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Research Article

Bacterial profiles and multi-drug resistance patterns in bacterial isolates associated with freshwater fish infections

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ABSTRACT

Fish and fish products can support 40% of world diets, which meets 60% of the animal protein requisites in Bangladesh. Infections of fishes, along with the continuously elevated emergence of microbial resistance, are the major drawbacks to the massive milestone forward. This investigation aimed to reveal the antimicrobial resistance patterns of the pathogens associated with diverse fish infections. According to Bergey's manual of bacteriological classification, isolated pathogens were provisionally identified at genera levels based on their cultural, morphological, and biochemical characteristics. The Kirby-Bauer (Cockerill and CLSI, 2013) disc diffusion method was exploited to determine the antimicrobial resistance. Pathogenic growths were found in 150 (83.34%) out of 180 samples by *Aeromonas* spp. (39.33%), *Vibrio* spp. (16.67%), *Flavobacter* spp. (14.67%), *Edwardsiell* spp (12.67%), *Pseudomonas* spp. (9.33%), *Streptococcus* spp. (5.55%), and *Citrobacter* spp. (2%) in Shing (*Heteropneustes* spp.), Pangus (*Pangasius* spp.), Pabda (*Ompok* spp.), Gulsha (*Mystus cavasius*), Tilapia (*Oreochromis niloticus*), Koi (*Cyprinus* spp.), Magur (*Clarias batrachus*), and Tengra (*Mystus tengara*). Pathogens showed resistance against Amoxicillin (136/150; 90.67%), Chlortetracycline (135/150; 90%), and Erythromycin (134/150; 89.33%), whereas Levofloxacin (138/150; 92%), Ciprofloxacin (123/150; 82%), Neomycin (120/150; 80%), and Colistin (117/150; 78%), exhibited potential effectiveness. A huge frequency of 60% (90 out of 150) of pathogens exhibited as high as 21 antimicrobial resistance patterns towards a minimum of 4 antibiotics and a maximum of 8 antibiotics, whereas *Aeromonas spp.* isolates were the most prominent. The investigation would provide substantial guidance to veterinarians and animal husbandmen involved in fish cultivation to design therapeutics against infections. Regular and vigorous investigation and implementation of the acquired knowledge would be the only possible solution to halt the rapid increase of antimicrobial resistance.

Introduction

The increasing population of today's world demands significantly augmented production of diverse food substances to satisfy nutritional requirements. Fisheries and aquaculture are major contributors to the nutritional requisites providing animal protein with a substantial frequency of 20% of the human diet covering the world's 40% with a remarkable increase in global fish production to 156.4 million tonnes in 2018 from 21.8 million tonnes in 1960, 69% of which has been provided by Asian countries

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(FAO, 2020). Countries of the Indian subcontinent, such as Bangladesh, India, Nepal, and Pakistan, comprise a major contributing group in this aquaculture production industry, significantly increasing yearly (Nayak, 2020).

Bangladesh, a realm of diversified wetlands of various open water bodies, facilitates in attaining an irrevocable resource of fisheries production to accomplish 60% of the animal protein requirement of her vast population's diet, attaining fifth position in overall aquatic production and ranking third in freshwater fisheries production in inland areas all over the world (DoF, 2018; FAO, 2020). Bangladesh is home to 265 freshwater indigenous fish species under 55 families and 154 genera inhabiting various rivers, canals, haors, baods, beels, lakes, ponds, and floodplains, which corroborates the ancient proverb *'Mache Vate Bangali*,' meaning 'fish and rice make a Bengali', delivered after the fish and rice dominance and irreplaceability in their regular daily diets (Rahman, 2005). The most common indigenous freshwater fishes include Shing (*H. fossilis*), Pangus (*Pangasius* spp.), Shol (*Channa striata*), Pabda (*Ompok* spp.), Gulsha (*Mystus cavasius*), Tilapia (*O. niloticus*), Koi (*Cyprinus* spp.), Magur (*C. batrachus*), Tengra (*M. tengara*) and so on, which is considered as one of the most inexpensive sources of protein production (Pandit *et al.*, 2021).

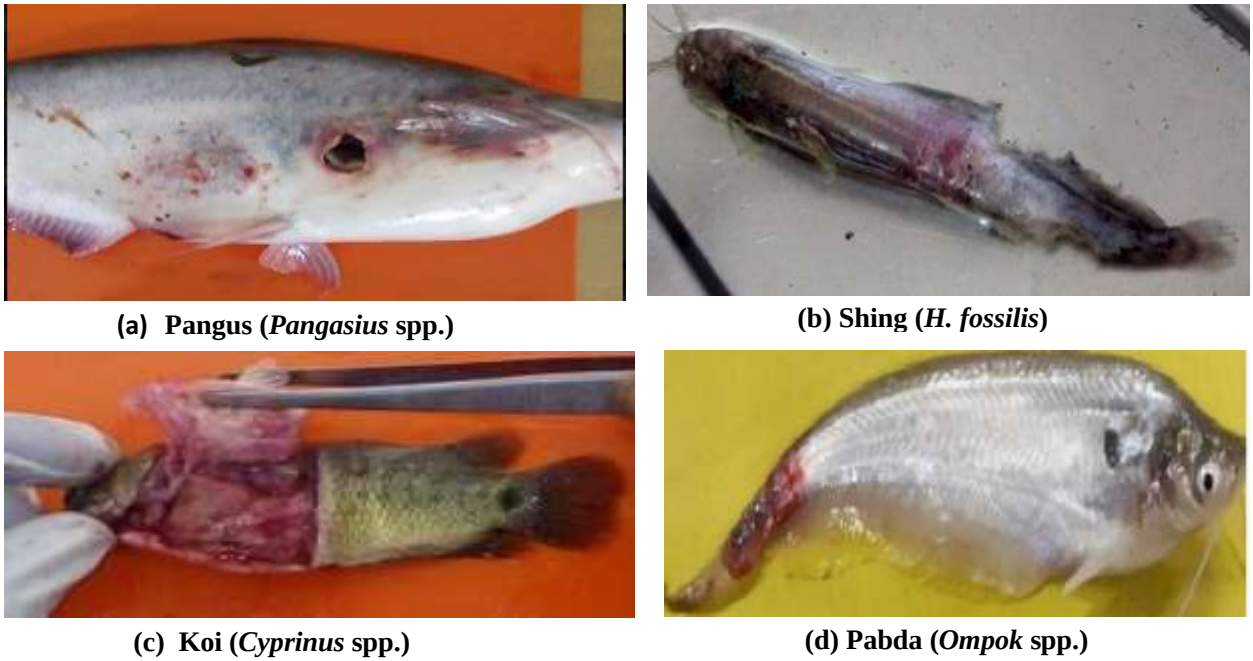
However, this three-penny protein production has encountered considerable drawbacks in infections by diverse pathogens. *Aeromonas spp., Edwardsiella spp., Flavobacterium spp., Pseudomonas* spp., *Streptococcus spp., Vibrio* spp., *Yersinia ruckeri,* etc. are the most frequent bacterial fish pathogens (Miller and Harbottle, 2018). To control those pathogens, various chemotherapeutics are involved by applying antimicrobial agents to fish species' habitats and food supplements. Unapparent application of such antimicrobials with little or no expertise in selecting antibiotics and determining dose concentrations by veterinarians is enumerated as an affair of egregious perturbation, as it might potentially lead to the attainment of antimicrobial resistance by the pathogens, resulting in further therapeutic failure of certain antibiotics. Genetic exchange of those fish pathogens with terrestrial pathogens might support the potential transfer of determinants of resistance to human and animal pathogens, resulting in antimicrobial resistance (AMR) strains, which might also be facilitated by the antimicrobial leaching into the surroundings from the cultivated area, providing enormously diluted agents to the zoonotic and animal pathogens, resulting in the development of antimicrobial resistance (AMR).

Taking the concerns of infections and therapeutic measures of indigenous fish species into account, this current project was designed in particular to perform thorough research to unearth the frequent pathogens causing abnormalities in cultivated local fish species by isolating and identifying the etiological agents, and to determine the reliable responsiveness patterns of those pathogens against commonly applied antimicrobials as therapeutics for selecting substantially effective antibiotics against the pathogens meticulously.

Materials and Methods

Sampling and transport

A total of 180 infected randomly selected fishes of different species, including 83 Shing (*H. fossilis*), 33 Pangus (*Pangasius* spp.), 18 Pabda (*Ompok* spp.), 17 Gulsha (*M. cavasius*), 16 Tilapia (*O. niloticus*), 5 Koi (*Cyprinus* spp.), 5 Magur (*C. batrachus*), and 3 Tengra (*M. tengara),* with diverse infections, were collected along with water samples from different cultivated areas (Figure 1). All the precautions were maintained during the collection of both water and fish samples. The specimens were preserved within an ice box to provide a cool chain and transported to the Quality Aqua Laboratory, Quality Feeds Limited, Mymensingh, for further exploration of the samples.



(a) Pangus (*Pangasius* spp.) **(b) Shing (*H. fossilis*)**

(c) Koi (*Cyprinus* spp.) **(d) Pabda (*Ompok* spp.)**

Fig. 1. Deep ulcerative lesions in different indigenous freshwater fishes (a) Pangus at the top left, (b) Shing at the top right, (c) Koi at the bottom left, and (d) Pabda at the bottom right.

Processing, enrichment, and isolation of bacteria

The solid specimens, i.e., infected fishes, potentially needed to be processed and made suitable for the enrichment culture method. Three different parts of the sampled fish species, such as skin, gill, and intestine, were separated and collected aseptically to avoid potential contamination. Those specimens were placed on a sterile, clean chopping board to mince and grind together. After mincing and grinding, 10 gm of the processed samples were added and mixed homogeneously with 90 ml of freshly prepared 0.1% (w/v) peptone water. The homogeneous mixture samples were exploited for further investigation of isolating bacterial pathogens by inoculating 0.1 ml of those blend compositions onto various selective and non-selective media, including Rimler Shotts Medium Base Agar (selective for *Aeromonas spp.*), Thiosulfate Citrate Bile Salt Sucrose (TCBS) Agar (selective for *Vibrio* spp.), *Pseudomonas* Base Agar (selective for *Pseudomonas* spp.), Tryptic Soy Agar (TSA) (for enrichment of bacterial isolates), Brain Heart Infusion (BHI) Agar (for fastidious organisms), Blood Agar, and MacConkey Agar following standard microbiological technique and procedure, and finally incubated for 24 h at 37°C.

Identification of bacterial isolates

Cultural, morphological, and biochemical characteristics of the isolated pathogens were taken into account to detect and identify the species of the pathogens. Colony characteristics, including color, size, shape, texture, and surface appearance on culture media, namely chromogenic agar, MacConkey agar, TCBS agar, SS agar, and Manitol salt agar, were recorded to use further for identification. Bacterial pathogens isolated from selective and non-selective media were exploited for microscopic study to determine cellular shapes and arrangements. The bacterial isolates were differentiated into gram-positives and gram-negatives via a staining technique. Biochemical tests including acid-alkaline reaction, hydrogen sulfide (H_2S) production test, TSI (Triple Sugar Iron) test, MIU (motility, indole, urease) test, gas production test, catalase test, oxidase test, Simmons citrate test, MR (Methyl Red)-VP (Voges-Proskauer) test were performed to determine the biochemical characteristics of the isolated pathogens. Combining all the cultural, morphological, and biochemical characteristics, the genus of the pathogens was identified

according to Bergey's manual of bacteriological classification (Chauhan and Jindal, 2020).

Determination of antimicrobial resistance patterns

The disc diffusion method by Kirby-Bauer (Cockerill and CLSI, 2013) was exploited to determine the antimicrobial resistance patterns of the fish pathogen isolates against Amoxicillin (10 µg), Erythromycin (15 µg), Enrofloxacin (5 µg), Chlortetracycline (30 µg), Ciprofloxacin (5 µg), Neomycin (30 µg), Colistin (25 µg), Cotrimoxazole (25 µg), Levofloxacin (5 µg), Doxycycline (30 µg). Bacterial cultures were homogeneously mixed with phosphate buffer saline (PBS) to obtain a bacterial suspension, and the suspensions were adjusted to 0.5 MacFarland's turbidity standard. Following the standard microbiological method, the suspensions of bacterial cultures were inoculated by streaking with sterile cotton swab steak, and the plates were supplied with various antibiotic discs. The disc-containing plates were subjected to a 40°C temperature for 1 hour to facilitate proper diffusion of antimicrobials, followed by a 24-hour incubation period at 37°C. The overnight culture plates were investigated to observe and measure the diameter of the clear zone of inhibition by the antibiotics, and the antimicrobials were interpreted as resistant, sensitive, and intermediate in their activity against all bacterial isolates. For quality control of bacterial culture studies and antimicrobial sensitivity testing, standard cultures of *Aeromonas hydrophila* (ATCC 7966), *Flavobacterium columnare* (ATCC 23463), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Vibrio cholerae* (ATCC 14035) were exploited throughout the study.

Result

A total of 180 infected fish of different species were collected as specimens. In this study, 6 (3.33%) of the samples were found to be growth negative, while 174 (96.67%) were found to be growth positive, including 150 (83.34%) pathogenic growths and 24 (13.33%) normal florae (Fig. 2).

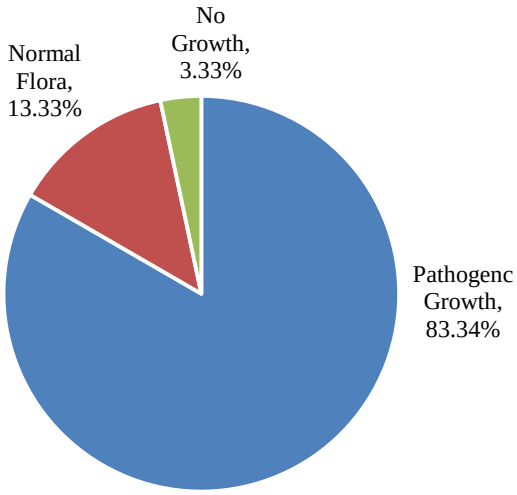


Fig. 2. Bacterial growth pattern of collected specimens.

Diverse species of fish with deleterious infections were randomly sampled for the study. Among the wide range of fish species available in the sampling area, Shing (*H. fossilis*) was vindicated to be widely susceptible to pernicious infections with an exceedingly elevated frequency of 46.1% (83 infected species), followed respectively by Pangus (*Pangasius* spp.) with 18.33% (33), Pabda (*Ompok* spp.) with 10% (18), Gulsha (*M. cavasius*) with 9.44% (17), Tilapia (*O. niloticus*) including 8.89% (16), Koi (*Cyprinus* spp.) and Magur (*C. batrachus*) both sharing a harmonious frequency of 2.78% (5), and Tengra (*M. tengara*) accommodating 1.67% (3) infections **(**Fig. 3**).**

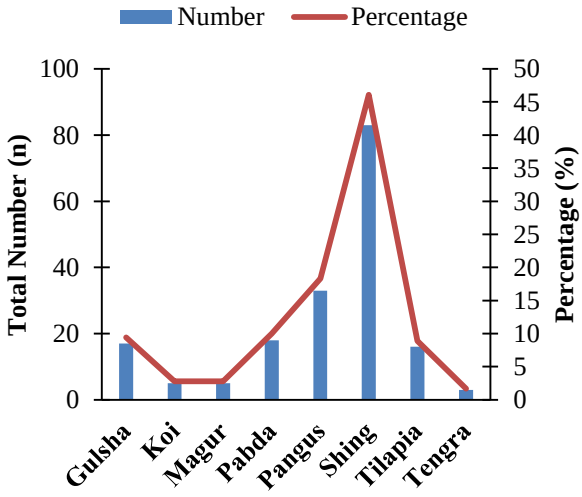


Fig. 3. Frequency of infected fish species.

All the multiplex fish species had remarkably pathogenic microbial communities. *Aeromonas spp.* was unearthed as the most frequent pathogen to infect the fish with a considerably high frequency of 39.33% (59 infections), sequentially followed by *Vibrio* spp., *Flavobacterium spp.*, *Edwardsiella spp.*, *Pseudomonas spp.*, *Streptococcus* spp., and *Citrobacter spp.*, inhabiting 16.67% (25), 14.67% (22), 12.67% (19), 9.33% (14), 5.33% (8), and 2% (3) of the pathogen contaminated fishes respectively **(**Fig. 4**).**

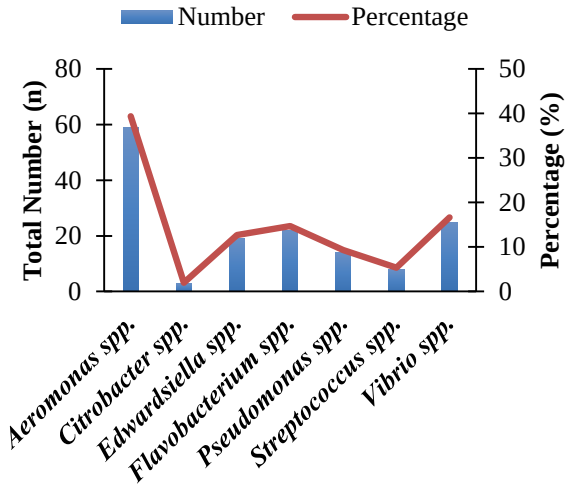


Fig. 4. Frequency of the pathogens contaminating the fish species.

Further exploration of the host-pathogen relation ships in our current investigation revealed a convincing specificity of host contamination by the pathogens. *According to the statistics, Aeromonas spp.*, the most prevalent etiological agent infecting 59 fish species, exhibited the highest frequency of 76.27% (45 infections) of poisoning Shing (*H. fossilis*), whereas its proclivity to pollute other fishes was significantly subordinate with a steeply low frequency of 11.86% (7 infections) in Pangus (*Pangasius* spp.), sequentially succeeded by Tilapia (*O. niloticus*), Pabda (*Ompok* spp.), Gulsha (*M. cavasius*), and Magur (*C. batrachus*) covering a recurrence of 5.08% (3), 3.39% (2), and 1.69% (1), respectively. Moreover, the extremely common pathogen *Aeromonas spp.* caused no harm to Koi (*Cyprinus* spp.) or Tengra (*M. tengara*). *Vibrio* spp., second in the catalog of etiological agents contaminating 25 fish species, manifested its towering infectivity towards Gulsha and Pabda with a parallel incidence of 36%(9 infections). Furthermore, *Flavobacterium spp.* colonized 22 fish species to develop diseases, among which Pangus was supremely infected by the pathogen with an occurrence consistency of 45.45% (10 infections). Nineteen infections of *Edwardsiella spp.* were recorded to populate the Pangus with an excellent frequency of 73.68% (14 infections).

Shing was the most susceptible to *Pseudomonas spp.*, exhibiting a high prevalence of 64.29% (9 infections out of 14). Only 8 *Streptococcus* spp. were isolated from infected pond-cultivated fishes, displaying the highest infection rate against Tilapia, with a frequency of 62.5% (5 infections). *In our current findings, Citrobacter spp.* was the least prevalent pathogen of pond-cultivated fish infections (3 infections). The pathogen was manifested to disease Gulsha and Pabda, obtaining a frequency of 66.67% (2 infections) and 33.33% (1 infection). In contrast, no pathogen contamination was observed against Koi, Magur, Shing, Pangus, Tengra, and Tilapia **(**Fig. 5, Table 1**).**

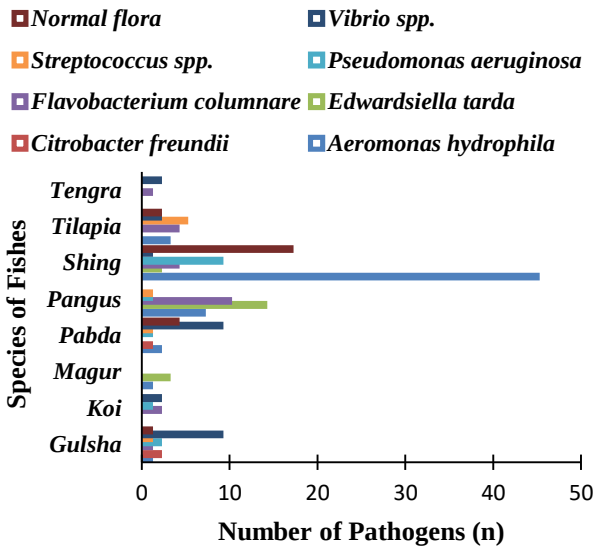


Fig. 5. Allocation of pathogens among diverse fish species

Table 1. Distribution of pathogens among infected fishes.

Pathogens	Total infections (%)	Infected fishes (150 out of 180)	Frequency (%)
Aeromonas spp.	59 (33.91)	Shing (*Heteropneustes fossilis*)	45 (76.27)
		Pangus (*Pangasius* spp.)	7 (11.86)
		Tilapia (*Oreochromis niloticus*)	3 (5.09)
		Pabda (*Ompok* spp.)	2 (3.40)
		Gulsha (*Mystus cavasius*)	1 (1.69)
		Magur (*Clarias batrachus*)	1 (1.69)
Vibrio spp.	25 (14.37)	Gulsha (*Mystus cavasius*)	9 (36.00)
		Pabda (*Ompok* spp.)	9 (36.00)
		Koi (*Cyprinus* spp.)	2 (8.00)
		Tilapia (*Oreochromi sniloticus*)	2 (8.00)
		Tengra (*Mystus tengara*)	2 (8.00)
		Shing (*Heteropneustes fossilis*)	1 (4.00)
Flavobacterium spp.	22 (12.64)	Pangus (*Pangasius* spp.)	10 (45.45)
		Shing (*Heteropneustes fossilis*)	4 (18.18)
		Tilapia (*Oreochromis niloticus*)	4 (18.18)
		Koi (*Cyprinus* spp.)	2 (9.09)
		Gulsha (*Mystus cavasius*)	1 (4.55)
		Tengra (*Mystus tengara*)	1 (4.55)
Edwardsiella spp.	19 (10.92)	Pangus (*Pangasius* spp.)	14 (73.68)
		Magur (*Clarias batrachus*)	3 (15.79)
		Shing (*Heteropneustes fossilis*)	2 (10.53)
Pseudomonas spp.	14 (8.05)	Shing (*Heteropneustes fossilis*)	9 (64.29)
		Gulsha (*Mystus cavasius*)	2 (14.29)
		Koi (*Cyprinus* spp.)	1 (7.14)
		Pabda (*Ompok* spp.)	1 (7.14)
		Pangus (*Pangasius* spp.)	1 (7.14)
Streptococcus spp.	8 (4.6)	Tilapia (*Oreochromis niloticus*)	5 (62.50)
		Gulsha (*Mystus cavasius*)	1 (12.50)
		Pabda (*Ompok* spp.)	1 (12.50)
		Pangus (*Pangasius* spp.)	1 (12.50)
Citrobacter spp.	3 (1.72)	Gulsha (*Mystus cavasius*)	2 (66.67)
		Pabda (*Ompok* spp.)	1 (33.33)
Normal flora	24 (13.79)	Shing (*Heteropneustes fossilis*)	17 (70.83)
		Pabda (*Ompok* spp.)	4 (16.67)
		Tilapia (*Oreochromis niloticus*)	2 (8.33)
		Gulsha (*Mystus cavasius*)	1 (4.17)

Amoxicillin, Chlortetracycline, Ciprofloxacin, Colistin, Cotrimoxazole, Doxycycline, Enrofloxacin, Erythromycin, Levofloxacin, and Neomycin were used for antibiotic responsiveness testing. Aeromonas *spp.* exhibited the peaked resistance against Amoxicillin(96.61%), sequentially succeeded by Chlortetracycline (91.53%), Erythromycin (91.53%), Cotrimoxazole (62.71%) (Fig. 6).

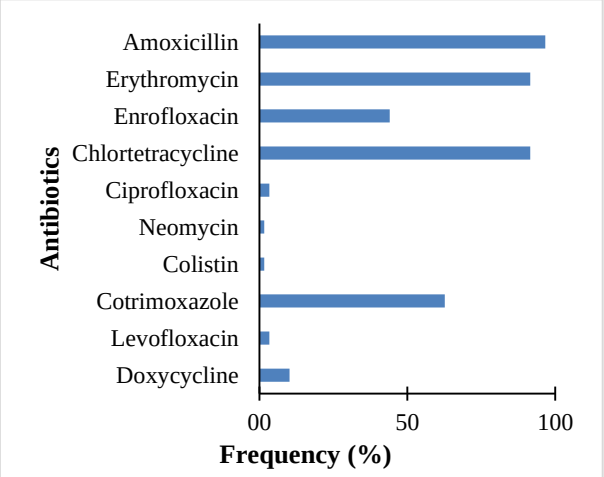


Fig. 6. Antibiotic resistance pattern of *Aeromonas* spp.

Vibrio spp. possessed resistance against Erythromycin (88%), Amoxicillin (80%), Chlortetracycline (72%), and Cotrimoxazole (56%), whereas no resistance was developed against Ciprofloxacin, Colistin, Levofloxacin, or Neomycin (Fig. 7).

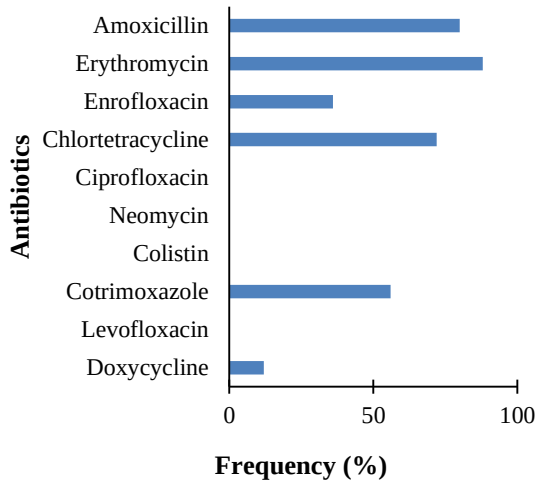


Fig. 7. Antibiotic resistance pattern of *Vibrio*

Flavobacterium spp. was discovered to be resistant to Chlortetracycline (90.91%), Erythromycin (90.91%), Amoxicillin (72.73%), and Cotrimoxazole (54.55%), but not to Colistin, Doxycycline, Levofloxacin, or Neomycin. Doxycycline and Levofloxacin exhibited a uniform effectiveness of 95.45% against *Flavobacterium spp*. **(**Fig. 8**).**

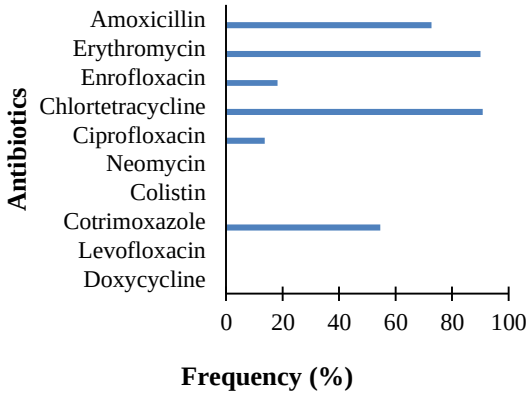


Fig. 8. Antibiotic resistance pattern of *Flavobacterium* spp.

Edwardsiella spp. exhibited supreme resistance to Amoxicillin (100%) and Chlortetracycline (100%), followed sequentially by Erythromycin (94.74%) and Cotrimoxazole (57.89%), whereas no resistance was recorded against Ciprofloxacin, Doxycycline, Levofloxacin, and Neomycin (Fig. 9).

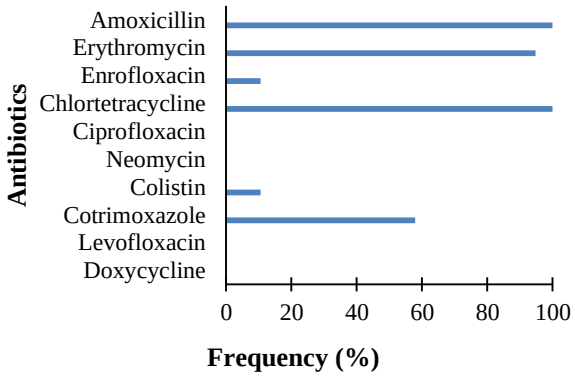


Fig. 9. Antibiotic resistance pattern of *Edwardsiella* spp.

During the treatment of *Pseudomonas spp.* infections, there was a massive resistance to Chlortetracycline (100%), Amoxicillin (92.86%), and Erythromycin (78.57%), but no resistance to

Ciprofloxacin, Colistin, Doxycycline, or Levofloxacin was observed. *Pseudomonas spp*. exhibited considerable susceptibility against Ciprofloxacin (100%), Levofloxacin (100%), Doxycycline (85.71%), Colistin (64.29%), and Neomycin (57.14%) (Fig.10).

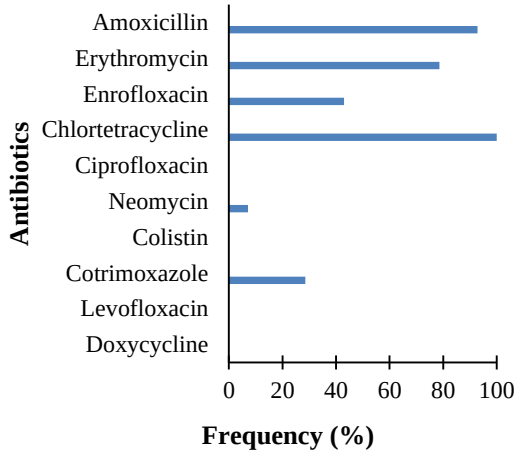


Fig. 10. Antibiotic resistance pattern of *Pseudomonas* spp.

Streptococcus spp. was recorded to have resistance to Amoxicillin (100%), Chlortetracycline (100%), Cotrimoxazole (75%), and Erythromycin (75%), whereas no resistance was displayed against Colistin and Neomycin **(**Fig. 11).

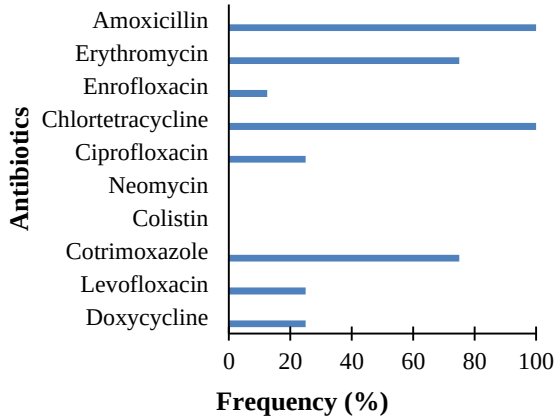


Fig. 11. Antibiotic resistance pattern of *Streptococcus* spp.

The least prevalent pathogen isolated in our study, *Citrobacter* spp., exhibited resistance against Amoxicillin (100%), Erythromycin (100%), Chlortetracycline (66.67%), and Cotrimoxazole (66.67%), whereas no resistance was found against Ciprofloxacin, Colistin, Doxycycline, Levofloxacin, and Neomycin. On the other hand, supreme effectiveness (100%) was possessed against the pathogen by Ciprofloxacin, Colistin, and Levofloxacin separately **(**Fig. 12**)**.

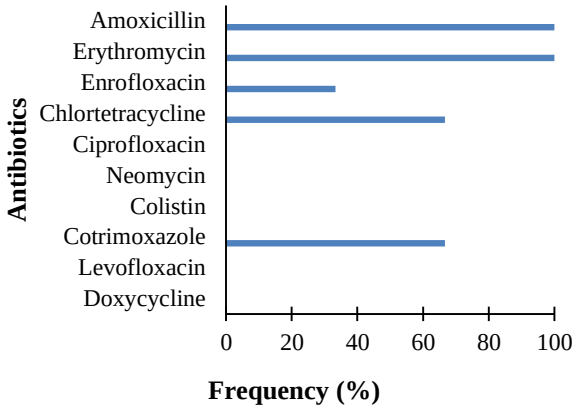


Fig. 12. Antibiotic resistance pattern of *Citrobacter* spp.

The entire pathogen communities were recorded to show resistance mostly to Amoxicillin (90.67%), Chlortetracycline (90%), Erythromycin (89.33%), and Cotrimoxazole (57.33%)**.** On the contrary, the effectiveness of the antibiotics was led by Levofloxacin (92%), subsequently succeeded by Ciprofloxacin (82%), Neomycin (80%), Colistin (78%), and Doxycycline (65.34%) **(**Fig. 13**)**.

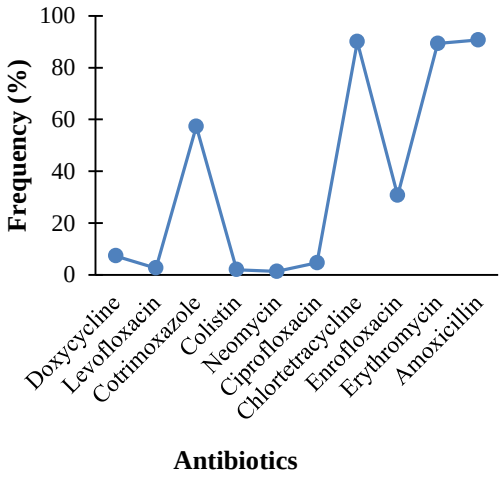


Fig. 13. Overall antibiotic resistance pattern of fish pathogens.

The antimicrobial resistance patterns exhibited by the pathogens isolated from various fish infections were enormously diverse. Patterns of resistance to single, double, triple, and up to eight different antibiotics were observed. Twenty-one types of patterns of resistance were established, taking a limit of a minimum of 4 antibiotics into account, where a single isolate of *Aeromonas spp. was* found resistant to 8 antibiotics (AMX-CIP-COT-CT-DO-ERY-LEV) and another isolate of the same species evolved resistance mechanisms to 7 antibiotics (AMX-CIP-COT-CT-ENR-ERY-LEV). Fourteen isolates of *Aeromonas spp.* were uncovered to be resistant to AMX-COT-CT-ENR-ERY (5 antibiotics), and 12 isolates of the same species were found to be resistant to AMX-COT-CT-ERY (4 antibiotics), which was undoubtedly imitated by ten isolates of *Edwardsiella spp.* Moreover, a total of 90 out of 150 pathogenic isolates (60%) shared those 21 types of resistance patterns towards a minimum of 4 different antimicrobial agents (Table 2).

Discussion

Fish are major contributors to the production of required animal protein worldwide. Freshwater fish culture facilitates the enormous growth and supply of animal protein through vast fish production. One of the most serious drawbacks of that process is the frequent infection of freshwater fish species. Our current project uncovered 83.33% (150 fish samples) of fish out of 180 randomly selected fish species with visible physical impurities being infected by diverse types of pathogens, which would be substantial evidence of the significance of that drawback. The study by Abedin et al. (2021a) also exhibited a higher frequency of 92.16% of fish infections by pathogenic bacteria. Another study on wild, small freshwater fish stated 66.2% of positive cases of infections (Labony et al., 2020). 70% of 500 samples of Nile Tilapia fish were found to be infected by *Aeromonas* spp. (Saleh et al., 2021).

A total of 180 infected fish species were randomly sampled as hosts of diverse pathogens where Shing, Pangus, Pabda, Gulsha, Tilapia, Koi, Magur, and Tengra were found to be contaminated by disease signs with a respective frequency of 46.1%, 18.33%, 10%, 9.44%, 8.89%, 2.78%, 2.78%, 1.67%. A similar pattern of infected fish host sequence in the context of prevalence was observed by Abedin *et al.* (2020a), who exhibited the Shing as the most frequently poisoned fish species, followed by Pangus and Pabda with a respective frequency of 56.9%, 14.7%, and 7.3%. Thai Koi, Shol, Magur, Tilapia, and Tengara fish were also found to be infected in that investigation.

Aeromonas spp., Vibrio spp., Flavobacterium spp., Edwardsiella spp., Pseudomonas spp., Streptococcus spp., and Citrobacter spp. were identified as etiological agents that contaminated and infected fish at rates of 39.33% (59 infections), 16.67%(25), 14.67%(22), 12.67%(19), 9.33%(14), 5.33%(8), and 2%(3), respectively. Among 36(42.9%) *Aeromonas spp.*, 15(17.9%) *Pseudomonas* spp., 7(8.3%) *Vibrio* spp., 9(10.7%) *Staphylococcus* spp., 7(8.3%) *Flavobacterium* spp., 7(8.3%) *Edwardsiella* spp., and 3(3.6%) *Citobacter* spp. and *Enterobacter* spp. were documented as fish pathogens by Abedin et al. (2020a), which is closely related to our investigation with *Aeromonas* spp. as the most frequent source of impurities in fish species. Another study by Saleh *et al.* (2021) displayed 53.4% (187 out of 350 positive growths) *Aeromonas spp.* infections, which elucidates the towering prevalence of *Aeromonas spp.* infections in fish. Miller and Harbottle (2018) described *Aeromonas spp., Edwardsiella spp., F. branchiophilum, Mycobacterium* spp., and *Pseudomonas* spp., *as* the most typical and most frequent bacterial fish pathogens, which showed significant resemblance to our analyzed pathogens. Austin and Austin (2012) and Pridgeon and Klesius (2012) stated the pathogenicity of *Aeromonas spp., Edwardsiella spp., Flavobacterium spp., Francisella spp., Photobacterium spp., Piscirickettsia, Pseudomonas, Vibrio, Yersinia, Lactococcus,* and *Streptococcus* towards fish species in distinct studies. Unlike ours, *M. marinum* was described as a well-known pathogen of fish skin and soft tissue infection by Aubry et al. (2017). *Aeromonas spp., Edwardsiella spp., Enterobacter cloacae, and Acinetobacter junii* exhibited pathogenicity towards Koi. Goldfish were revealed to be infected by pathogenic bacterial isolates, including *Klebsiella aerogenes, Enterobacter cancerogenus, Citrobacter freundii, Edwardsiella tarda, and Acinetobacter nosocomial's*, which corroborate our current research in the context of pathogens with a high percentage of resemblance (Preena et al., 2019).

Table 2. Multi-antibiotic resistance (MAR) patterns of pathogenic isolates exhibit tolerance to at least four antibiotics.

Pattern Type	Antibiotic (Minimum 4) Resistance Pattern	Pathogens	No of Isolates
I	AMX-CL-CT-ERY	*Edwardsiella spp.*	1
II	AMX-COT-CT-ENR	*Aeromonas spp.*	1
III	AMX-COT-CT-ERY	*Aeromonas spp.*	12
		Edwardsiella spp.	10
		Flavobacterium spp.	6
		Vibrio spp.	5
		Pseudomonas spp.	4
		Streptococcus spp.	3
		Citrobacter spp.	1
IV	AMX-COT-ENR-ERY	*Vibrio spp.*	3
V	AMX-CT-ENR-ERY	*Pseudomonas spp.*	2
		Vibrio spp.	2
		Aeromonas spp.	2
		Citrobacter spp.	1
		Edwardsiella spp.	1
VI	AMX-CT-ERY-NEO	*Pseudomonas spp.*	1
VII	COT-CIP-CT-ERY	*Flavobacterium spp.*	1
VIII	COT-CT-ENR-ERY	*Aeromonas spp.*	1
IX	COT-DO-ENR-ERY	*Vibrio spp.*	1
X	AMX-COT-CT-ENR-ERY	*Aeromonas spp.*	14
		Streptococcus spp.	1
		Vibrio spp.	1
XI	AMX-COT-CT-DO-ENR	*Vibrio spp.*	1
XII	AMX-CIP-COT-CT-ERY	*Flavobacterium spp.*	2
XIII	AMX-CIP-CT-DO-LEV	*Streptococcus spp.*	1
XIV	AMX-COT-CT-DO-ERY	*Aeromonas spp.*	2
XV	AMX-CL-CT-ENR-ERY	*Aeromonas spp.*	1
		Edwardsiella spp.	1
XVI	AMX-CT-DO-ENR-ERY	*Aeromonas spp.*	2
XVII	AMX-CIP-COT-CT-DO-LEV	*Streptococcus spp.*	1
XVIII	AMX-COT-CT-DO-ENR-ERY	*Aeromonas spp.*	1
		Vibrio spp.	1
XIX	AMX-COT-CT-ENR-ERY-NEO	*Aeromonas spp.*	1
XX	AMX-CIP-COT-CT-ENR-ERY-LEV	*Aeromonas spp.*	1
XXI	AMX-CIP-COT-CT-DO-ENR-ERY-LEV	*Aeromonas spp.*	1
		Total isolates	**90 (60%)**

Note: AMX = Amoxicillin; CIP = Ciprofloxacin; CL = Colistin; COT = Cotrimoxazole; CT = Chlortetracycline; DO= Doxycycline; ENR = Enrofloxacin; ERY= Erythromycin; LEV= Levofloxacin;

The pathogens isolated in our current study revealed no significant host specificity as all the isolates individually showed pathogenicity towards a wide range of fish species. For example, *Aeromonas spp.* was parasitic towards a wide range of hosts, including Shing (*H. fossilis*), Pangus (*Pangasius* spp.), Tilapia (*O. niloticus*), Pabda (*Ompok spp*.), Gulsha (*M. cavasius*), and Magur (*C. batrachus*), and analogous features in the context of a wide variety of hosts were exhibited by the whole pathogenic communities. Individual hosts were also susceptible to a wide range of pathogens. Resemblance to our study on host-pathogen interaction was previously described in several studies by Preena et al., (2019), Abedin *et al.* (2020a), Abedin et al. (2020b), Abedin et al. (2021a). Further exploration of the host-pathogen relationships in our current investigation revealed a convincing specificity of contaminating the hosts by the pathogens, where *Aeromonas spp.* displayed significantly high pathogenicity towards Shing (*H. fossilis*), *Vibrio* spp. towards Gulsha (*M. cavasius*) and Pabda (*Ompok* spp.), *Flavobacterium spp*.towards Pangus (*Pangasius* spp.), *Edwardsiella spp*.towards Pangus (*Pangasius* spp.), *Pseudomonas spp*. towards Shing (*H. fossilis*), *Streptococcus* spp. towards Tilapia (*O. niloticus*), and normal flora towards Shing (*H. fossilis*).

The most common isolate, *Aeromonas spp.*, was resistant to Amoxicillin (96.61%), Chlortetracycline

(91.53%), and Erythromycin (91.53%) but susceptible to Levofloxacin (91.53%) and Ciprofloxacin (83.05%). Likewise, *Aeromonas spp.* was found supremely resistant to Amoxicillin (100%) and sensitive to Levofloxacin (57%) through an investigation by Abedin et al. (2021b) with the highest intermediate activity towards Chlortetracycline. The highest resistance of 67.4% towards Chloramphenicol and a maximum susceptibility of 90.9% to Meropenem were conferred by *Aeromonas spp.*, whereas the highest intermediate efficiency of 32.6% was shown by Ceftriaxome (Saleh et al., 2021). A total of 12 varieties of resistance patterns (resistant to a minimum of 4 antibiotics) were revealed in *Aeromonas spp.*, where specific isolates were found tolerant to a maximum of 8 different antibiotics. Saleh et al. (2021) stated about 9 different antibiotic resistance patterns along with 10 resistant isolates of *Aeromonas spp*. against 8 different antibiotics. Erythromycin (88%) and Amoxicillin (80%) were resistant to *Vibrio* spp., whereas Levofloxacin (92%), Ciprofloxacin (88%), Colistin (84%), and Neomycin (80%) were found effective to treat *Vibrio spp.* infections and Enrofloxacin (44%) with an intermediate response. In a study by Deng et al. (2020), they described Vancomycin (95.71%) as the highest and Chloramphenicol (4.29%) as the lowest resistant antibiotic against *Vibrio* spp. isolated from marine fish infections in the South China Sea. Chlortetracycline (90.91%) and Erythromycin (90.91%) were resistant, whereas Doxycycline (95.45%) and Levofloxacin (95.45%) were sensitive to *Flavobacterium spp.* Besides, Enrofloxacin (81.82%) was uncovered as an intermediately effective agent against the pathogen. In a particular study by Declercq et al. (2021), *Flavobacterium spp.* isolated from Koi fish exhibited resistance towards both 1st and 2nd generation Quinolone in a certain way. *Edwardsiella spp. had complete resistance to Amoxicillin (100%) and Chlortetracycline (100%) but complete susceptibility to Ciprofloxacin (100%) and Levofloxacin* (94.74%). Moreover, tolerable intermediate activity against the pathogen was recorded in Enrofloxacin (89.47%). Like ours, a study by Abedin et al. (2021b) stated Ciprofloxacin (67%) was the most effective and Amoxicillin (100%) was ineffective against *Edwardsiella* spp. infections in Pangas (*P. pangasius*). Chlortetracycline (100%) and Amoxicillin (92.86%) possessed resistance towards *Pseudomonas spp.*, whereas Ciprofloxacin (100%), Levofloxacin (100%), and Doxycycline (85.71%) were sensitive. A past study by Abedin et al. (2021c) described Amoxicillin (100%), Ciprofloxacin (100%), and Erythromycin (100%) as resistant, and Enrofloxacin (100%) as the most effective antibiotic against *Pseudomonas* spp. fish infections. *Streptococcus*

spp. was resistant to Amoxicillin (100%), Chlortetracycline (100%), and Chlortetracycline (100%) and was sensitive to Colistin (100%). *Citrobacter spp.* exhibited 100% resistance against Amoxicillin and Erythromycin, whereas 100% effectiveness against the pathogen was by Ciprofloxacin, Colistin, and Levofloxacin.

In this study, Amoxicillin (90.67%), Chlortetracycline (90%), and Erythromycin (89.33%) exhibited resistance toward entire fish pathogen communities. Divyashree et al. (2020) described Ampicillin (40.78%), Tetracycline (40.22%), and Nitrofurantoin (29.05%) as resistant to pathogens isolated from fish processing effluent. Wu et al. (2019a) found fish pathogens isolated from marine fish farming areas resistant to Oxytetracycline. Amoxicillin (76.47%) and Erythromycin (54.9%) were resistant to fish pathogens, according to Abedin et al. (2021a), which displayed a resemblance to our current study. Pathogens isolated from fish cage culture were exhibited by Wu et al. (2019b) with resistance towards Amoxicillin (27.67%), Erythromycin (23.31%), and Gentamicin (37.32%). As resembling our research, Abedin et al. *(*2020a) stated that Amoxicillin (100%) exhibits supreme resistance towards the pathogens, followed by Erythromycin (64.3%). On the contrary, Levofloxacin (92%), Ciprofloxacin (82%), Neomycin (80%), and Colistin (78%) were effective against fish pathogens, according to our current study. We found that Enrofloxacin (97.8%), Cotrimoxazole (97.3%), and Ciprofloxacin (77%) were potent against the fish pathogens (Abedin et al., 2021c), which displayed some resemblance to our investigation with a huge imparity as Enrofloxacin was noticed to be intermediately effective (62.67%) against the fish pathogens in our study. Twenty-one resistance patterns (resistant to a minimum of 4 antibiotics) were displayed by 90 out of 150 (60%) pathogens isolated in our current study, with the considerable participation of almost every pathogen. *Aeromonas spp. exhibited a wide range of resistance patterns, including tolerance to up to eight antibiotics (AMX-CIP-COT-CT-DO-ENR-ERY-LEV).* Fourteen isolates of *Aeromonas spp.* were uncovered to be resistant towards AMX-COT-CT-ENR-ERY (5 antibiotics), and 12 isolates of the same species were found to be resistant against AMX-COT-CT-ERY (4 antibiotics), which was undoubtedly imitated by 10 isolates of *Edwardsiella spp.* Saleh et al. (2021) also stated that *Aeromonas spp.* has 9 different antibiotic resistance patterns and 10 tolerant isolates towards 8 different antibiotics.

Conclusion

Fish production, the major contributor to animal protein supply worldwide, encounters a massive hindrance of infection by a wide range of pathogens of viruses, bacteria, and fungi, gradually worsening due to the rampant development of resistance towards antimicrobials by the pathogens. Among 180 randomly sampled infected fishes, 6 (3.33%) showed no growth, and 174 (96.67%) displayed culture positivity, including 150 (83.34%) pathogenic growth and 24 (13.33%) normal flora. The presence of *Aeromonas spp., Vibrio spp., Flavobacterium spp., Edwardsiella spp., Pseudomonas spp., Streptococcus spp*., and *Citrobacter spp*. in bacterial communities was linked to 39.33%(59), 16.67%(25), 14.67%(22), 12.67%(19), 9.33%(14), 5.33%(8), and 2%(3) infections in Shing (*H. fossilis*), Pangus (*Pangasius* spp.), Pabda (*Ompok* spp.), Gulsha (*M. cavasius*), Tilapia (*O. niloticus*), Koi (*Cyprinus spp.*), Magur (*C. batrachus*), Tengra (*M. tengara*) accommodating a frequency of 46.1%(83), 18.33%(33), 10%(18), 9.44%(17), 8.89%(16), 2.78%(5), 2.78%(5), and 1.67%(3) fish species respectively as hosts with no particular host-pathogen specificity. The entire communities of pathogens were resistant to Amoxicillin (90.67%), Chlortetracycline (90%), Erythromycin (89.33%), and Cotrimoxazole (57.33%). On the contrary, Levofloxacin (92%), Ciprofloxacin (82%), Neomycin (80%), Colistin (78%), and Doxycycline (65.34%) exhibited potential effectiveness against the pathogens, whereas Enrofloxacin was found to have an intermediate (62.67%) response in our study. A huge frequency of 60% of pathogens exhibited 21

resistance patterns towards a minimum of 4 antibiotics and a maximum of 8 antibiotics, whereas *Aeromonas spp.* isolates were the most prominent. The discoveries about fish pathogens, their prevalence, and antimicrobial responsiveness patterns to pathogens would be a valuable guide to veterinarians in treating a wide range of infections in aquaculture. Elevated resistance towards antimicrobials by fish pathogens might be an alarming issue as the potential pathogenic gene transfer can drive the environmental isolates into a potential threat. Further studies on the resistance genes or transposons of the pathogenic isolates are required, as well as regular investigations into fish pathogens and their responses to antimicrobials.

References

Abedin MZ, Aktar MB, Zaman MS, Hasan R, Jarin L, Karim MR, Rahman MS, Islam R and Uddin ME. Occurrence and antimicrobial susceptibility profiling of bacteria isolated from cultured Pangas Catfish (Pangasius pangasius) and Climbing Perch (Anabas testudineus) Fishes. *J. Mar. Biol. Aquac.* 2020a; 6(1): 7-12.

Abedin MZ, Rahman MS, Hasan R, Shathi JH, Jarin L and Zaman MS. Isolation, identification, and antimicrobial profiling of bacteria from aquaculture fishes in pond water of Bangladesh. *Amer. J. Pure Appli. BioSci.* 2020b; 2(3): 39-50.

Abedin MZ, Yeasmin F, Mehfuz A, Koly FA, Arfat ME, Farnaz NEK, Sadiqur R, Khairujjaman, Islam MI, and Emdadul HM. Enumeration of emergent bacterial pathogen isolated from small indigenous fish species: Pabda (*Ompok* spp) and Gulsha (*Mystus cavasious)*. *Int. J. Oceanogra. Aquacu.* 2021a; 5(4): 000213.

Abedin MZ and Islam MI. Microbiological screening and antibiotics responsiveness of pathogenic bacteria isolated from pond cultivated pangas fishes (Pangasius hypothallus) in Bangladesh. *Sci. Arch.* 2021b; 2(3): 238-244.

Abedin MZ, Hasan R, Rahman MS, Jarin L, Shilpi RY, Islam R and Rahman MA. 2021c. Detachment, distinguishing proof of bacterial pathogens from infected Shing (*Heteropneustes fossilis*) cultured in freshwater ponds in Bangladesh. *Int. J. Biomo. Biome.* 2021c; 12(3): 10-16

Aubry A, Mougari F, Reibel F and Cambau E. Mycobacterium marinum. In: *Tuberculosis and Nontuberculous Mycobacterial Infections*. Schlossberg D (ed.), 7th edition, Chap. 43. John Willy & Sons, 2017. pp. 735-752.

Austin B and Austin DA. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. 4th edition, Springer, Dordrecht, The Netherlands, 2012. p. 552.

Chauhan A and Jindal T. Microbiological methods for environment, food and pharmaceutical analysis. *Springer Nature*, 2020; p. 487.

Cockerill FR. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement; [... provides updated tables for... M02-A11, M07-A9, and M11-A8]. National Committee for Clinical Laboratory Standards; 2013.

Declercq AM, Tilleman L, Gansemans Y, De Witte C, Haesebrouck F, Van Nieuwerburgh F, Smet A and Decostere A. Comparative genomics of Flavobacterium columnare unveils novel insights in virulence and antimicrobial resistance mechanisms. *Vet. Res.* 2021; 52: 18.

Deng Y, Xu L, Chen H, Liu S, Guo Z, Cheng C, Ma H and Feng J. Prevalence, virulence genes, and antimicrobial resistance of Vibrio species isolated from diseased marine fish in South China. *Sci.. Rep.* 2020; 10(1): 14329.

Divyashree M, Kumar DV, Ballamoole KK, Shetty V, Chakraborty A and Karunasagar I. Occurrence of antibiotic resistance among gram-negative bacteria isolated from effluents of fish processing plants in and around Mangalore. *Int. J. Environ. Health Res.* 2019; 30: 653-60.

Divyashree M, Kumar DV, Ballamoole KK, Shetty A V, Chakraborty A and Karunasagar I. Occurrence of antibiotic resistance among Gram negative bacteria isolated from effluents of fish processing plants in and around Mangalore. *Int. J. Environ. Health Res.* 2020; 30(6): 653-660.

DoF. Yearbook of fisheries statistics of Bangladesh 2017-2018. Fisheries Resources Survey System (FRSS), Department of Fisheries, Ministry of Fisheries and Livestock: Dhaka, Bangladesh. 2018; 35: p. 129.

FAO. The state of world fisheries and aquaculture 2020: sustainability in action. In: The State of World Fisheries and Aquaculture (SOFIA); FAO: Rome, Italy. 2020; p. 244.

Labony SS, Alim MA, Hasan MM, Hossain MS, Islam A, Alam MZ, Tsuji N and Anisuzzaman. Fish-borne trematode infections in wild fishes in Bangladesh. *Pathog. Glob. Health.* 2020; 114(2): 91-98.

Miller RA and Harbottle H. Antimicrobial Drug Resistance in Fish Pathogens. *Microbiol. spectr*. 2018; 61: 1-20.

Nayak SK. Current prospects and challenges in fish vaccine development in India with special reference to Aeromonas hydrophila vaccine. *Fish Shellfish Immunol*. 2020; 100: 283-299.

Pandit D, Saha S, Kunda M and Harun-Al-Rashid A. Indigenous freshwater ichthyofauna in the Dhanu River and surrounding wetlands of Bangladesh: species diversity, availability, and conservation perspectives. *Conserv.* 2021; 1(3): 241-257.

Preena PG, Arathi D, Raj NS, Arun Kumar TV, Arun Raja S, Reshma RN and Raja Swaminathan T. Diversity of antimicrobial resistant pathogens from a freshwater ornamental fish farm. *Lett. Appl. Microbiol.*, 2019; 71(1): 108-116.

Pridgeon JW and Klesius PH. Major bacterial diseases in aquaculture and their vaccine development. *CABI Reviews.* 2012; 7: 1-16.

Rahman AKA. Freshwater Fishes of Bangladesh, 2nd ed. Zoological Society of Bangladesh, Department of Zoology, University of Dhaka: *Dhaka, Bangladesh*. 20005; p. 263

Saleh A, Elkenany R and Younis G. Virulent and multiple antimicrobial resistance aeromonas hydrophila isolated from diseased nile tilapia fish (Oreochromis niloticus) in Egypt with sequencing of some virulence-associated genes. *Biocontrol. Sci.*, 2021; 26(3): 167-176.

Sørum H. Antimicrobial drug resistance in fish pathogens. In: *Antimicrobial Resistance in Bacteria of Animal Origin*. Chap. 13, Aarestrup FM (ed.) 2005; 213-238.

Wu J, Su Y, Deng Y, Guo Z, Mao C, Liu G, Xu L, Cheng C, Bei L and Feng J. Prevalence and distribution of antibiotic resistance in marine fish farming areas in Hainan, China. *Sci. Total Environ.*, 2019a; 653: 605-611.

Wu J, Mao C, Deng Y, Guo Z, Liu G, Xu L, Bei L, Su Y, Feng J. Diversity and abundance of antibiotic resistance of bacteria during the seedling period in marine fish cage-culture areas of Hainan, China. Mar. Pollut. Bull. 2019b; 141: 343-349.