Prevalence of Pangasius Fish Disease in Nepal

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ABSTRACT

Objectives: This study was conducted to identify bacterial pathogens in both symptomatic and asymptomatic enteric septicemic infection of *Pangasiaus hypophthalmus* and evaluate their susceptibility to antibiotics.

Methods: The totals of 120 kidney and liver swab samples of fish were collected from different places of Nepal. The samples were processed to identify the causative agent using pure culture of bacteria through streak plate techniques and different biochemical tests. To assess the susceptibility of the isolated bacteria to antibiotics Ciprofloxacin, Tetracycline, Doxycycline, Gentamycin, Ampicillin/sulbactam, and Tobramycin were used.

Results: The estimated prevalence of pathogenic bacteria is 27 per 10000 populations. Three pathogenic bacteria i.e. *Edwardsiella ictaluri* 57.7% (56/97), *Aeromonas hydrophila* 24.7% (24/97), and *Pseudomonas fluorescence* 17.5% (17/97) were identified in the studied asymptomatic and symptomatic Pangas fish disease. *E. ictaluri* was major isolates which was causative agent for the disease, enteric septicemia of catfish. Among the total bacterial isolates 100% bacterial isolates were sensitive to Ciprofloxacin and 84% to Gentamycin. Whereas 100% were resistance to Ampicillin and 84% to Tetracycline. And Tobramycin (60.8%) and Doxycycline (64.9%) showed intermediate result.

Conclusion: Ciprofloxacin and Gentamycin could be administered to control enteric septicemia of catfish in Pangas. This study provides information to strategies to reduce economic losses to farmer and reduce possible health hazards.

Keywords: Pangas catfish, bacteria culture, antibiotics susceptibility test.

INTRODUCTION

Aquaculture is one of the fastest growing agricultural subsectors in Nepal. Due to landlocked in nature, Nepal depends only on inland aquaculture with finfish farming. Climatic condition favors cultivation of both warm and cold-water species. The most common species under cultivation are indigenous and exotic carp. Pangas catfish and Rainbow trout. Institutional development of aquaculture in Nepal was started almost seven decades ago but its development place was rather slow. Nevertheless, the progress achieved by this sector in last decade is highly commendable.

Government programs like fish mission, one village one product, and resource center establishment programmes etc. are the key factors in the development of this sector. Fish consumption in Nepal is rather low compared to poultry, pork, buff and mutton. Increasing health awareness among people has led to rise in fish consumption has demanded more aquaculture industries. Pangas (*Pangasiandon hypophthalmus*) belonging to the family Pangasiidae under the order Siluriformes, is a newly introduced exotic fish species in Nepal. This fish is commonly known as "Pangas" or "Baikhi" in Nepal. The origin of Pangas catfish was from the Mekong River of Vietnam to Chao Phraya River of Thailand and distributed to other countries such as Malaysia, Indonesia and China (FAO, 2016).

Date of Submission: November 9, 2023
Date of Acceptance: December 16, 2023
Published Online: December, 2023
DOI: https://doi.org/10.3126/tujm.v10i1.60645

Commercial culture and production of Pangas has recently been expanded dramatically in some Asian countries especially in China, Thailand, Vietnam and Bangladesh. This fish is sold to more than 130 countries globally, mainly in the form of white fillets. Pangas is now considered as the third most important freshwater fish group within the aquaculture sector (FAO, 2016). This species gained popularity because of its omnivorous feeding habit, fast growth rate, high stocking capacity, easy culture system, high disease resistance, good market demand and tolerance to a wide range of environmental change (Sarkar et al. 2007; Ali et al. 2005; Rohul Amin et al. 2005). Pangas can be cultured in high stocking density as this fish has a higher number of erythrocytes than any other fish, plus an additional respiratory organ, and can breathe through bubbles and skin which help it tolerate an environment short of dissolved oxygen (Shrestha et al. 2015). There is a huge demand for Pangas in Nepalese markets due to lower market price and presence of fewer spines inside body. Moreover, the vast majority of people consume this fish due to its delicacy and taste with high fat content. It indicates that this fish can make a significant contribution in increasing fish production, poverty alleviation and livelihoods support in Nepal. Pangas are generally cultured completely on supplemental feed in intensive aquaculture system.

Presently, this fish is considered as one of the important fishes in aquaculture of Nepal because of its fast growth, year-round production, and high productivity. The climbing perch fish (*Anabas testudineus*) is one of the important small indigenous spp. (SIS) fresh water fish of Nepal, which is locally known as koi fish. In Southeast Asian region, this fish is native and often found in fresh water sources of east India and south China. But in Nepal, it is normally found in open water (streams, lakes, floodplain and beels), paddy fields, swamps and its habitats are heavily-vegetated and stagnant waters. Pathogenic microorganisms are a serious threat to fish production in all over the world due to high economic importance of diseases they cause. A number of bacterial pathogens have been found to cause diseases in fish worldwide. In fresh water fishes, bacteria were particular importance including *Aeromonas* spp., *Pseudomonas* spp, *Streptococcus* spp., *Flavobacterium* spp., *Edwardsiella* spp., *Vibrio* spp., and so on. The bacterial infections which are caused by antibiotic-resistant bacteria increasing all over the world. The main objective of this work was to isolate and identify bacteria from climbing perch (*Anabas testudineus*) and pangas catfish (Pangasius pangasius) cultivated in the pond water of different areas in Nepal and to determine the level of antibiotic susceptibility rates of the isolated bacteria against seven antibiotics.

MATERIALS AND METHODS

Study area

This cross-sectional study involved collection of both symptomatic and asymptomatic fish samples of Pangas catfish from all over the Nepal from November 2021 to April 2022. A total of 120 liver and kidney swab samples of both asymptomatic and symptomatic Pangas fish were taken aseptically and transported to disease laboratory of National Fisheries Research Centre, Godawari, Lalitpur, Nepal in ice box maintaining cool chain.

Isolation of pathogenic bacterial isolates

The enrichment of swab samples were done in BHI(Brain Heart Infusion) broth and selective isolation for the suspected pathogens were done Rimler shots for *Aeromonas hydrophila*, Edwardsiella ictaluri medium for *Edwardsiella ictaluri* and Pseudomonas F-base agar for *Pseudomonas fluorescence* further morphological characterization ,Gram staining and biochemical characterization were performed such as IMViC test, catalase test, oxidase test and oxidative fermentative test to identify the bacteria from pangas (*P. hypophthalmus*) and assessment of antibiotic susceptibility test of isolates were performed by using the following antibiotics: Ciprofloxacin (CIP5mcg), Gentamycin (GEN 10mcg) Tobramycin (TOB 10mcg); Doxycyline(DO 30mcg); Tetracycline (TE 15mcg); Ampicillin/sublactam(A/S 10/10 mcg).

RESULTS

General prevalence of bacterial disease in Pangas fish

Out of 36000, fish samples the estimated prevalence of bacterial disease in pangas fish was 27 per 10000 populations shown in figure 1.

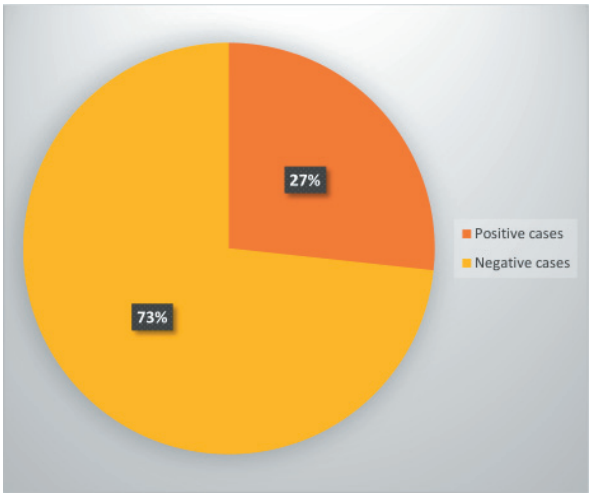

Figure 1: Prevalence of bacterial disease in Pangas fish

Farm-wise prevalence of bacteria

The study revealed that Farm 4, was most infected by bacterial diseases in pangas fish with a prevalence rate of 0.3%. Farm 1, was the least with the prevalence rate of 0.23% as compared to other four farms examined.

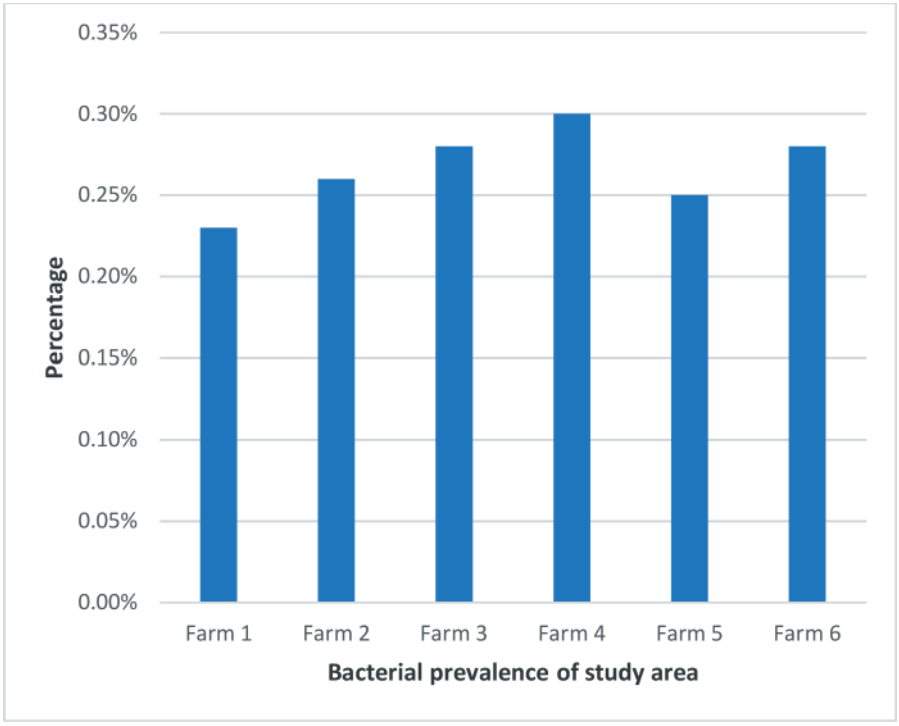

Figure 2: Farm-wise prevalence of bacteria

Distribution of isolates based on type of sample

Out of 120 swab samples examined, 60 samples were of liver and 50 samples were of kidney. Out of 60 samples examined from liver, 76.6% (46/60) samples were found to be positive with bacterial isolates among which 47.8% (22/46) were isolated from asymptomatic samples and 52.1% (24/46) was from symptomatic swab sample. Likewise, out of 60 samples examined from kidney, 85% (51/60) samples were found to be positive for bacterial isolates.43.1% (22/51) positive samples were from asymptomatic swab samples and 56.8% (29/51) was from symptomatic swab samples.

Table 2: Distribution of bacterial isolates based on type of sample

Sample	Total examined samples	Total positive cases	Asymptomatic samples	Symptomatic sample
			Positive cases	Positive cases
Liver	60	46(77%)	22(47.8%)	24(52.1%)
Kidney	60	51(85%)	22(43.1%)	29(57%)
Total	**120**	**97(80%)**	**44(45.3%)**	**53(55%)**

Identification of isolates from Pangas fish samples

Out of 97(80%) positive samples, 57.7% (56/97) were infected with *Edwardsiella ictaluri*, followed by 24.7% (24/97) for *Aeromonas hydrophila*, and 17.5% (17/97) for *Pseudomonas fluorescence*. Out of 56 *Edwardsiella ictaluri* positive samples, 39.2% (22/56) were from asymptomatic sample and 60.7% (34/56) was from Symptomatic samples. Similarly, out of 24 *Aeromonas hydrophila* positive samples 54.1% (13/24) were from asymptomatic samples and 45.8% (11/24) was from symptomatic samples. Likewise, 17 *Pseudomonas fluorescence* positive samples 52.9% (9/17) were from asymptomatic and 47% (8/17) was from symptomatic samples.

Table 3: Identification of isolates from pangas fish samples

Species of bacteria identified	Total no. of bacteria found	Asymptomatic samples	Symptomatic samples
Edwardsiella ictaluri	56(57.7%)	22(39.2%)	34(60.7%)
Aeromonas hydrophila	24(24.7%)	13(54.1%)	11(45.8%)
Pseudomonas fluorescence	17(17.5%)	9(52.9%)	8(47%)

Antibiogram profile of bacterial isolates

In this study, all of the isolated pathogenic bacteria from both symptomatic and asymptomatic were 97/97(100%) resistant to Ampicillin, and 82/97(84.5%) resistant to tetracycline and all the strain showed sensitive to Ciprofloxacin 97/97(100%) and 84/97 (86.5%) sensitive to Gentamicin and intermediate 59/97 (60.8%) to Tobramycin and 63/97(64.9%) to Doxycycline.

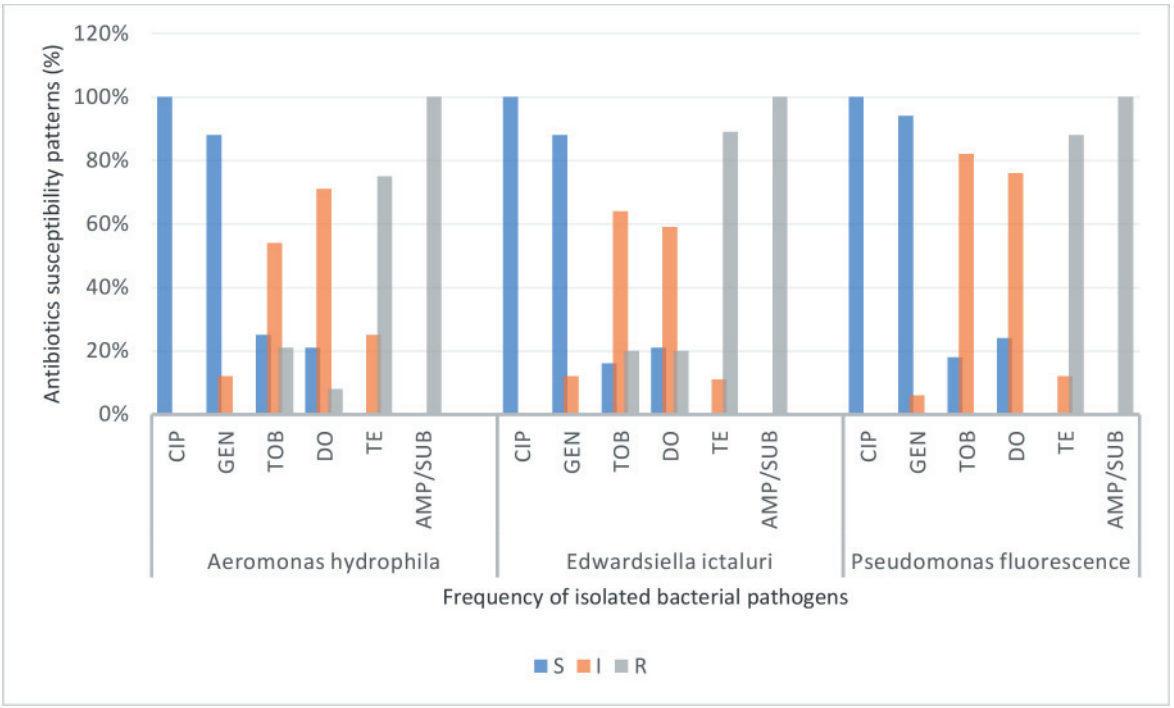

Figure 2: Antibiogram profile of bacterial isolates (CIP= Ciprofloxacin; GEN =Gentamycin; TOB=Tobramycin; DO= Doxycyline; E=Tetracycline; AMP/SUB=Ampicillin/sublactam; S = Sensitive; I = Intermediate; R= Resistance)

DISCUSSION

The present study indicated that the prevalence of bacterial diseases in Pangas fish was remarkable. Out of 36,000 fish of targeted population, altogether a total of 120 samples were examined which showed the prevalence of bacterial disease in 27 per 10000 population. The study revealed that farm 4 (Siraha Province 2) was highly infected (0.3%) by bacterial diseases in pangas fish and farm 1 (Chitwan Bagmati Province), was the least (0.23%) as compared to other four farms examined. The reason behind this may be due to the handling and sanitization protocol of fish farming. There is another farm from Chitwan Bagmati Province farm 6 which was showing second highest infection rate (0.28%) among all six farms.

According to our results, out of 120 fish samples, 97 were found to be positive with different bacterial isolates. *Edwardsiella ictaluri* 56(57.7%) was the most prevalent bacteria isolated compared to two other isolates. *A. hydrophila* 24(24.7%) and *P. fluorescence* 17(17.5%). Overall prevalence rate among the Pangas showed comparatively higher than several studies which showed 50% prevalence rate (Sreedharan *et.al*. 2013; Eissa et al. 2015; Hassan et al. 2017).

Pangasius fish faces different types of problems like diseases caused by bacterial, viral and fungal pathogens which leads to high mortality of *P. hypophthalmus* in cultured ponds. However, clinical symptoms, similar to the other studies (Hawke et al. 2002) were loss of equilibrium, skin lesions, hemorrahages, body and tail rots, congestion and enlargement with hemorrhage of the internal organs such as body cavity and abdomen. The bacteria isolated in this from the fish samples were *Edwardsiella* spp, *Aeromonas* spp, and *Pseudomonas* spp which were also similar results reported as (Md. Abdullah et al. 2020).

The study on bacterial diseases of pangasiandon cultured in earthen pond of Vietnam clearly showed that the majority of infection was caused by *Edwardsiella ictaluri*counting for 87.9% of all isolates (Ho et al. 2008) which is similar to this study with the majority isolates for *Edwardsiella* of 57.73% and slightly different in biochemical tests, *E. ictaluri* was first reported by WHO had isolated and described this bacteria from ponds in U.S.A and named this disease "Enteric Septicemia of Catfish" (Am et al. 1966). ESC was believed to be infectious only to ictaluids. The susceptibility was high which indicated that *P. hypophthalmus* was highly susceptible to these enteric bacteria (Plumb et al. 1983). This disease was firstly reported in *Pangasius* spp. In Vietnam in 2001, including several species of bacteria from affected fish i.e. *Bacillus* causing the diseases "bacillary necrosis" (Fergusn et al. 2001). The same pathogen was isolated in Vietnam with slightly different biochemical characteristics from UK (Crumlish et al. 2002). Both reports indicated the same clinical sign affected fish. According to our results, *E. ictaluri* is the most prevalent bacteria isolated. However, in a study conducted by (Sreedharan et al. 2013) the most prevalent isolates were *A. veronii* in Panga which is not well reported.

Aeromonas hydrophila was second highest isolated pathogenic bacteria that could cause diseases in *P. hypophthalmus*, but the prevalence of this Aeromonads septicemia was low in this study i.e. 24.7% which is comparatively low than *E. ictaluri*. In a study conducted by (Md. Abdullah et al. 2020) the prevalence was very low that is (3.03%) with similar biochemical results. The external and internal clinical signs of these two diseases were quite similar including hemorrhage in the infected organs, and swollen eyes (Angka et al. 1995). In this study, some *Pseudomonas* strains were also isolated and identified from farmed fishes in Nepal which counted for 17.4%. In a study by (Khatun et al. 2011) several Pseudomonad isolates were identified from diseased fish from water bodies. It showed more or less similar morphology and biochemical characteristics results similar to diseased shing fish. (Monir et al. 2017).

The test organisms used were resistance to the antibiotics such as Ampicillin, and Tetracycline. These antibiotics are commonly used to treat pathogens in isolated (CLSI Guideline 2017). The organisms showed to be resistant to these antibiotics with visibly no zone of inhibition. And sensitive to the ciprofloxacin and Gentamicin with clear zone of inhibition. In a study by (Md. Abdullah et al. 2020) all the isolates showed sensitive to Ciprofloxacin (100%), intermediate to Azithromycin (70%), Erythromycin (65%) and resistance to tetracycline an ampicillin. Azithromycin was intermediate to all isolates except *Pseudomonas* spp which were sensitive against it.

In disk diffusion of antibiotic assay *Aeromonas* spp, *Edwardsiella* spp, and *Pseudomonas* spp isolates were found to be sensitive to Ciprofloxacin (100%) and

Gentamicin (86.5%) and intermediate to Tobramycin (60.8%) and Doxycycline (64.9%) and resistance to tetracycline (84.5%) and Ampicillin (100%). *Edwardsiella* was highly resistance to the tetracycline compared to other isolate and sensitive to Gentamicin. In a study by Khatun et al. 2011, *A. hydrophila, F. coumnare, Edwardsiella* spp and *Pseudomonas* spp. from diseased Shing (*H. fossilis*) were found sensitive to Ciprofloxacin and moderately sensitive to gentamycin and ampicillin/sulbactam, but resistance to tetracycline and doxycycline.

In another study, *S. agalactiae, F. columnare, E. tarda, Aeromonas* spp isolated from Nile tilapia showed sensitive to tetracycline and Ciprofloxacin, moderately sensitive to Azithromycin and Streptomycin and resistant to Ampicillin (Hamom et al. 2020).

CONCLUSIONS

The findings of this study showed that pathogenic bacteria such as *E. ictaluri, A. hydrophila,* and *Pseudomonas fluorescence* are the major cause of bacterial diseases of this species. Ciprofloxacin (5µg) and Gentamycin (10µg) could be administrated to control of diseases in *P. hypophthalmus.*

ACKNOWLEDGEMENTS

We would like to express our gratitude to entire team of National Fisheries Research Center, who made available every material and place for our study and financial support. Also like to thank Microbiology Department, D.A.V. College for support throughout the work.

CONFLICT OF INTREST

Authors declared no conflict of interest.

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