

Original Research Article

Characterization of enteric pathogens and aerobic bacterial flora associated with catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) from tropical freshwater farms

Abstract

BACKGROUND: Recently *Pangasianodon hypophthalmus* has become an emerging fish species cultured in India. Understanding bacterial flora association in the fish meant for food consumption is a precursory to various processing, in the Indian context the study has not been carried out.

OBJECTIVE: Determination of aerobic bacterial flora and antimicrobial resistance in enteric pathogens associated with the farm raised catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) species cultured in freshwater ponds in Andhra Pradesh, India.

METHODS: Enumeration of aerobic plate count, Pseudomonads, Aeromonas, Enterobacteriaceae and Staphylococcus aureus were determined as per standard methods of examination of food. Bacterial identification was carried out with analytical profile index 20E (APE20E) and 16s rDNA sequencing analysis. Antimicrobial resistance in *E. coli*, *S. aureus* and *Aeromonas* sp were carried out by disk diffusion assay.

RESULTS: The aerobic bacterial flora on catfish muscle tissue comprised of H₂S producing bacteria, Enterobacteriaceae, *Pseudomonas*, *Aeromonas* and *B. thermosphacta*. The dominant genera were identified as *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, *Aeromonas*, *Klebsiella* and *Psychrobacter*. Among enteric pathogens, multidrug-resistant strains of enterotoxigenic *E. coli* and *Aeromonas hydrophila* were recovered from farmed catfish. The prevalence of multidrug resistance in *A. hydrophila*, the etiological agent of red disease that is widely occurring in farmed catfish in Andhra Pradesh (India) is a potential concern.

CONCLUSION: The study indicates the microbiological risk posed by the presence of *Aeromonas* spp. and enterotoxigenic *E. coli* in the farmed catfish species in fresh water ponds. This may act as a vehicle for spread of pathogenic and antibiotic resistant determinants to

diverse habitats and food chain. Efforts are needed in finding effective biopreservatives to control pathogens in processed *Pangasius* products.

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

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.¹ Aquaculture of this fast growing fish is gaining momentum in India and recent production amounts for 18,000-20,000 m.t.² 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 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*.³ The increase in incidence of diseases has led to increased use of antimicrobials/ antibiotics in fish farms⁴ 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 bacteria⁵. Bacterial resistance to antibiotics has been widely spread in aquaculture environments, which has been reported in many studies.⁶⁻¹⁰ Antimicrobial resistance has long been reported widespread in *Aeromonas* species in aquacultures, and recently an upward trend in the resistance has been observed.¹¹⁻¹³ These resistant bacteria may be transferred to humans either through the food supply or by direct contact with animals.^{14, 15}

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.^{7, 11}

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.

MATERIALS AND METHODS

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.

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.¹⁶ 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.¹⁷ *Aeromonas* spp. were enumerated on Starch Ampicillin Agar at 30°C for 2 days and amylase positive yellow to honey coloured colonies were counted.¹⁸

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 H₂S -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 H₂S were enumerated after 2–3 days.¹⁹

Escherichia coli enumeration was performed in Tergitol-7 Agar (T-7 Agar, Himedia M616) was used, incubated at 37°C 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.^{20, 21} *Brochothrix thermosphacta* was determined on Streptomycin sulfate-Thallos acetate – Actidione Agar (STAA, HiMedia, India) after incubation at 20°C for 4 days.²²

Staphylococcus aureus counts were estimated on Baird Parker Agar (BP, Oxoid) incubated at 37°C for 2 days and typical colonies were confirmed by coagulase test.²³ Percentages of confirmed colonies were used to correct the results of the counts obtained. Enterococci numbers were estimated on KF Agar (Difco) incubated at 37°C for 2 days. Five typical *Enterococcus* colonies were identified by checking growth at 45± 1°C and growth in Dextrose Azide Broth containing 6.5% sodium chloride incubated at 35 ± 2°C and confirmed by biochemical tests.²⁰

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.

Bacterial identification using 16S rRNA sequencing analysis

Bacterial isolates (18 nos) were subcultured 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'.²⁴ 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.

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.^{19, 25-28} 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 cytotoxic enterotoxin (*ast*, *alt*) genes.^{29, 30}

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).^{21, 31}

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.³² 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.³³ For quality control, Mueller Hinton agar was tested with *Escherichia coli* ATCC 25922.

RESULTS AND DISCUSSION

Bacterial flora of farmed catfish

The aerobic bacterial flora comprised of H₂S producing bacteria, Enterobacteriaceae *Pseudomonas*, *Aeromonas* and *B. thermosphacta* (Table 1). The levels indicator bacteria such as *E. coli* and Enterococci were in the range of 2.1- 2.7 log₁₀ cfu g⁻¹ and *S. aureus* counts were

$\leq 2.0 \log_{10} \text{ cfu g}^{-1}$. The total aerobic bacterial counts were in the range of $10^4 - 10^5 \text{ cfu/g}$. Total viable count of $<10^4 \text{ cfu g}^{-1}$ in *P. hypophthalmus* from Bangladesh farms.³⁵ *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.³⁶ High levels of faecal coliforms and Enterococci were previously recorded from freshwater farms and prawns in India.³⁷

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 H₂S 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*.

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 diarrhoea (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.^{38, 39} The first outbreak of ETEC mediated diarrhoea in India was reported from Ahmedabad, Gujarat and majority of the strains expressed multidrug resistance.⁴⁰ The antimicrobial resistance has emerged and evolved in many bacterial genera due to the excessive

use of antimicrobials in human and aquaculture systems.⁴ Most enteric pathogens easily share genes for antimicrobial resistance.⁷ It has been demonstrated that once acquired by *E. coli* the resistance genes are not rapidly lost.⁴¹

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 cytotoxic enterotoxins, *alt* and *ast* have all been implicated as important virulence factors in diarrhoeal disease.^{42, 43} *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).⁴⁴ *Aeromonas* is also recognized as aetiological agents in extra-intestinal infections such as septicaemia, wound infections, cellulitis.⁴⁵ 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.⁴⁶ The increasing levels of resistance displayed by both clinical and environmental *Aeromonas* spp. isolates⁴⁷ and also by isolates from aquaculture poses a growing challenge in the treatment of *Aeromonas* infections in fish as well as in humans.⁴⁸ *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.^{49, 50} *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.³ 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, Amoxicillin clavulanic acid, Co-trimoxazole and Gentamycin) was found in two strains.³⁴ Among the other 8 *A. hydrophila* isolates, two showed resistance to two

antibiotics (cefepodoxime and Amoxicillin clavulanic acid) and six showed resistance to only Amoxicillin clavulanic acid. When tested for other antibiotics, resistance to ceftiofur, Amoxicillin clavulanate, cefepodoxime, nalidixic acid was also observed.

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.

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 biopreservatives to control pathogens in processed products.

ETHICS STATEMENT

The study does not involve any animals.

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

Bacteriological parameters	Microbial count (log ₁₀ cfu g ⁻¹)	
	Farm1	Farm2
Aerobic mesophilic bacteria 30°C	4.54±0.221*	5.36 ± 0.057
H ₂ S producing bacteria	2.84±0.057	3.17± 0.095
Enterobacteriaceae	3.06±0.053	3.46 ± 0.045
<i>Pseudomonas</i>	3.18±0.104	2.91 ± 0.131
<i>Aeromonas</i>	3.07±0.073	3.49 ± 0.111
<i>Brochothrix thermosphacta</i>	3.17±0.171	2.94 ± 0.098
<i>E. coli</i>	2.49±0.111	2.15 ± 0.107
<i>S aureus</i>	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
<i>Pseudomonas fluorescens</i>	5	7.5
<i>Pseudomonas poae</i>	2.5	5
<i>Burkholderia cepacia</i>	5	7.5
<i>Burkholderia gladioli</i>	7.5	7.5
<i>Stenotrophomonas maltophilia</i>	5	5
<i>Aeromonas sobria</i>	7.5	10
<i>Aeromonas caviae</i>	7.5	7.5
<i>Aeromonas hydrophila</i>	5	2.5
<i>Klebsiella pneumoniae</i>	7.5	7.5

<i>Acinetobacter</i>	7.5	2.5
<i>Psychrobacter faecalis</i>	7.5	5
<i>Staphylococcus kloosi</i>	7.5	5
<i>Staphylococcus saprophyticus</i>	7.5	7.5
<i>Bacillus pumilus</i>	5	5
<i>B. thuringiensis</i>	NI	2.5
<i>B. cereus</i>	NI	2.5
<i>B. licheniformis</i>	10	5
<i>Micrococcus luteus</i>	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 fluoroquinolones	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