

ISOLATION AND CHARACTERIZATION OF SELECTED GUT BACTERIAL FAUNA OF GOLDEN MAHSEER (TOR PUTITORA)

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Abstract

Background: The golden mahseer (*Tor putitora*) is an endangered fish of the Himalayan rivers and the national fish of Pakistan. It is now bred in culture facilities so that young fish can be released back into rivers. Very little is known about the bacteria that live in its gut. Some of these bacteria are harmless in a healthy fish but cause disease when the fish is under stress, so it is useful to know which species are present and which antibiotics still work against them.

Methods: Nine apparently healthy golden mahseer samples were collected in Attock District, Punjab, between July and November 2025. Five were obtained from the Mahseer Fish Hatchery at Garyala and four were caught from tributaries of the River Indus. The gut of each fish was removed under sterile conditions and homogenized in normal saline. The homogenate was diluted to 10^{-5} and spread on MacConkey agar and blood agar, then incubated at 37 °C for 24 to 48 hours. Pure isolates were studied for colony appearance and Gram staining and were identified with the catalase test, oxidase test, sugar fermentation test, urease test, indole test and citrate test. Identifications were confirmed with the API 20E kit. An Antibiotic Sensitivity Test (AST) was carried out by the Kirby-Bauer disc diffusion method using six antibiotics.

Results: Seventeen isolates were obtained, eight from cultured fish and nine from wild fish. All were Gram negative rods. Three species were found: *Aeromonas hydrophila* (9 of 17, 52.9%), *Klebsiella pneumoniae* (7 of 17, 41.2%) and *Pseudomonas* sp. (1 of 17, 5.9%). In cultured fish *A. hydrophila* was the most common at 62.5%, followed by *K. pneumoniae* at 37.5%. In wild fish, *A. hydrophila* and *K. pneumoniae* were equally common at 44.44% each and *Pseudomonas* sp. made up the remaining 11.11%. The AST showed that all 17 isolates were resistant to erythromycin, penicillin and ampicillin. Three isolates (17.6%) were also resistant to doxycycline. All 17 were sensitive to ceftazidime and cefepime.

Conclusion: Healthy wild and cultured golden mahseer carry gut bacteria

that can turn harmful under stress, and these bacteria are already resistant to several common antibiotics. Ceftazidime and cefepime still worked against every isolate. Regular bacterial checks, good water quality and careful use of antibiotics are needed in the culture facilities that breed this endangered fish.

INTRODUCTION

Fish is a cheap source of good quality protein and matters a great deal for food security in many countries. Aquaculture has grown quickly over the last few decades because wild catches can no longer meet demand. That growth has brought problems with it. Crowding, poor water quality and heavy use of antibiotics have all made disease more common on fish farms (FAO, 2024; Naylor et al., 2021). Freshwater fish farming is expanding in Pakistan, but there is still little information on the bacteria that farmed fish carry, and this makes disease management harder than it needs to be.

The golden mahseer (*Tor putitora*) lives in the fast flowing rivers of the Himalayan region and is the national fish of Pakistan. It grows to a large size, travels long distances upstream to breed and feeds on insects, plants and small aquatic animals. Its numbers have fallen steadily. Dams and barrages block the breeding migrations, overfishing removes the large breeding adults, and farm and factory waste has damaged the habitat (Bhatt and Pandit, 2016). Because of this decline the species is now bred in culture facilities and the young fish are released into rivers.

The gut of a fish holds a large and mixed population of bacteria. These bacteria help digest food, produce vitamins and enzymes, and take up space and nutrients that harmful bacteria would otherwise use (Nayak, 2010; Butt and Volkoff, 2019). What lives there depends on what the fish eats, on the quality of the water and on whether the fish lives in a river or in a culture system (Sullam et al., 2012; Ghanbari et al., 2015). Wild fish take a mixed natural diet and meet many bacteria in their surroundings. Cultured fish are given a fixed feed and kept in a controlled water

supply, so their gut bacteria are often different (Tan et al., 2019).

Some gut bacteria are harmless while the fish is healthy but cause disease once it is stressed (Austin and Austin, 2016). *Aeromonas hydrophila* is the best known of these. It causes motile aeromonas septicaemia, yet it is also found in the gut of normal freshwater fish (Janda and Abbott, 2010; Wu et al., 2013). Kumar et al. (2016) infected golden mahseer with this bacterium and produced fin haemorrhage together with a rise in cortisol. *Klebsiella pneumoniae* has been isolated from diseased Indian major carp (Das et al., 2018), and *Pseudomonas koreensis* was recovered from eye lesions of golden mahseer (Shahi and Mallik, 2014).

Antibiotic resistance makes such bacteria harder to deal with. Resistance in fish farm bacteria follows the way antibiotics are used and is expected to increase as water temperatures rise (Reverter et al., 2020; Ferri et al., 2022). Khurana et al. (2020) studied the gut of *Tor putitora* by shotgun metagenomic sequencing. Proteobacteria dominated the community, with *Aeromonas*, *Klebsiella* and *Escherichia* all present, and the same work detected antibiotic resistance genes and virulence genes in these bacteria.

Even so, there is almost no culture based work on the gut bacteria of *Tor putitora* in Pakistan, and no study that compares wild and cultured fish from the same area. Culture methods are still worth using because they give living isolates that can be tested against antibiotics and kept for later work (Hossain et al., 2022). This study was carried out to isolate gut bacteria from healthy wild and cultured golden mahseer in Attock, to identify them by morphological and biochemical characterization with API 20E confirmation, and to test their sensitivity to six antibiotics.

Materials And Methods

Study area and sample collection

The work was done at the Aquaculture and Fisheries Laboratory, Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi. Fish were collected between July and November 2025. Wild golden mahseer were caught from tributaries of the River Indus in the Attock region, and cultured fish were obtained from the Mahseer Fish Hatchery, Garyala, Attock. Nine fish were collected in all, four wild and five cultured. All of them looked healthy, with no ulcers, fin erosion or abnormal swimming. Live fish were caught with a cast net. Before dissection the total length of each fish was measured in centimeters with a ruler and the body weight was recorded in grams on a sensitive balance.

Dissection of fish gut

Each fish was washed with sterile distilled water to remove sand, dirt and the bacteria sticking to the body surface, and was then wiped with ethanol swabs. The gut was taken out with a sterile surgical blade. Gut samples were carried to the laboratory in an ice box so that they would not spoil on the way.

Serial dilution of gut homogenate

Sterile conical tubes were arranged in a rack, five for the cultured samples and four for the wild samples, each holding 9 ml of sterile normal saline. From each gut 1 g of intestine was cut out, washed two to three times with sterile normal saline, put into 9 ml of sterile normal saline and vortexed for 1 minute. This suspension was then diluted up to 10^{-5} . One millilitre was taken from the first tube into the second and vortexed and the same step was repeated up to the fifth tube.

Isolation and purification of bacteria

From each dilution 0.1 ml was spread on MacConkey agar and blood agar plates. The plates were incubated at 37 °C for 24 to 48 hours. Colonies that differed from one another were streaked again on fresh plates and this was repeated until pure cultures were obtained.

Morphological characterization

The size, shape, colour, margin and elevation of the colonies were recorded for every pure isolate and haemolysis was noted on blood agar.

Biochemical tests

Isolates were identified using the standard biochemical tests described by Cappuccino and Sherman (2017) and MacFaddin (2000).

Gram staining

A colony was picked from the plate and a thin smear was made on a glass slide and heat fixed. The smear was flooded with crystal violet for 1 minute and washed with water. Iodine solution was added for 1 minute and rinsed off. The smear was decolorized with ethanol for 20 seconds, washed again, and counterstained with safranin for 1 minute. The slide was dried and examined under a light microscope.

Catalase test

A small part of a colony was placed on a clean glass slide and one or two drops of hydrogen peroxide were added to it. Bubbles were recorded as a positive result and no change as a negative result.

Oxidase test

Oxidase reagent was placed on a clean filter paper and a colony was rubbed onto it. A dark purple or blue colour was recorded as positive and no colour change as negative.

Sugar fermentation test

Fermentation of glucose, lactose, sucrose and mannitol was checked in phenol red sugar broth containing inverted Durham tubes. A small part of a colony was added with a sterile loop. A yellow broth with gas collected in the Durham tube was recorded as positive, while a red or pink colour was recorded as negative.

Urease test

A colony was inoculated into urea broth and incubated at 37 °C for 24 to 48 hours. A pink

medium was recorded as positive and a yellow or orange medium as negative.

Indole test

A colony was transferred into tryptone broth with a sterile loop and incubated for 24 to 48 hours. Kovac's reagent was then added. A red or pink ring at the top of the broth was recorded as positive and no ring as negative.

Citrate test

A colony was streaked on a Simmon's citrate agar slant and incubated at 37 °C for 24 to 48 hours. A dark blue medium was recorded as positive and a green medium as negative.

API 20E

Isolates were confirmed with the API 20E kit (bioMérieux, France) after the morphological and biochemical work was complete. A pure colony was suspended in sterile saline and this suspension was used to fill the microtubes of the strip, each of which holds a dried substrate. The strips were placed in incubation trays and kept at 37 °C for 18 to 24 hours. Colour changes in the tubes showed which reactions were positive and which were negative, and a few tests needed reagents to be added after incubation. Each reaction was recorded and the pattern was converted into a numerical profile to find the most likely species (MacFaddin, 2000).

Antibiotic Sensitivity Test (AST)

The AST was carried out by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. A 24 hour broth culture of each isolate was spread on the plate and antibiotic discs were placed on the surface. Six antibiotics were used: erythromycin (15 µg), doxycycline (30 µg), penicillin (10 µg), ampicillin (10 µg), ceftazidime (30 µg) and cefepime (30 µg). The plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured and each isolate was recorded as sensitive or resistant.

Data handling

Results are given as counts and percentages. The share of each species is the percentage of the

isolates obtained from that group of fish. AST results are given as the number and percentage of isolates that were sensitive or resistant to each antibiotic. Only nine fish were sampled, so no statistical test was applied between the two groups.

RESULTS

Characterization of microbes with Gram staining

Seventeen isolates were obtained in pure culture, eight from the five cultured fish and nine from the four wild fish. On MacConkey agar the colonies were either pink or pale, and on blood agar they were grey, creamy, pale or clear. All 17 isolates were Gram negative rods and appeared pink to red under the microscope. Gram negative bacteria have a thin peptidoglycan layer and an outer membrane rich in lipid, so the ethanol step washes out the crystal violet and iodine complex and the cells then take up the safranin counterstain (Cappuccino and Sherman, 2017). No Gram positive bacteria were recovered.

Biochemical characterization of isolates from cultured fish

Eight isolates were obtained from cultured fish and two species were identified among them. The full results are given in Table 1.

Aeromonas hydrophila made up 62.5% of the cultured isolates. It gave pale colonies on MacConkey agar and grey haemolytic colonies on blood agar, and the cells were Gram negative rods. It was positive for the catalase test, oxidase test, indole test and citrate test. It fermented glucose, mannitol and sucrose but not lactose, and the urease test was negative.

Klebsiella pneumoniae made up the remaining 37.5%. It gave pink colonies on MacConkey agar and creamy non-haemolytic colonies on blood agar, and the cells were Gram negative rods. It was positive for the catalase test, urease test and citrate test and fermented all four sugars tested, including lactose. The oxidase test and the indole test were negative.

Biochemical characterization of isolates from wild fish

Nine isolates were obtained from wild fish and three species were identified among them. The full results are given in Table 2.

Aeromonas hydrophila accounted for 44.44% of the wild isolates and gave the same reactions as the cultured isolates of this species. The colonies were pale on MacConkey agar and grey and haemolytic on blood agar, and the cells were Gram negative rods. The catalase test, oxidase test, indole test and citrate test were positive. Glucose, mannitol and sucrose were fermented but lactose was not, and the urease test was negative.

Klebsiella pneumoniae also accounted for 44.44% of the wild isolates. It produced pink colonies on MacConkey agar and creamy non-haemolytic colonies on blood agar. The catalase test, urease test and citrate test were positive and all four sugars were fermented, while the oxidase test and indole test were negative.

Pseudomonas sp. made up the remaining 11.11% and was found only in wild fish. It gave pale colonies on MacConkey agar and clear haemolytic colonies on blood agar, and the cells were Gram negative rods. The catalase test, oxidase test, urease test and citrate test were positive. The indole test was negative and none of the four sugars was fermented, which set this species apart from the other two.

Table 1:- Biochemical characterization of gut bacterial isolates from cultured golden mahseer (*Tor putitora*).

Characteristic feature	1	2	3	4	5	6	7	8
Colony appearance on MacConkey agar	Pale	Pink	Pale	Pale	Pink	Pale	Pink	Pale
Colony appearance on blood agar	Grey	Creamy	Grey	Grey	Creamy	Grey	Creamy	Grey
Haemolysis	H	NH	H	H	NH	H	NH	H
Gram stain	-	-	-	-	-	-	-	-
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	+	-	+	+	-	+	-	+
Glucose test	+	+	+	+	+	+	+	+
Mannitol test	+	+	+	+	+	+	+	+
Sucrose test	+	+	+	+	+	+	+	+
Lactose test	-	+	-	-	+	-	+	-
Citrate test	+	+	+	+	+	+	+	+
Indole test	+	-	+	+	-	+	-	+
Urease test	-	+	-	-	+	-	+	-

Characteristic feature	1	2	3	4	5	6	7	8
Organism identified as	<i>Aeromonas hydrophila</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas hydrophila</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>

(+) Positive; (-) Negative; H = Haemolytic; NH = Non-haemolytic. All the reactions shown agree with the API 20E results given in the text.

Table 2:- Biochemical characterization of gut bacterial isolates from wild golden mahseer (*Tor putitora*).

Characteristic feature	9	10	11	12	13	14	15	16	17
Colony appearance on MacConkey agar	Pink	Pale	Pink	Pale	Pale	Pink	Pale	Pink	Pale
Colony appearance on blood agar	Creamy	Grey	Creamy	Grey	Clear	Creamy	Grey	Pale	Grey
Haemolysis	NH	H	NH	H	H	NH	H	NH	H
Gram stain	-	-	-	-	-	-	-	-	-
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase test	+	+	+	+	+	+	+	+	+
Oxidase test	-	+	-	+	+	-	+	-	+
Glucose test	+	+	+	+	-	+	+	+	+
Mannitol test	+	+	+	+	-	+	+	+	+
Sucrose test	+	+	+	+	-	+	+	+	+
Lactose test	+	-	+	-	-	+	-	+	-
Citrate test	+	+	+	+	+	+	+	+	+
Indole test	-	+	-	+	-	-	+	-	+
Urease test	+	-	+	-	+	+	-	+	-
Organism identified as	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Pseudomonas</i> sp.	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>

(+) Positive; (-) Negative; H = Haemolytic; NH = Non-haemolytic; Clear = no pigmentation on blood agar. All the reactions shown agree with the API 20E results given in the text.

Biochemical characterization through API 20E

The identifications made from the conventional tests were confirmed with the API 20E kit. All the isolates of one species gave the same reactions on the strip.

Aeromonas hydrophila was positive for beta-galactosidase (ONPG), arginine dihydrolase (ADH), lysine decarboxylase (LDC), citrate utilization (CIT), indole production (IND), gelatinase (GEL), glucose (GLU), mannitol (MAN), sucrose (SAC), melibiose (MEL), amygdalin (AMY) and oxidase (OX). It was negative for ornithine decarboxylase (ODC), hydrogen sulphide production (H₂S), urease (URE), tryptophan deaminase (TDA), Voges-Proskauer (VP), inositol (INO), sorbitol (SOR), rhamnose (RHA) and arabinose (ARA).

Klebsiella pneumoniae was positive for Onpg, Ldc, Cit, Ure, Vp, Glu, Man, Ino, Sor, Rha, Sac, Mel, Amy And Ara, And Negative For Adh, Odc, H₂S, Tda, Ind, Gel And Ox.

Pseudomonas sp. was positive for ADH, CIT, URE, GEL and OX only. It was negative for every other test on the strip, including GLU and SAC, which agrees with its conventional results because it fermented none of the sugars.

The API 20E reactions matched the conventional biochemical results for all three species. ONPG was positive in *A. hydrophila* although lactose was not fermented on conventional media within 48 hours, which is the usual pattern for this species because it ferments lactose slowly.

Percentage distribution of bacterial isolates

Of the 17 isolates, 9 (52.9%) were *A. hydrophila*, 7 (41.2%) were *K. pneumoniae* and 1 (5.9%) was *Pseudomonas* sp. Figure 1 compares the two groups of fish. The cultured fish were dominated by one species, *A. hydrophila*, at 62.5% of their isolates. The wild fish carried *A. hydrophila* and *K. pneumoniae* in equal numbers and also gave the only *Pseudomonas* sp. isolate, so their bacteria were more evenly spread across three species.

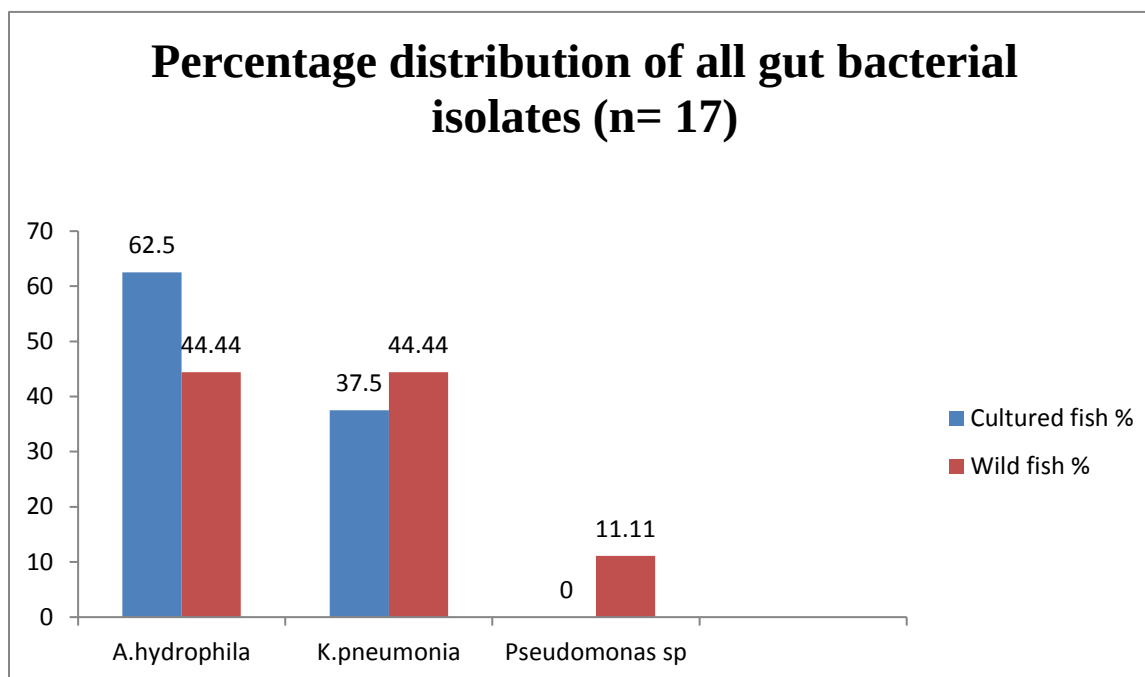


Figure 1:- Percentage distribution of bacterial isolates from cultured and wild golden mahseer (*Tor putitora*).

Antibiotic Sensitivity Test (AST)

Tables 3 and 4 give the AST results for the cultured and wild isolates, and Figure 2 shows the overall picture for all 17. Every isolate was resistant to erythromycin, penicillin and ampicillin. Three isolates were resistant to doxycycline as well, and all three came from wild fish: the single *Pseudomonas* sp. isolate and two of

the four wild *K. pneumoniae* isolates. The other 14 isolates (82.4%) were sensitive to doxycycline, including every isolate from cultured fish. All 17 isolates were sensitive to ceftazidime and cefepime. Each isolate in the study was therefore resistant to at least three of the six antibiotics tested.

Table 3:- Antibiotic Sensitivity Test (AST) for cultured bacterial isolates.

S. No.	Antibiotic	Disc concentration (µg)	1	2	3	4	5	6	7	8
1	Erythromycin	15	R	R	R	R	R	R	R	R
2	Doxycycline	30	S	S	S	S	S	S	S	S
3	Penicillin	10	R	R	R	R	R	R	R	R
4	Ampicillin	10	R	R	R	R	R	R	R	R
5	Ceftazidime	30	S	S	S	S	S	S	S	S
6	Cefepime	30	S	S	S	S	S	S	S	S

(S) Sensitive; (R) Resistant. Isolates 1 to 8 were recovered from cultured fish.

Table 4:- Antibiotic Sensitivity Test (AST) for wild bacterial isolates.

S. No.	Antibiotic	Disc concentration (µg)	9	10	11	12	13	14	15	16	17
1	Erythromycin	15	R	R	R	R	R	R	R	R	R
2	Doxycycline	30	S	S	S	S	R	R	S	R	S
3	Penicillin	10	R	R	R	R	R	R	R	R	R
4	Ampicillin	10	R	R	R	R	R	R	R	R	R
5	Ceftazidime	30	S	S	S	S	S	S	S	S	S
6	Cefepime	30	S	S	S	S	S	S	S	S	S

(S) Sensitive; (R) Resistant. Isolates 9 to 17 were recovered from wild fish.

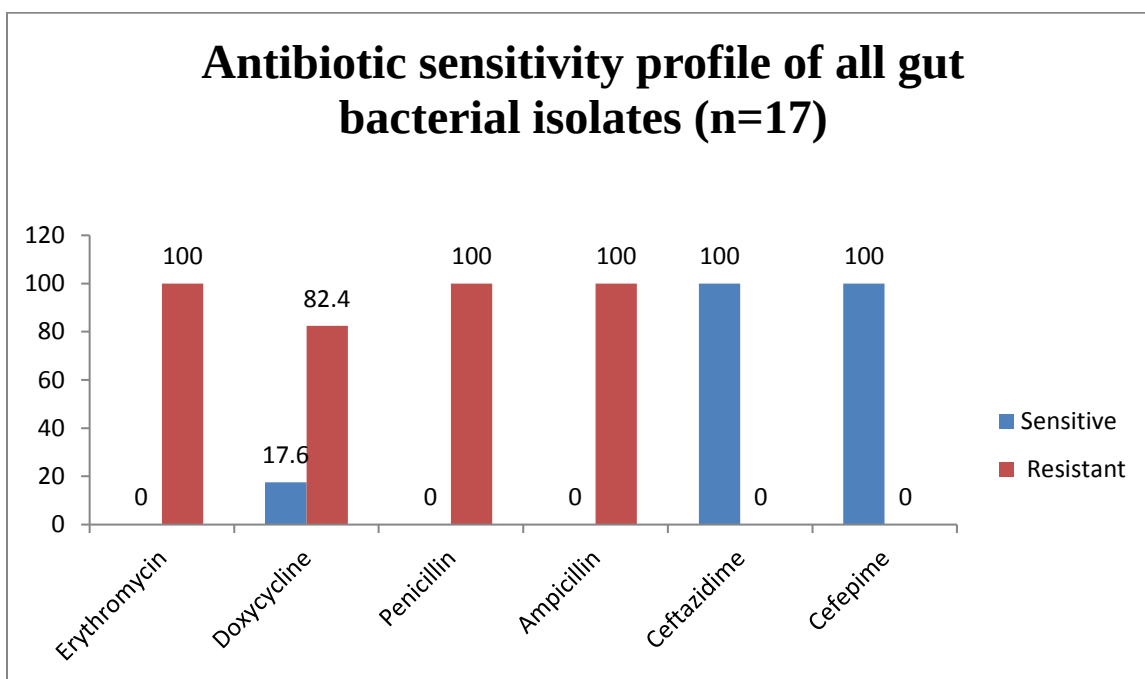


Figure 2:- Antibiotic sensitivity profile of gut bacterial isolates of golden mahseer (*Tor putitora*) (n = 17).

DISCUSSION

All the fish in this study looked healthy, yet every one of them carried bacteria that are able to cause disease. *A. hydrophila* was the most common isolate in both groups, at 62.5% in cultured fish and 44.44% in wild fish. This is not a strange result. *A. hydrophila* is not a bacterium that appears only in sick fish. It lives in the gut of

normal freshwater fish and turns harmful when the host is weakened by stress (Janda and Abbott, 2010; Fernández-Bravo and Figueras, 2020; Semwal et al., 2023). It can certainly cause disease in this host. Kumar et al. (2016) infected golden mahseer with *A. hydrophila* and saw fin haemorrhage along with a rise in cortisol and antioxidant enzyme activity. Khurana et al. (2020)

later showed that *Aeromonas* is the main genus in the gut of *Tor putitora* and that the bacteria there carry virulence genes, including a complete type III secretion system operon. The same thing is seen in other cyprinids. Wu et al. (2013) described *Aeromonas* as a normal part of the grass carp gut, and Mohammed et al. (2023) found *Aeromonas sobria* to be the commonest isolate in farmed common carp in Iraq.

K. pneumoniae came second in cultured fish at 37.5% and was as common as *A. hydrophila* in wild fish at 44.44%. It is usually thought of as a human pathogen, but it has also been isolated from diseased *Labeo rohita* in West Bengal, where the strains carried a high multiple antibiotic resistance index (Das et al., 2018; Das et al., 2019). Khurana et al. (2020) detected *Klebsiella* in the gut of *Tor putitora* as well. Finding it here in healthy fish from both sources suggests that it belongs to the normal gut fauna of this species rather than being a chance contaminant. It also means that people who handle these fish may be exposed to a bacterium that can infect humans.

Pseudomonas sp. was found only in wild fish, where it made up 11.11% of the isolates, and none were recovered from cultured fish. *Pseudomonas* species grow on many different substrates and adjust easily to their surroundings, which is why they act as opportunists rather than as pathogens of one particular host (Silby et al., 2011). Wild fish live in open rivers and meet a wider range of bacteria than fish kept in a controlled water supply, and this is the likely reason for the difference. The genus can still cause disease in this species. Shahi and Mallik (2014) recovered *Pseudomonas koreensis* from eye lesions of golden mahseer in Uttarakhand.

Both groups of fish carried the same two main species but in different proportions. Cultured fish were dominated by a single opportunist, while wild fish had a more even mix and one extra genus. Two points are worth keeping in mind. All the fish were healthy, so the difference lies in the normal bacterial load and not in disease status. The pattern is also not the same in every cyprinid. Tan et al. (2019) found that wild

Malaysian mahseer (*Tor tambroides*) had a more diverse gut community than captive fish, which agrees with the present result, while Pingle and Khandagle (2023) found the opposite in four Indian carp species and explained it by the varied feeds used on farms. Diet and habitat have a strong effect on the gut bacteria of fish (Sullam et al., 2012; Ghanbari et al., 2015), so the result here should be read as applying to the Indus tributaries and the Garyala culture facility that were sampled.

Identification in this study rested on two sets of tests. The conventional biochemical results matched the usual characters for each genus: oxidase positive, indole positive and lactose negative for *Aeromonas*; oxidase negative, urease positive and lactose positive for *Klebsiella*; and oxidase positive with no sugar fermentation for *Pseudomonas*. The API 20E reactions agreed with all of these. Taken together the two methods give reasonable confidence in the identifications, but they do not replace sequencing. Culture methods only recover the bacteria that will grow, and the media and temperature used here were chosen for Gram negative opportunists rather than for a full survey of the gut. Incubation at 37 °C also favours bacteria that grow at body temperature over those suited to cold hill streams, so the isolates reported here are a selected group and not the whole gut flora. Khurana et al. (2020) used shotgun metagenomics on the same host and obtained a much fuller picture, including resistance and virulence genes that biochemical tests cannot show. Adding 16S rRNA gene sequencing to culture work (Muyzer and Smalla, 1998) would give a more complete list of species while still providing living isolates.

The AST results are the most useful part of this work for day to day management. All 17 isolates came from healthy fish that had not been treated for any disease, yet all of them were resistant to erythromycin, penicillin and ampicillin, and three wild isolates were resistant to doxycycline as well. Only ceftazidime and cefepime worked against every isolate. Part of this is expected, because the outer membrane of Gram negative

bacteria keeps out many beta lactam antibiotics and limits the action of macrolides such as erythromycin (Fernández-Bravo and Figueras, 2020). What matters is that the resistance was already present in fish that had never been given antibiotics. Khurana et al. (2020) detected antibiotic resistance genes in the gut *Aeromonas* of *Tor putitora*, which would account for this. High levels of multidrug resistance have also been reported in farmed common carp (Mohammed et al., 2023) and in *Aeromonas* from Nile tilapia broodstock (Sherif and Kassab, 2023). Long standing use of antibiotics in fish farming is thought to lie behind much of this, and the problem is expected to grow as waters warm (Reverter et al., 2020; Ferri et al., 2022; Afroze et al., 2025).

This matters for the conservation of golden mahseer. The species is already endangered because of overfishing, dam construction, habitat loss and pollution (Bhatt and Pandit, 2016). The stresses that act on it, such as low river flow, warmer water, crowding in tanks and handling during breeding and stocking, are exactly the conditions that turn these gut bacteria into pathogens. Disease in cultured golden mahseer has been recorded before (Shahi et al., 2018; Khangembam et al., 2023; Kala et al., 2024). The high share of *A. hydrophila* in cultured fish is a particular worry for breeding facilities, where fish are held at high density and an outbreak would spread quickly, and with the resistance found here such an outbreak would also be hard to treat. Three things follow from this. Bacterial checks should become part of routine health management for cultured *Tor putitora*. Water quality, stocking density, feeding and handling should be managed so that stress stays low. Antibiotics should be chosen on the basis of sensitivity testing rather than by guesswork. Probiotic bacteria taken from mahseer themselves may also be worth trying (Hossain et al., 2022; Arya et al., 2025).

This study has clear limits. Only nine fish were sampled, from two sites in one district and in a single season, so the results describe the fish that

were examined rather than the species as a whole. No statistical comparison was possible between the wild and cultured groups at this sample size, so the difference between them should be treated as descriptive. Identification rested on biochemical tests alone, which means the species names and the *Pseudomonas* identification are not confirmed at the molecular level. Water quality was not measured at the two sites, so the differences cannot be tied to particular conditions. Sampling more fish across seasons, adding 16S rRNA gene sequencing, recording water parameters and screening for resistance genes would fill these gaps.

CONCLUSION

Healthy wild and cultured golden mahseer from Attock District carry gut bacteria that can become harmful once the fish is stressed. *Aeromonas hydrophila* and *Klebsiella pneumoniae* were found in both groups of fish, and *Pseudomonas* sp. only in wild fish. *A. hydrophila* was more common in cultured fish, while wild fish carried a more even mix of species. The AST showed that every isolate was resistant to erythromycin, penicillin and ampicillin, that three wild isolates were also resistant to doxycycline, and that ceftazidime and cefepime were effective against all of them. Resistance is therefore already present in the normal gut bacteria of fish that have never been treated. For a species that now depends on breeding and stocking programmes, this points to a need for regular bacterial checks, good water quality, low stress and careful use of antibiotics, alongside the protection of river habitat on which the recovery of *Tor putitora* finally depends.

DECLARATIONS

Conflict of interest

The authors declared that there is no conflict of interest.

Author contributions

MK and SR collected the samples did the laboratory work and wrote the first draft. MZA planned and design the study, supervised the study and revised the manuscript. AR, MM and

SUR helped with the experimental work. All authors read and approved the final manuscript.

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Ethical Statement

Ethical approval was not required for this study.

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