/2024/v24i11864.*

Objective: This study aims to determine the aerobic bacterial flora and antimicrobial resistance in enteric pathogens associated with farmed *Pangasianodon hypophthalmus* cultured in freshwater ponds in Kerala, India.
Methods: The study included enumeration of aerobic plate counts, *Pseudomonads, Aeromonas, Enterobacteriaceae* and *Staphylococcus aureus* using standard methods. Bacterial identification was performed using analytical profile index 20E (APE20E) and 16s rDNA sequencing. Antimicrobial resistance was assessed in *E. coli*, *S. aureus* and *Aeromonas* species using disc diffusion assays.
Results: The aerobic bacterial flora in catfish muscle tissue included H_2S-producing bacteria, *Enterobacteriaceae, Pseudomonas, Aeromonas*, and *B. thermosphacta*. Dominant genera identified were *Pseudomonas, Burkholderia, Stenotrophomonas, Aeromonas, Klebsiella*, and *Psychrobacter*. Multidrug-resistant strains of enterotoxigenic *E. coli* and *A. hydrophila* were recovered, raising concerns about the prevalence of multidrug resistance in *A. hydrophila*, an etiological agent of red disease in farmed catfish.
Conclusion: This study highlights the microbiological risks posed by *Aeromonas* spp. and enterotoxigenic *E. coli* in farmed catfish, emphasising their potential as vectors for pathogenic and antibiotic-resistant determinants in the food chain. Recommendations for future research should focus on the development of effective biopreservatives to control these pathogens in processed Pangasius products.

Keywords: Enteric pathogens; aerobic bacterial flora; Pangasianodon hypophthalmus; 16s rDNA analysis.

1. INTRODUCTION

Catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878], locally known as tra catfish/ sutchi catfish, has recently become very popular food fish and valuable aquaculture species in South-East Asia including India (APHA. WPCF, 1998). Aquaculture of this fast growing fish is gaining momentum in India and recent production amounts for 18,000-20,000 m.t. (Austin and Al-Zahrani, 1988, Milijasevic et al., 2024). The cultured and harvested *P. hypophthalmus* viz., whole and gutted, are chilled and packed and transported by land to West Bengal and North Eastern states where demand for fish is the highest. In 2011, India imported 716 tonnes of Pangasius fillet from Vietnam. A large number of restaurants across major metros like Mumbai and Delhi serve the fish, and supermarkets in major cities are selling it sliced and packed. Globally, food safety is a major concern of public health.

There are reports of diseases in Pangasius cat fish caused by *Edwardsiella ictaluri* and *Aeromonas hydrophila*. Due to increase in the production, the risk of disease outbreak is also high. Red spot disease is the widely occurring disease in *P. hypophthalmus* culture ponds of Kaikaluru and Mudinepalli mandals in Krishna districts of Andhra Pradesh and causative agents were identified as *A. hydrophila, A. caviae and A. sobria*. (Austin and Adams, 1996). The increase in incidence of diseases has led to increased use of antimicrobials/ antibiotics in fish farms (Bauer et al., 1966) and the selective pressure exerted by the use of antimicrobial agents in food animals promotes the emergence and dissemination of antimicrobial-resistant bacteria: animal pathogens, human pathogens with food animal reservoirs, and commensal bacteria (Cabello, 2006). Bacterial resistance to antibiotics has been widely spread in aquaculture environments, which has been reported in many studies (Cantas et al., 2013, Chakraborty et al., 2001, CLSI, 2012, CLSI, 2010, Enne et al., 2005). Antimicrobial resistance has long been reported widespread in *Aeromonas* species in aquacultures, and recently an upward trend in the resistance has been observed (FDA, 1998, Galindo and Chopra, 2007, Gardner, 1985). These resistant bacteria may be transferred to humans either through the food supply or by direct contact with animals (Gram et al., 1987, Griffith et al., 2010). The clinical implications of growing antimicrobial resistance among zoonotic enteric pathogens include increased enteric illness and more treatment failures. *Aeromonas* strains are able to transmit and share AMR determinants with such bacteria as Escherichia coli isolated from humans (Chakraborty et al., 2001, FDA 1998).

In spite of the increase in aquaculture of *P. hypophthalmus* in the country, little is known on the microbial profile, and antimicrobial resistance in enteric pathogens of farmed catfish in the country. Understanding the antimicrobial

resistant bacteria prevalent in catfish farms is important to devise effective disease treatment strategies and to assess potential risks to public health. Hence the present study was carried out to determine the bacterial flora associated with farmed *P. hypophthalmus* catfish from tropical grow out farms and to characterize the enteric pathogens with a view to developing effective disease treatment strategies.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Pangasianodon hypophthalmus were collected aseptically from tropical freshwater farms thrice during grow out phase and monitored for the bacterial microflora and enteric pathogens. Fish were fed on animal/plant protein sources like live stock processing waste, brewery waste and rice bran. Fish samples collected were between 600-800 g and they were transported to the laboratory aseptically on ice in ice box. On arrival at the laboratory, samples were removed from ice for bacteriological analysis. Four live catfish were randomly selected from the catch at each sampling time.

2.2 Bacteriological Analysis

A 25g portion of the fish (skin with muscle) was aseptically weighed and transferred to a stomacher bag (Seward Medical, London, UK); 225 ml of sterile physiological saline (Nacl,0.85% w/v) was added, and the mixture was homogenized for 60s in a stomacher (Lab Blender 400; Seward Medical, UK). Samples (0.5 ml) of serially decimal diluted fish homogenates were spread plated onto appropriate media in duplicate petridishes such as Tryptone Soya Agar (TSA Oxoid, UK) for enumeration/ determination of aerobic mesophilic count incubated at 30°C for 2 days (Holmberg et al., 1987). For enumerating Pseudomonads, Cetrimide-fusidin-cephaloridine agar (code CM 559, supplemented with SR 103; Oxoid, Basingstoke, United Kingdom) were used and kept incubated at 20°C for 3 days (ICMSF, 2005). *Aeromonas* spp. were enumerated on Starch Ampicillin Agar at 30°C for 2 days and amylase positive yellow to honey coloured colonies were counted (Janda and Abbott, 2010).

Enumeration of Enterobacteriaceae was performed in Violet Red Bile Glucose Agar (VRBGA) (Oxoid code CM 485, Basingstoke, UK) at 30 °C for 2 days and H_2S -producing bacteria (including *Shewanella putrefaciens*) Iron Agar (IA, Oxoid code CM 867, Basingstoke,UK), respectively. 1.0ml sample was inoculated into 10ml of molten media (45°C). After setting, a 10ml overlay of molten medium was added. For the former (VRBGA), incubation was carried out at 30 °C for 24h with the large colonies with purple haloes were counted while for the later Iron agar plates incubation were at 20°C for 5days; black colonies formed by the production of H_2S were enumerated after 2–3 days (Kemper, 2008).

Escherichia coli enumeration was performed in Tergitol-7 Agar (T-7 Agar, Himedia M616) was used, incubated at 37ºC for 24h. Five typical colonies were identified by streaking onto Eosin Methylene Blue Agar (EMB Agar, Difco, Detroit, Michigan) and characteristic *E. coli* colonies were confirmed by biochemical tests (Kingombe et al., 1999, Kong et al., 1999) *Brochothrix thermosphacta* was determined on Streptomycin sulfate-Thallous acetate – Actidione Agar (STAA, HiMedia, India) after incubation at 20°C for 4 days (Krieg and Holt, 1984).

Staphylococcus aureus counts were estimated on Baird Parker Agar (BP, Oxoid) incubated at 37ºC for 2 days and typical colonies were confirmed by coagulase test (Lalitha and Surendran 2006). Percentages of confirmed colonies were used to correct the results of the counts obtained. Enterococci numbers were estimated on KF Agar (Difco) incubated at 37ºC for 2 days. Five typical *Enterococcus* colonies were identified by checking growth at 45± 1ºC and growth in Dextrose Azide Broth containing 6.5% sodium chloride incubated at 35 ± 2ºC and confirmed by biochemical tests (Kingombe et al., 1999).

2.3 Composition of the Dominant Bacterial Flora

Composition of the dominating aerobic mesophilic microflora was determined by isolating and identifying 20% of the colonies from TSA (30°C) plates. A total of 80 bacterial isolates (40 isolates from each farm) were randomly selected from TSA plates to represent the different colony morphology types found in fish samples and characterized. Bacterial isolates characterized were grouped into 18 and were identified using 16S rRNA.

2.4 Bacterial Identification Using 16S *rRNA* Sequencing analysis

Bacterial isolates (18 nos) were sub cultured and maintained on Trypticase soy agar until processed for 16S rRNA amplification. The isolates were identified by 16S rRNA gene sequence analysis. Colonies were suspended in 100 µl of sterile distilled water, the suspension was boiled and centrifuged, and the supernatant was used as template DNA for PCR with 18 isolates. Amplification of 16S rRNA gene was performed with the combination of 27F 5'(GAGTTTGATCCTGGCTCAG)3' and 1544R 5'(AGAAAGGAGGTGATCCAGCC)3' (Maqsood and Benjakul 2010). Polymerase chain reaction was performed in 50µl reaction mix with 1x concentration of Onetaq Quick load master mix with GC buffer (NEB, Cat.No.M0487), 0.5µM concentration of each primer (Sigma), 5µl of crude DNA prepared by cell lysate method and the Cycling conditions was standardized for one taq master mix as 94°C for 2min initial denaturation, followed by 30 to 35 cycles of denaturation (94°C for 30sec), annealing (50°C for 1 min) and extension (68°C for 1.5 min) and a final extension of 68°C for 7 min. Amplicon of size 1500bp were purified using Gen Elute gel extraction kit (Sigma, NA1111) and sent for sequencing with same primers. Blast analysis was performed in NCBI to determine the homology and identification upto genus/species level.

2.5 Characterization of Bacteria Isolated from Selective Media

Enteric bacterial (30) isolates from VRBGA, H2S producing bacterial colonies (30) from IA plates, typical colonies (6-8 each) from Starch Ampicilin Agar and CFC Agar plates were isolated, identified using key schemes of Bergey's Manual of Systematic Bacteriology and schemes proposed by several authors for identification (Kemper, 2008, Mead, 1985, Miranda et al., 2013, Moli and Ternstrom, Msangi et al., 2013). About 5% of the isolates were cross checked for identification using analytical profile index 20E (API 20E, bioMerieux).

A. hydrophila (10) and *A. veronii* biovar *sobria* isolates (2) recovered from catfish were characterized by PCR assays for virulence genes such as hemolysin gene (aerolysin) *aerA,* enterotoxin genes such as cytotoxic enterotoxin (*act*) and cytotonic enterotoxin (*ast*, *alt*) genes (Mukhopadhyay et al., 2008, Murugadas et al., 2016).

Escherichia coli isolates (20) obtained in the study were tested for enterotoxigenicity using multiplex PCR for *phoA gene* (Genus specific primers, 903bp), *st1* gene (specific for heat stable enterotoxin, 175bp) and *Lt1, Lt2* genes (specific for heat labile enterotoxins, 275bp and 720bp) Kong et al., 1999, Palu et al., 2006).

2.6 Antimicrobial Susceptibility Testing of Enteric Pathogens

Antimicrobial susceptibility of enteric pathogens such as *E. coli* (20)*, A. hydrophila* (10), *A. veronii* biovar *sobria* (2) and *S. aureus* (8) isolates obtained during this study were assessed by the agar diffusion method using Mueller Hinton agar (Difco, 225250, USA) as a test medium and commercially available antibiotic test discs (Palumbo et al., 1985). For *E. coli*, Icosa G-I-Minus (Cat.No.IC003, Himedia) and for *A hydrophila* and *A. sobria*, ICOSA G-II Minus (Cat.No.IC008, Himedia) were used. Icosa G-I-Minus and ICOSA G-II Minus consists of Imipenem (IPM) 10µg, Ciprofloxacin (CIP) 5µg, Tobramycin (TOB) 10µg, Moxifloxacin (MO) 5µg, Ofloxacin (OF) 5µg, Levofloxacin (LE) 5µg, Norfloxacin (NX) 10µg, Co-Trimoxazole (COT) 25µg, Colistin (CL) 10µg, Nalidixic acid (NA) 30µg, Augmentin (AMC) 30µg, Gatifloxacin (GAT) 5µg, Gentamicin (GEN) 10µg, Amikacin (AK) 30µg, Ceftriaxone (CTR) 30µg, Cefpodoxime (CPD) 10µg. In addition, Icosa G-I-Minus consists of Sparfloxacin (SPX) 5µg, Kanamycin (K) 30µg, Streptomycin (S) 10µg, and Ticarcillin (TI) 75µg where as ICOSA G-II Minus consists of Ceftazidime (CAZ) 30µg, Cefoxitin (CX) 30µg, Aztreonam (AT) 30µg and Nitrofurantoin (NIT) 300µg.

For *S. aureus,* Icosa G-I Plus (Cat.No.IC002, Himedia) were used which consists of Cephalothin (CEP) 30µg, Clindamycin (CD) 2µg, Co-Trimoxazole (COT) 25µg, Erythromycin (E) 15µg, Gentamicin (GEN) 10µg, Ofloxacin (OF) 5µg, Penicillin (P) 10Unit, Vancomycin (VA) 30µg, Ampicillin (AMP) 10µg, Chloramphenicol (C) 30µg, Oxacillin (OX) 1µg, Linezolid (LZ) 30µg, Azithromycin (AZM) 15µg, Amikacin (AK) 30µg, Clarithromycin (CLR) 15µg, Teicoplanin (TEI) 10µg, Methicillin (MET) 5µg, Amoxyclav (AMC) 30µg, Novobiocin (NV) 5µg, Tetracycline (TE) 30µg.

Test plates were incubated for 16 to 18 h at 35±2 °C under aerobic conditions. Results were interpreted (Petersen et asl., 2002). For quality control, *Escherichia coli* ATCC 25922 was used.

3. RESULTS AND DISCUSSION

3.1 Bacterial Flora of Farmed Catfish

The aerobic bacterial flora comprised of H_2S producing bacteria, Enterobacteriaceae *Pseudomonas, Aeromonas* and *B. thermosphacta* (Table 1) (Phani et al., 2013). The levels indicator bacteria such as *E. coli and* Enterococci were in the range of 2.1- 2.7 $\log_{10}$ cfu g^{-1} and *S. aureus* counts were ≤ 2.0 $\log_{10}$ cfu g^{-1}. The total aerobic bacterial counts were in the range of 10^4 – 10^5 cfu/g. Total viable count of $<10^4$ cfu g^{-1} in *P. hypophthalmus* from Bangladesh farms (Piotrowska and Popowska, 2014) *Escherichia coli* counts in farmed catfish exceeded the limit (m) count of 11 g^{-1}. However, the count did not exceed the maximum (M) limit (500 g^{-1}) for acceptability (Rhodes et al., 2000). High levels of faecal coliforms and Enterococci were previously recorded from freshwater farms and prawns in India. (Roberts, 1997).

3.2 Composition of the Microflora

A total of 80 bacterial isolates were characterized from *P. hypophthalmus*. The dominant microflora (65-68%) included motile, Gram-negative, non-fermentative rods comprising of Genera *Pseudomonas, Burkholderia*, *Stenotrophomonas, Aeromonas, Klebsiella* and *Psychrobacter.* Gram positive flora belonged to *Staphylococcus, Bacillus* and *Micrococcus.* Enterobacteriaceae isolates from VRBGA belonged to *Providencia stuartii, P. rettgeri*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Citrobacter freundii*.

The H_2S producing bacteria were identified as *Aeromonas sobria, A. hydrophila*, *A. caviae, Shewanella putrefaciens* and *Stenotrophomonas maltophilia.* Bacterial isolates from CFC agar were identified as *P. fluorescens, P. putida, P. aureofaciens, P. poae, Burkholderia gladioli* and *B. cepacia.*

3.3 Characterization of Enteric Pathogens

In farmed catfish, 15 *E. coli* isolates were identified as enterotoxigenic and were found to harbor st1 gene (175bp) specific for ETEC pathotype. These enterotoxigenic *E. coli* isolates exhibit resistance to a broad array of antibiotics including β-lactam antibiotics, carbapenems, cephalosporins, quinolones, fluoroquinolones, aminoglycosides and TMP-SMX. 85% strains exhibited resistance to Augmentin and Cefpodoxime (Table 3). Out of 15 ETEC isolates tested, 6 isolates were multidrug resistant (Table 4). ETEC is the major etiologic agent of diarrhea (traveler's diarrhea and infant diarrhea) due to their expression of either heat-labile (LT) or heat-stable (ST) or both the enterotoxins. Farm workers in fish production systems are more prone to contamination by MDR *E. coli* of fish origin and they become more frequently carriers of MDR *E. coli* from fish. The development of multi-drug resistance in E. coli is of major concern worldwide (Sáenz, 1997, Schmidt et al., 2001). The first outbreak of ETEC mediated diarrhea in India was reported from Ahmedabad, Gujarat and majority of the strains expressed multidrug resistance (Sha et al., 2002). The antimicrobial resistance has emerged and evolved in many bacterial genera due to the excessive use of antimicrobials in human and aquaculture systems (Bauer et al., 1966, Santos and Ramos, 2018). Most enteric pathogens easily share genes for antimicrobial resistance (Chakraborty et al., 2001, Santos and Ramos 2018). It has been demonstrated that once acquired by *E. coli* the resistance genes are not rapidly lost Shah et al., 2012, Vaiyapuri et al., 2021).

In this study, *Aeromonas* isolates in catfish comprised of *A. hydrophila, A. veronii* biovar *sobria* and *A. caviae* which are known to cause infections in humans. The virulence genes were screened for the *A. hydrophila* and *A. veronii* biovar *sobria* isolates. Among the four enterotoxin and haemolysin/aerolysin genes (*act, alt, ast* and *aer)* tested, all the four genes were detected in four *A. hydrophila* isolates, *act, alt* and *aer* were detected in three *A. hydrophila* isolates. In three *A. hydrophila* isolates, only *alt* and *act* genes were found. In *A. veronii* biovar *sobria* isolates, only *act* gene was detected. The cytotoxic enterotoxin, *act/hlyA/aerA* and the cytotonic enterotoxins, *alt* and *ast* have all been implicated as important virulence factors in diarrhoeal disease (Shigematsu et al., 2009, Sinha et al., 2004). *A. caviae*, *A. hydrophila* and *A. veronii* biovar *sobria* were associated with acute diarrhoea at least in 57% patients admitted to the Infectious Diseases Hospital and the B.C.Roy Memorial Hospital, Kolkata (India) (Smith et al., 1994). *Aeromonas* is also recognized as aetiological gents in extra-intestinal infections such as septicaemia, wound infections, cellulitis (Sneath et al, 1986). Cases of extra-intestinal infections caused by *A. hydrophila* were reported in eight patients fishermen or women by occupation from the

south Karnataka coastal region, India (Sørum, 2006). The increasing levels of resistance displayed by both clinical and environmental *Aeromonas* spp. isolates (Wang et al., 2003) and also by isolates from aquaculture poses a growing challenge in the treatment of *Aeromonas* infections in fish as well as in humans (Witte, 1998). *A. hydrophila* was the causative agent of diverse pathologic conditions such as septicaemia, tail and fin rot and epizootic ulcerative syndrome (EUS) in freshwater fish (Wong, et al., 1998, Zhang et al., 2006). *A. hydrophila, A. sobria,* and *A. caviae* are reported to be associated with red disease in Pangasid catfish from farms located in Andhra Pradesh, India (Austin and Adams 1996). In view of the findings of this study, continuous monitoring is needed to determine the diversity of these pathogens and their antibiotic resistance profile to assess the health risk for humans and farmed fish.

Among the recommended antibiotics for *Aeromonas*, only imipenam resistance was observed in *A. veronii* biovar *sobria* isolates whereas in *A.hydrophila*, multidrug-resistance (Tobramycin, Amikacin, Amoxycilin clavulanic acid, Co-trimoxazole and Gentamycin) was found in two strains (Phani and Ramulu 2013). Among the other 8 *A.hydrophila* isolates, two showed resistance to two antibiotics (cefpodoxime and Amoxycillin clavulanic acid) and six showed resistance to only Amoxycillin clavulanic acid. When tested for other antibiotics, resistance to cefoxitin, Amoxycillin clavulanate, cefpodoxime, nalidixic acid was also observed.

Table 1. Aerobic Bacterial load on farmed catfish *Pangasianodon hypophthalmus* from tropical freshwater farms

Bacteriological parameters	Microbial count (log $_{10}$ cfu g^{-1})	
	Farm 1	**Farm 2**
Aerobic mesophilic bacteria 30°C	4.54±0.221*	5.36 ± 0.057
H_2S producing bacteria	2.84±0.057	3.17± 0.095
Enterobacteriaceae	3.06±0.053	3.46 ± 0.045
Pseudomonas	3.18±0.104	2.91 ± 0.131
Aeromonas	3.07±0.073	3.49 ± 0.111
Brochothrix thermosphacta	3.17±0.171	2.94 ± 0.098
E. coli	2.49±0.111	2.15 ± 0.107
S aureus	1.39±0.088	1.15 ± 0.151
Enterococci	2.4±0.013	2.76 ± 0.023

**- standard deviation*

Table 2. Dominant Bacterial flora of catfish *Pangasianodon hypophthalmus* from tropical farms

Bacterial (Genera/ species)	Percentage composition of the Aerobic flora	
	Farm 1	**Farm 2**
Pseudomonas fluorescens	5	7.5
Pseudomonas poae	2.5	5
Burkholderia cepacia	5	7.5
Burkholderaia gladioli	7.5	7.5
Stenotrophomonas maltophilia	5	5
Aeromonas sobria	7.5	10
Aeromonas caviae	7.5	7.5
Aeromonas hydrophila	5	2.5
Klebsiella pneumoniae	7.5	7.5
Acinetobacter	7.5	2.5
Psychrobacter faecalis	7.5	5
Staphylococcus kloosi	7.5	5
Staphylococcus saprophyticus	7.5	7.5
Bacillus pumilus	5	5
B. thuringiensis	NI	2.5
B. cereus	NI	2.5
B. licheniformis	10	5
Micrococcus luteus	2.5	5

NI-Not Identified

Table. 3. Resistance profile of *E. coli* strains isolated from *P. hypophthalmus*

Antibiotic group	Antibiotic	% Resistance
Penicillin	Augmentin (AMC)	85
	Ticarcillin (Ti)	25
Cephalosporin	Cefpodoxime (CPD)	85
	Ceftriaxone (CTR)	10
Quinolone and fluroquinolones	Nalidixic acid (NA)	25
	Moxifloxacin (MO)	20
	Sparfloxacin (SPX)	20
	Ciprofloxacin (CIP)	15
	Norfloxacin (NO)	10
	Ofloxacin (OF)	5
Aminoglycoside	Kanamycin (K)	10
	Streptomycin (S)	5
Folate Pathway inhibitors	Co-trimoxazole (COT)	10
Lipopeptide	Colistin (CL)	5

Table 4. Multidrug resistance profile of enterotoxigenic *E. coli* strains isolated from *P. hypophthalmus*

ETEC strains	Resistance profile	No. of Group
ETEC-1	AMC- CPD – MO/SPX- NA	4
ETEC-2	AMC- CPD- NA-COT	4
ETEC-3	AMC/Ti – CPD- CIP/Mo/SPX – NA	4
ETEC-4	AMC/Ti- CPD- S- COT	4
ETEC-5	AMC/Ti- CPD-K- CIP/OF/NO/SPX/MO - NA	5
ETEC-6	AMC- CPD- CIP/OF/NO/SPX/MO- NA	4

Among *S. aureus* isolates recovered from catfish, all the strains were resistant only to any two antibiotic group each namely penicillin, ampicillin, amoxicillin clavulanate, erythromycin, clarithromycin, cephalothin.

The presence of multidrug resistance in *Aeromonas hydrophila* and ETEC isolates in farmed catfish may also result from the use of antimicrobial agents in the fish culture or/and from their former presence in aquatic environment, since fish farms are indirectly exposed to water potentially contaminated by run-off waters from agricultural activities or by urban sewage. Exposure to these resistant bacterial strains can occur while handling farmed catfish or working in farm environments. Hence it is important to identify these environmental sources to suggest measures to prevent the transmission or spread of these strains from farmed fish and farm environment. Antibiotic resistance among commensal and pathogenic bacteria is a concern for food safety worldwide. These antibiotic resistant bacteria can transfer antibiotic resistance to other pathogenic and commensal bacteria. There has been a rapid worldwide increase in pathogenic bacteria that are resistant to multiple antibiotics. For controlling the development of AMR in the food sector and its spread shall be dealt in a multifaceted holistic approach called “one health approach” with suitable alternative to antibiotics usage across the system like human, animal, food and environment (Vaiyapuri et al., 2021, Mothadaka et al., 2023).

4. CONCLUSION

Our results demonstrate that *Aeromonas* spp. such as *A. hydrophila, A. sobria* and *A. caviae* that harbour virulence genes, are prevalent in catfish and fresh water farms, a finding that has public health implications for farm and processing workers and consumers. The study revealed presence of multidrug-resistant enterotoxigenic *E. coli* and *A. hydrophila* strains in farmed *P. hypophthalmus.* Thus, farmed catfish and catfish products may be a vehicle for spread of these virulent strains and antibiotic – resistant determinants to diverse habitats and food chain. In order to address the problem of multi-drug resistant bacteria in food environment, new antibiotics / novel antimicrobial agents have to be developed. Recently Antimicrobial peptides have gained attention as antimicrobial

alternatives to chemical food preservatives and commonly used antibiotics. Hence major research efforts need to be directed towards finding effective antimicrobial agent /drug(s) from Lactic acid bacteria/ Actinobacteria for bio-control in Pangasius farms, as food additive and as bio-preservatives to control pathogens in processed products.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENT

The authors sincerely thank the funding support provided by ICAR, India to conduct this study and also the staff of the Microbiology fermentation and Biotechnology division of CIFT in the conduct of this study

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

APHA, & WPCF. (1998). *Standard methods for the examination of water and wastewater*. Part 9000-9221: 20:48-59.

Austin, B., & Al-Zahrani, A. M. (1988). The effect of antimicrobial compounds on the gastrointestinal microflora of rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology, 33*(1), 1-4.

Austin, B., & Adams, C. (1996). Fish pathogens. In B. Austin, M. Altwegg, P. J. Gosling, & S. Joseph (Eds.), *The genus Aeromonas* (pp. 197–243). John Wiley & Sons.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotics susceptibility testing by a single disc method. *American Journal of Clinical Pathology, 45*, 493.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*(7), 1137-1144.

Cantas, L., Shah, S. Q., Cavaco, L. M., Manaia, C., Walsh, F., Popowska, M., Garelick, H., Bürgmann, H., & Sørum, H. (2013). A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. *Frontiers in Microbiology, 4*, 96.

Chakraborty, S., Deokule, J. S., Garg, P., Bhattacharya, S. K., Nandy, R. K., Nair, G. B., Yamasaki, S., Takeda, Y., & Ramamurthy, T. (2001). Concomitant infection of enterotoxigenic *Escherichia coli* in an outbreak of cholera caused by *Vibrio cholerae* O1 and O139 in Ahmedabad, India. *Journal of Clinical Microbiology, 39*(9), 3241-3246.

Clinical and Laboratory Standards Institute (CLSI). (2012). *Performance standards for antimicrobial susceptibility tests* (11th ed.). CLSI document M02-A11. Wayne, PA, USA.

CLSI. (2010). *Methods for antimicrobial dilution and disk susceptibility of infrequently isolated or fastidious bacteria* (2nd ed.). CLSI document M45-A2.

Enne, V. I., Delsol, A. A., Davis, G. R., Hayward, S. L., Roe, J. M., & Bennett, P. M. (2005). Assessment of the fitness impacts on *Escherichia coli* of acquisition of antibiotic resistance genes encoded by different types of genetic element. *Journal of Antimicrobial Chemotherapy, 56*, 544-551.

Food and Drug Administration (FDA). (1998). *FDA bacteriological analytical manual* (8th ed., Rev. A). AOAC International.

Galindo, C. L., & Chopra, A. K. (2007). *Aeromonas* and *Plesiomonas* species. In *Food Microbiology: Fundamentals and Frontiers* (3rd ed., pp. 381-400). American Society of Microbiology.

Gardner, G. A. (1985). Streptomycin-thallous acetate-actidione (STAA) agar: A medium for the selective enumeration of *Brochothrix thermosphacta*. *International Journal of Food Microbiology, 2*(1-2), 69-70.

Gram, L., Trolle, G., & Huss, H. H. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. *International Journal of Food Microbiology, 4*(1), 65-72.

Griffith, D. P., van Khanh, T. T., & Trong, T. Q. (2010). *Cultured aquatic species information programme: Pangasius hypothalamus* (Sauvage, 1878). Fisheries & Aquaculture Department, FAO.

Holmberg, S. D., Solomon, S. L., & Blake, P. A. (1987). Health and economic impacts of antimicrobial resistance. *Reviews of Infectious Diseases, 9*(6), 1065-1078.

International Commission on Microbiological Specifications for Foods (ICMSF). (2005). *Microorganisms in Foods 6* (2nd ed.). Kluwer Academic/Plenum Publishers.

Janda, J. M., & Abbott, S. L. (2010). The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews, 23*, 35–73.

Kemper, N. (2008). Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators, 8*, 1-13.

Kingombe, C. I., Huys, G., Tonolla, M., Albert, M. J., Swings, J., Peduzzi, R., & Jemmi, T. (1999). PCR detection, characterization, and distribution of virulence genes in *Aeromonas* spp. *Applied and Environmental Microbiology, 65*(12), 5293-5302.

Kong, R. Y., So, C. L., Law, W. F., & Wu, R. S. (1999). A sensitive and versatile multiplex PCR system for the rapid detection of enterotoxigenic (ETEC), enterohaemorrhagic (EHEC), and enteropathogenic (EPEC) strains of *Escherichia coli*. *Marine Pollution Bulletin, 38*(12), 1207-1215.

Krieg, N. R., & Holt, J. G. (1984). *Bergey's manual of systematic bacteriology*. Yi Hsien Publishing Co.

Lalitha, K. V., & Surendran, P. K. (2006). Microbiological changes in farm reared freshwater prawn (*Macrobrachium rosenbergii* de Man) in ice. *Food Control, 17*(10), 802-807.

Maqsood, S., & Benjakul, S. (2010). Synergistic effect of tannic acid and modified atmospheric packaging on the prevention of lipid oxidation and quality losses of refrigerated striped catfish slices. *Food Chemistry, 121*(1), 29-38.

Mead, G. C. (1985). Enumeration of pseudomonads using cephaloridine-fucidin-cetrimide agar (CFC). *International Journal of Food Microbiology, 2*(1-2), 21-26.

Miranda, C. D., Tello, A., & Keen, P. L. (2013). Mechanisms of antimicrobial resistance in finfish aquaculture environments. *Frontiers in Microbiology, 4*, 233.

Moli, G., & Ternstrom, A. (1982). Numerical taxonomy of psychrotrophic pseudomonads. *Journal of General Microbiology, 128*, 1249-1264.

Msangi, S., Kobayashi, M., Batka, M., Vannuccini, S., Dey, M. M., & Anderson, J. L. (2013). *Fish to 2030: Prospects for fisheries and aquaculture*. World Bank Report, 83177(1), 102.

Mukhopadhyay, C., Chawla, K., Sharma, Y., & Bairy, I. (2008). Emerging extra-intestinal infections with *Aeromonas hydrophila* in coastal region of southern Karnataka. *Journal of Postgraduate Medicine, 54*, 199-202.

Murugadas, V., Joseph, T. C., & Lalitha, K. V. (2016). Distribution of pathotypes of *Escherichia coli* in seafood from retail markets of Kerala. *Indian Journal of Fisheries, 63*(1), 152-155.

Palu, A. P., Gomes, L. M., Miguela, M. A. L., Balassiano, I. T., Queiroz, M. L. P., Almeida, A. C. F., & de Oliveira, S. S. (2006). Antimicrobial resistance in food and clinical *Aeromonas* isolates. *Food Microbiology, 23*, 504–509.

Palumbo, S. A., Maxino, F., Williams, A. C., Buchanan, R. L., & Thayer, D. W. (1985). Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. *Applied and Environmental Microbiology, 50*(4), 1027-1030.

Petersen, A., Andersen, J. S., Kaewmak, T., Somsiri, T., & Dalsgaard, A. (2002). Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Applied and Environmental Microbiology, 68*(12), 6036-6042.

Phani Kumar, M., & Sree Ramulu, K. (2013). Percentage composition of various species of *Aeromonas* in different organs of *Pangasius hypophthalmus* in culture ponds of Kaikaluru and Mudinepalli mandals in Krishna districts of Andhra Pradesh. *International Journal of Research in Fisheries and Aquaculture, 3*, 30-33.

Piotrowska, M., & Popowska, M. (2014). The prevalence of antibiotic resistance genes among *Aeromonas* species in aquatic environments. *Annals of Microbiology, 64*(3), 921-934.

Rhodes, G., Huys, G., Swings, J., Mcgann, P., Hiney, M., Smith, P., & Pickup, R. W. (2000). Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn1721 in dissemination of the tetracycline resistance determinant Tet A. *Applied and Environmental Microbiology, 66*(9), 3883-3890.

Roberts, R. J. (1997). Epizootic ulcerative syndrome (EUS): Progress since 1985. In T. W. Flegel & I. H. MacRae (Eds.),

Diseases in Asian Aquaculture III (pp. 125–128). Asian Fisheries Society.

Sáenz, Y., Brinas, L., Domínguez, E., Ruiz, J., Zarazaga, M., Vila, J., & Torres, C. (2004). Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrobial Agents and Chemotherapy, 48*(10), 3996-4001.

Schmidt, A. S., Bruun, M. S., Dalsgaard, I., & Larsen, J. L. (2001). Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. *Applied and Environmental Microbiology, 67*(12), 5675-5682.

Sha, J., Kozlova, E. V., & Chopra, A. K. (2002). Role of various enterotoxins in *Aeromonas hydrophila*-induced gastroenteritis: Generation of enterotoxin gene-deficient mutants and evaluation of their enterotoxic activity. *Infection and Immunity, 70*(4), 1924-1935.

Shah, S. Q., Colquhoun, D. J., Nikuli, H. L., & Sørum, H. (2012). Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. *Environmental Science & Technology, 46*(16), 8672-8679.

Shigematsu, T., Hayashi, M., Kikuchi, I., Ueno, S., Masaki, H., & Fujii, T. (2009). A culture-dependent bacterial community structure analysis based on liquid cultivation and its application to a marine environment. *FEMS Microbiology Letters, 293*(2), 240-247.

Sinha, S., Shimada, T., Ramamurthy, T., Bhattacharya, S. K., Yamasaki, S., Takeda, Y., & Nair, G. B. (2004). Prevalence, serotype distribution, antibiotic susceptibility, and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. *Journal of Medical Microbiology, 53*(6), 527-534.

Smith, P., Hiney, M. P., & Samuelsen, O. B. (1994). Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. *Annual Review of Fish Diseases, 4*, 273-313.

Sneath, P. H., Mair, N. S., Sharpe, M. E., & Holt, J. G. (1986). *Bergey's manual of systematic bacteriology* (Vol. 2). The Williams and Wilkins Co.

Sørum, H. (2006). Antimicrobial drug resistance in fish pathogens. In F. M. Aarestrup (Ed.), *Antimicrobial Resistance in Bacteria of Animal Origin* (pp. 213-238). American Society for Microbiology.

Wang, G., Clark, C. G., Liu, C., Pucknell, C., Munro, C. K., Kruk, T. M., Caldeira, R., Woodward, D. L., & Rodgers, F. G. (2003). Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. *Journal of Clinical Microbiology, 41*(3), 1048-1054.

Witte, W. (1998). Medical consequences of antibiotic use in agriculture. *Science, 279*, 996-997.

Wong, C. Y., Heuzenroeder, M. W., & Flower, R. L. (1998). Inactivation of two haemolytic toxin genes in *Aeromonas hydrophila* attenuates virulence in a suckling mouse model. *Microbiology, 144*(2), 291-298.

Zhang, R., Eggleston, K., Rotimi, V., & Zeckhauser, R. J. (2006). Antibiotic resistance as a global threat: Evidence from China, Kuwait, and the United States. *Globalization and Health, 2*(1), 6.

Milijasevic, M., Veskovic-Moracanin, S., Babic Milijasevic, J., Petrovic, J., Nastasijevic, I. (2024). Antimicrobial resistance in aquaculture: Risk mitigation within the One Health context. *Foods, 13*, 2448. https://doi.org/10.3390/foods13152448.

Santos, L., & Ramos, F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents, 52*(2), 135–143. https://doi.org/10.1016/j.ijantimicag.2018.03.010.

Suyamud, B., Chen, Y., Quyen, D. T. T., Dong, Z., Zhao, C., & Hu, J. (2024). Antimicrobial resistance in aquaculture: Occurrence and strategies in Southeast Asia. *The Science of the Total Environment, 907*, 167942. https://doi.org/10.1016/j.scitotenv.2023.167942.

Vaiyapuri, M., Sebastian, A. S., George, I., Variem, S. S., Vasudevan, R. N., George, J. C., ... & Mothadaka, M. P. (2021). Predominance of genetically diverse ESBL *Escherichia coli* identified in resistance mapping of Vembanad Lake, the largest fresh-cum-brackishwater lake of India. *Environmental Science and Pollution Research, 28*, 66206-66222.

Vaiyapuri, M., Pailla, S., Rao Badireddy, M., Pillai, D., Chandragiri Nagarajarao, R., &

Prasad Mothadaka, M. (2021). Antimicrobial resistance in Vibrios of shrimp aquaculture: Incidence, identification schemes, drivers, and mitigation measures. *Aquaculture Research, 52*(7), 2923-2941.

Mothadaka, M. P., Vaiyapuri, M., Rao Badireddy, M., Nagarajrao Ravishankar, C., Bhatia, R., & Jena, J. (2023). *Handbook on antimicrobial resistance*. Springer Singapore. https://doi.org/10.1007/978-981-19-9279-7.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/125687