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Prevalence of diseases caused by *Flavobacterium* spp. and other opportunistic bacteria in carps of sewage-fed farms in West Bengal, India

Sudeshna Sarker • Thangapalam Jawahar Abraham • Avijit Patra

Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata - 700094, India

Correspondence
Prof Thangapalam Jawahar Abraham; Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata - 700094, India.
abrahamtj1@gmail.com

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Abstract

India is the second largest fish producing nation after China, contributing about 5.68% of the global fish production. The state West Bengal is occupying the second position in freshwater fish production after Andhra Pradesh. Although a number of constraints have been put forth, diseases and poor farm management are some of the most noticeable reasons for the reduced fish production in West Bengal. This study reports the prevalence of diseases caused by *Flavobacterium* spp. and other opportunistic bacteria in carps of sewage-fed farms in West Bengal. The bacteriological examination of the diseased carps revealed infections with different bacterial species and most of them were opportunistic pathogens. Flavobacteriosis, aeromoniasis, pseudomoniasis and other mixed bacterial infections, as confirmed by conventional biochemical tests and by VITEK 2 Compact system, were noted frequently. Antibiotic sensitivity of the opportunistic bacterial pathogens from the diseased carps indicated that some of these bacterial strains are resistant to potential human medicines like erythromycin, cotrimoxazole, oxytetracycline, nitrofurantoin, etc., which is a serious cause for concern. These results further present the fact that sewage-fed fish farms and the food fish from such systems may pose a serious public health risk from the antibiotic-resistant bacteria, if not handled properly.

Keywords: Sewage-fed fish farm; carps; opportunistic pathogens; *Flavobacterium* spp.; antibiotic sensitivity

1 | INTRODUCTION

Water quality strongly affects fish health in the aquaculture system. Deteriorated water quality and compromised biological factors would cause disease occurrence in cultured fish. Wastewater aquaculture practices are increasing in countries like China, India, Indonesia and Vietnam in large-scale systems mainly for carp culture (FAO 2018). The production of fish in sewage-fed aquaculture system is considered to be at least four times more than the production of fish in normal water (Ray-Chaudhuri *et al.* 2012). Yet, the investigations in wastewater fish culture wetlands revealed that fish growth and production are affected by various stressors (Das 2018). One of the major stressors is opportunistic bacteria, which will take advantage of poor water condition of the sewage-fed water body. Opportunistic bacterial pathogens are responsible for mixed bacterial infections and also mixed infections with parasites and fungi. *Flavobacterium* is the remarkable one among all opportunistic bacteria that cause gill disease in carps and other freshwater finfish (Sarker *et al.* 2017). Other than *Flavo-*

bacterium spp., *Aeromonas*, *Brevundimonas*, *Flectobacillus*, *Pseudomonas*, *Spingomonas*, *Shewanella* etc. also have an important role as opportunistic pathogens (Austin and Austin 2012). The present communication reports the diseases caused by *Flavobacterium* spp. and other opportunistic bacteria in carps of sewage-fed farms in West Bengal, India.

2 | METHODOLOGY

Healthy and diseased carp and other cyprinid samples for this study were collected from different sewage-fed fish farms located in Haripota, Kantipota, and Sonarpur of South 24 Parganas district, Mogra and Serampore of Hooghly district, Chakgaria, Patuli and Salt Lake of Kolkata district, West Bengal, India (Figure 1). A total of 39 sample lots of diseased, as well as healthy carps and cyprinids, *viz.*, *Labeo rohita* (*n* = 4), *Catla catla* (*n* = 10), *Cirrhinus mrigala* (*n* = 8), *L. bata* (*n* = 1), *Hypophthalmichthys molitrix* (*n* = 3), *Cyprinus carpio* (*n* = 4), and *Carassius auratus* (*n* = 9) from South 24 Parganas, Hooghly and Kolkata districts were investigated according to Heil (2009) between 2014 and 2015. The bacteriological samples from the gills, kidney and lesions were aseptically inoculated onto the Cytophaga agar (CA), Shieh agar (SA), selective Cytophaga agar (SCA) and selective Shieh agar (SSA) with or without antibiotics such as neomycin sulfate 5 µg/ml and polymyxin B 200 IU/ml (Farmer 2004; Sarker *et al.* 2017) followed by 24–48 h incubation at 30°C for the isolation of *Flavobacterium* spp. Tryptic soy agar (TSA) was also used to isolate other opportunistic bacterial pathogens. The colonies with distinct characteristics, pigmentation etc. were picked, purified and identified on the basis of biochemical characterization by conventional biochemical tests (Bertolini and Rohovec 1992; Griffin 1992; Collins *et al.* 2004; Austin and Austin 2012) and by an automated bacterial identification system (VITEK-2 compact, bioMérieux, France) per the manufacturer's protocol.

The genotypic characterization of select bacterial isolates (*n* = 6) was done by 16S rDNA gene sequencing. The genomic DNA of the bacterial isolates was extracted by using a genomic DNA isolation kit (Macherey-Nagel, Germany) as per the manufacturer's protocol. The universal primers (forward primer 8F 5'-AGAGTTTGAT CCTGGCTCAG-3' and reverse primer 1492R 5'-ACGGCTACCTTGTTACGACTT-3') of amplification size 1400 bp were used (Eden *et al.* 1991). The 16S rDNA gene was amplified through PCR reaction that was performed in a Master cycler Pro S system (Eppendorf, Germany) as per Sarker *et al.* (2016, 2017). The PCR amplified products were sequenced at the Genomics Division, Xcelris Labs Ltd, Ahmedabad, India. The edited sequences were compared against the GenBank database of the National Center for Biotechnology Information (NCBI) by using the BLAST (Basic Local Alignment Search Tool) program (http://blast.ncbi.nlm.nih.gov).

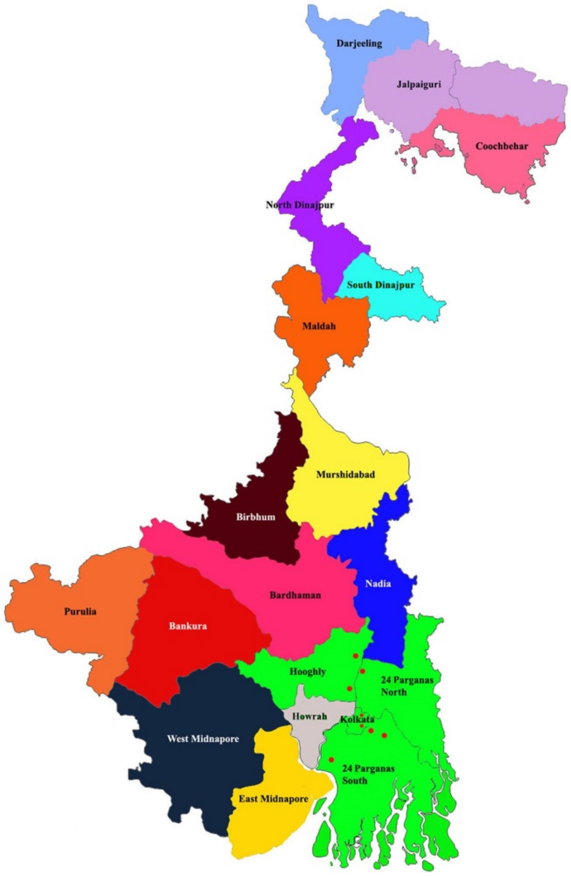

FIGURE 1 The location of the sampling areas (●) in different districts (light green) of West Bengal

Antibiotic sensitivity of select bacterial strains were tested against 12 antibiotic-impregnated discs, viz., chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), gentamicin (10 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg), co-trimoxazole (25 µg), vancomycin (30 µg), sulphafurazole (300 µg), gatifloxacin (5 µg), amoxyclav (30 µg) and erythromycin (15 µg) as per Bauer *et al.* (1966).

3 | RESULTS AND DISCUSSION

The diseased carps exhibited gross and clinical signs like white patches on the gills, excessive mucus secretion, caudal peduncle lesions, tail rot, focal cutaneous haemorrhages, body and opercula haemorrhages, exophthalmia, scale loss, dropsy, fluid accumulation in scale pockets, ulcers, skin discoloration, skin peeling, saddle back and emaciation. Several of these gross and clinical signs resembled the descriptions of flavobacteriosis and other related diseases (Bernardet and Bowman 2006; Loch and Faisal 2015).

The phenotypic characterization of the isolated bacteria through VITEK 2 and other conventional tests are tabulat-

ed in Tables 1–3. The identity of six bacterial strains was confirmed by molecular characterization (Table 4). The phenotypic identification by VITEK 2 contradicted with the molecular identification of the bacterial strains. According to Peix *et al.* (2003), the identification of non-clinical isolates is often wrong with the VITEK 2 system because of the lack of database in the system software. Therefore, the VITEK 2 data was used only to characterize the bacterial strains phenotypically.

TABLE 1 Biochemical characterization of bacterial isolates from flavobacteriosis and related diseased fish by conventional tests as per the criteria of Griffin (1992), and Bertolini and Rohovec (1992)

Bacterial strains	***Conventional tests***							
	Casein	***Chondroitinase sulphate***	***Congo red***	***Fibrinogen***	***Flexirubin pigment***	***Gelatin***	***Growth in selective media#***	***Colony colour and morphology***
Flavobacterium sp. CBH1	+	-	-	-	+	+	+	Yellow, round, convex
Brevundimonas dimunuta CC1WG2.1	+	-	-	-	-	-	-	Yellow, round, convex
Flavobacterium sp. CC2WG7	+	-	-	-	-	-	+	Yellow, round, uneven edges, glossy
Shewanella putrefaciens CWG5	+	-	ND	ND	-	+	-	Light orange cantered, round
Flavobacterium sp. KG3	+	-	+	-	-	-	+	Yellow, round, convex
Brevendimonas diminuta M2AH2	-	-	-	+	-	-	-	Light yellow, round, irregular edges, convex
Flavobacterium sp. M2AH4*	+	-	-	+	-	-	-	Light yellow, round, irregular edges, convex
Flavobacterium sp. MG1	-	-	-	-	-	-	-	Light greenish yellow, irregular edges, non-glossy
Rheinheimera sp. RCG1	+	-	ND	-	-	+	-	Light brownish orange centred, round, convex

*+, positive reaction; -, negative reaction; #, growth in presence of neomycin sulfate and polymyxin B; ND, not done; *, Vitek 2-Compact (bioMérieux, France) identified this strain as Aeromonas salmonicida*

TABLE 2 Biochemical characterization of Gram-negative bacteria isolated from flavobacteriosis and related diseased fish by conventional tests and Vitek 2-Compact system (*bioMérieux,* France)

Biochemical characteristics	***Bacterial strains and reactions***											
	CBH1	***KG3***	***CLC***	***MMU1***	***MMU3***	***MSG1***	***MSG2***	***MG1***	***M2AH2***	***M2AH4***	***TGB***	***CC1WG2.1***
Conventional tests												
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-
Morphology	R	LR	R	R	R	R	R	R	R	R	R	LR
Oxidase	+	-	-	-	-	-	+	-	-	-	+	+
O/F reaction	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Vitek 2-Compact system												
5-Keto D-gluconate (5KG)	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol (ADO)	-	-	-	-	-	-	-	-	-	-	-	-
Ala-Phe-Pro-arylamidase (APPA)	+	+	+	-	+	+	+	-	+	-	+	+
Alpha-galactosidase (AGAL)	-	-	-	+	-	+	-	-	-	-	-	-
Alpha-glucosidase (AGLU)	+	-	+	+	+	+	+	-	-	-	-	+
Beta-alanine arylamidase pNA (BAlap)	-	-	-	-	-	-	-	-	-	-	-	-
Beta-galactosidase (BGAL)	-	-	+	+	-	+	-	-	-	-	-	-

Continued

TABLE 2 Continued.

Biochemical characteristics	***Bacterial strains and reactions***											
	CBH1	***KG3***	***CLC***	***MMU1***	***MMU3***	***MSG1***	***MSG2***	***MG1***	***M2AH2***	***M2AH4***	***TGB***	***CC1WG2.1***
Beta-glucoronidase (BGUR)	-	-	-	-	-	-	-	-	-	-	-	-
Beta-glucosidase (BGLU)	(+)	+	+	+	-	+	-	-	-	-	-	+
Beta-xylosidase (BXYL)	-	-	-	-	-	-	-	-	-	-	-	-
Citrate (sodium) (CIT)	-	-	-	-	-	-	-	-	-	-	-	-
Coumarate (CMT)	+	-	(+)	+	+	+	-	-	-	+	-	-
D-Cellobiose (dCEL)	-	-	-	+	-	+	-	-	-	-	-	-
D-Glucose (dGLU)	+	-	-	+	+	+	-	-	-	-	-	-
D-Maltose (dMAL)	-	+	-	+	-	+	+	-	-	-	-	-
D-Mannitol (dMAN)	-	-	-	+	-	+	-	-	-	-	-	-
D-Mannose (dMNE)	-	-	-	-	-	-	-	-	-	-	-	-
D-Sorbitol (dSOR)	-	-	-	-	-	-	-	-	-	-	-	-
D-Tagatose (dTAG)	-	-	-	-	-	-	-	-	-	-	-	-
D-Trehalose (dTRE)	-	-	-	+	-	+	-	-	-	-	-	-
Ellman (ELLM)	-	-	+	-	-	-	+	-	-	-	-	-
Fermentation/ glucose (OFF)	-	-	-	-	-	-	-	-	-	-	-	-
Gamma-glutamyl transferase (GGT)	-	-	-	-	-	-	+	-	+	-	+	-
Glu-Gly-Arg-arylamidase (GGAA)	+	-	+	-	+	+	+	-	+	-	+	-
Glutamyl arylamidase pNA (AGLTp)	+	+	+	-	+	+	+	-	-	-	+	+
Glycine arylamidase (GlyA)	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production (H_2S)	-	-	-	-	-	-	+	-	-	-	+	-
L Pyrrolydonyl-arylamidase (PyrA)	-	+	(-)	+	-	-	+	-	-	-	-	-
L-Arabitol (IARL)	-	-	-	-	-	-	-	-	-	-	-	-
L-Histidine assimilation (IHISa)	-	-	-	-	-	-	-	-	-	-	-	-
Lipase (LIP)	-	(-)	+	-	-	-	-	-	+	-	-	+
L-Lactate alkalinisation (ILATk)	-	-	-	-	-	+	+	-	-	-	+	-
L-Lactate assimilation (ILATa)	-	-	-	-	-	-	-	-	-	-	-	-
L-Malate assimilation (IMLTa)	-	-	-	-	-	-	-	-	-	-	-	-
L-Proline arylamidase (ProA)	-	(-)	+	-	-	-	+	+	+	-	-	-
Lysine decarboxylase (LDC)	-	-	-	-	-	-	-	-	-	-	-	-
Malonate (MNT)	-	-	-	-	-	-	-	-	-	-	-	-
O/129 Resistance (O129R)	-	-	-	+	-	+	-	-	-	-	-	-
Orinithine decarboxylase (ODC)	-	-	-	-	-	-	+	-	-	-	-	-
Palatinose (PLE)	-	-	-	-	-	-	-	-	-	-	-	-
Phosphatase (PHOS)	(-)	+	+	-	-	-	+	-	+	-	+	+
Saccharose/Sucrose (SAC)	-	-	-	+	-	+	+	-	-	-	+	-
Succinate alkalinisation (SUCT)	-	-	-	-	-	-	+	-	-	-	+	-
Tyrosine arylamidase (TyrA)	+	+	+	+	+	+	+	+	-	-	+	+
Urease (URE)	-	-	-	-	-	+	-	-	-	-	-	-
β-N-acetyl-galactosaminidase (NAGA)	-	-	-	-	-	-	+	-	-	-	-	-
β-N-Acetyl-glucosaminidase (BNAG)	-	-	-	-	-	-	+	-	-	-	-	-

+, positive reaction; (+), weakly positive reaction; -, negative reaction; R, rod; LR, long rod; CBH1, Spingomonas paucimobilis; KG3, Spingomonas paucimobilis; CLC, Brevundimonas diminuta; MMU1, Spingomonas paucimobilis; MMU3, Spingomonas paucimobilis; MSG1, Spingomonas paucimobilis; MSG2, Shewanella putrefaciens; MG1, Flavobacterium sp.*; M2AH2, Brevundimonas diminuta; M2AH4, Aeromonas salmonicida; TGB, Shewanella putrefaciens; CC1WG2.1, Brevundimonas diminuta*

TABLE 3 Biochemical characterization of Gram-positive rod shaped bacteria isolated from diseased fish by conventional tests and Vitek 2-Compact system (*bioMérieux,* France)

Biochemical characteristics	*Alicyclobacillus acidoterrestris SG4W2*	*Lysinibacillus sphaericus GDH1*
Alamine Arylamidase (AlaA)	+	+
Ala-Phe-Pro-arylamidase (APPA)	+	+
Alpha-galactosidase (AGAL)	-	-
Alpha-Mannosidase (AMAN)	-	-
Beta-galactosidase (BGAL)	+	-
Beta-glucosidase (BGLU)	-	-
Beta-Mannosidase (BMAN)	-	-
Beta-xylosidase (BXYL)	-	-
Cyclodextrin (CDEX)	-	-
D- Galactose (dGAL)	-	-
D-Glucose (dGLU)	-	-
D-Mannitol (dMAN)	-	-
D-Mannose (dMNE)	-	-
D-Melezitose (dMLZ)	+	-
D-Ribose (dRIB)	-	-
D-Tagatose (dTAG)	-	-
D-Trehalose (dTRE)	-	-
Ellman (ELLM)	+	-
Esculin hydrolysis (ESC)	-	-
Glycine arylamidase (GlyA)	-	-
Glycogen (GLYG)	-	-
Growth in 6.5 % NaCl (NaCl 6.5%)	-	-
Inulin (INU)	-	-
Kanamycin resistance(KAN)	-	-
L Pyrrolydonyl-arylamidase (PyrA)	-	-
L-Aspartate Arylamidase (AspA)	-	+
Leucine-Arylamidase (LeuA)	+	+
L-Lysine-Arylamidase (LysA)	(-)	
L-Proline arylamidase (ProA)	+	-
L-Rhamnose (lRHA)	-	-
Maltotriose (MTE)	-	-
Methyl-A-D-Xyloside (MdG)	-	-
Methyl-D-xyloside (MdX)	-	-
Myo-Inositol (INO)	-	-
N-Acetyl-D-Glucosamine (NAG)	+	-
Oleandomycin resistance (OLD)	-	-
Palatinose (PLE)	-	-
Phenylalanine Arylamidase (PheA)	-	+
Phosphoryl choline (PHC)	-	-
Polymixin_B resistance (POLYB_R)	-	-
Putrescine assimilation (PSCNa)	-	-
Pyruvate (PVATE)	-	(+)
Tetrazolium red (TTZ)	-	-
Tyrosine arylamidase (TyrA)	(-)	+
β-N-Acetyl-glucosaminidase (BNAG)	-	+

+, positive reaction; (+), weakly positive reaction; -, negative reaction; (-), weakly negative reaction

Mixed bacterial infection was noticed in 48.72% of the samples screened. Gram-negative bacterial species with long rods such as *Flavobacterium* spp., Gram-negative short rods such as *Shewanella putrefaciens*, *Brevundimonas diminuta*, *Pseudomonas* spp., *Rheinheimera* spp., *Spingomonas paucimobilis*, *Acinetobacter lwoffii*, *Aeromonas sobria*, *A. tecta*, *A. caviae*, *A. veronii* biovar *sobria*, *A. sanavelli*, *A. hydrophila* and *A. veronii* biovar *veronii*, Gram-positive rods such as *Alicyclobacillus acidoterrestris*, *Bacillus megaterium* and *Lysinibacillus sphaericus* were identified from the diseased carps. Besides, few unidentified Gram-negative long rods, Gram-negative short rods and Gram-negative stout rods were also isolated from the diseased carps with or without mixed bacterial infection. Out of 39 cases, 23 were diagnosed as flavobacteriosis with or without mixed bacterial infection and the rest were gill rot or gill disease, aeromoniasis, pseudomoniasis and bacillus infection. These findings were clearly enlightening the opportunistic potentials of the *Flavobacterium* spp. and other bacteria in carps of sewage-fed fish farms. The present results corroborate several earlier studies (Marks *et al.* 1980; Inglis *et al.* 1993; Hawk and Thune 1992; Decostere *et al.* 1996; Abraham *et al.* 2017).

A total of 16 bacterial strains from the flavobacteriosis and other diseased carps were subjected to antibiogram against 12 antibiotics. As depicted in Figure 2, the bacterial flora exhibited varying degrees of sensitivity to different antibiotics. All of them were sensitive to gentamicin and ciprofloxacin. The sensitivity to gatifloxacin (88%), chloramphenicol (81%) and amoxyclav (75%) were observed among the majority of the bacterial strains. Likewise, Rajpakshe *et al.* (2012) recorded high sensitivity of bacterial flora towards gatifloxacin, moxifloxacin, ciprofloxacin and levofloxacin. The potential therapeutic role of gatifloxacin, gentamicin and others against bacterial pathogens has been amply documented (Ronald *et al.* 1999; Ibrahim *et al.* 2010) so also in this study (Figure 2). On the other hand, resistance to vancomycin, oxytetracycline, erythromycin and clindamycin was seen among 44–50% of the bacterial flora of diseased carps. Similarly, Mohammed and Arias (2014) reported the resistance of *Flavobacterium* strains to oxytetracycline. According to FAO/OIE/WHO (2006) oxytetracycline, an effective antibiotic in human medicine is one of the most commonly used antibiotics for the treatment of bacterial infections in commercially raised fish. Among the 16 strains tested, 10 strains including *Flavobacterium* spp., *Spingomonas paucimobilis*, *Shewanella putrefaciens* and *Brevendimonas diminuta* were observed as multiple antibiotic resistant (MAR), which is a serious cause for concern. Similarly, earlier studies also documented the involvement of MAR bacteria in disease occurrences (Bloch *et al.* 1997; Michel *et al.* 2005). Declercq *et al.* (2013) reported MAR

Flavobacterium strains; while Clark et al. (2006; 2009) documented several MAR strains of fish pathogenic *Flavobacterium*. These results, thus, indicated the prevalence of bacterial flora resistant to these antibiotics in the culture system itself or the entry of these bacteria resistant to human antibiotics through the waste/sewage (source) water or possible abuse of the human antibiotics in such systems to control fish diseases. Since the fish farming systems of the present study are mainly sewage-fed, possibly the sewage might be the major source of these antibiotic-resistant bacteria. These results further present the fact that sewage-fed fish farming system and the food fish from such a system may pose a serious public health risk from the antibiotic-resistant bacteria, if not handled properly.

TABLE 4 Molecular characterization of bacterial strains isolated from flavobacteriosis and related diseased fish

Fish species	*Clinical sign*	*Site of infection*	*Strain code*	*16S rDNA gene sequence length (bp)*	*NCBI GenBank Accession number*	*Identification*
Catla catla	Cutaneous lesions	Body surface	CBH1	1403	KP997178	*Flavobacterium* sp.
Catla catla	White patches on gill	Gill	KG3	1402	KP997186	*Flavobacterium* sp.
Catla catla	White patches on gill	Gill	CWG5	1307	KU851956	*Shewanella putrefaciens*
Cyprinus carpio	White patches on gill	Gill	CC1WG2.1	1339	KU851955	*Brevundimonas diminuta*
Labeo rohita	White patches on gill	Gill	RCG1	1431	Submitted	*Rheinheimera* sp.
Catla catla	Caudal peduncle lesions	Caudal peduncle	CLC	1154	Do	*Brevundimonas diminuta*

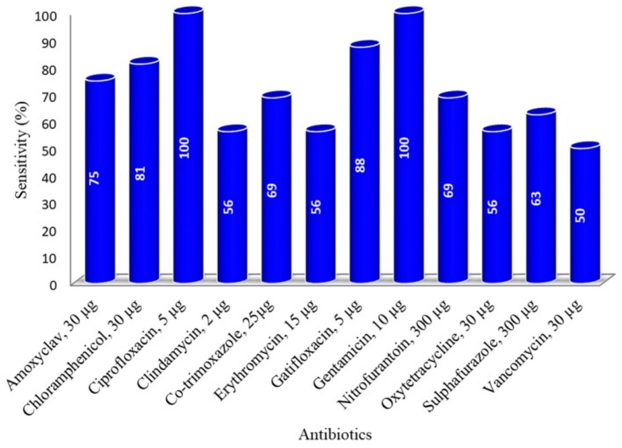

FIGURE 2 Antibiotic sensitivity (%) of the bacterial flora associated with flavobacteriosis diseased fish

4 | CONCLUSION

In general, the results of the present study provided a basis upon which we can understand the role of these opportunistic pathogens of flavobacteriosis and other related diseases and their management in sewage-fed aquaculture systems. The antibiogram results further revealed that the MAR bacteria are part of the normal flora of sewage-fed aquaculture system, possibly brought in to the pond through the contaminated incoming water. This is a serious cause for concern as these bacterial population spread through water circulation.

TABLE 5 Multiple antibiotic resistance (MAR) index of bacterial flora associated with flavobacteriosis diseased fish

Bacterial strains	*MAR Index*
Brevendimonas diminuta M2AH2	0.083
Brevundimonas diminuta CC1WG2.1	0.000
Brevundimonas dimunuta CLC	0.167
Flavobacterium sp. C1GH1	0.333
Flavobacterium sp. CC2WG6	0.000
Flavobacterium sp. CC2WG7	0.083
Flavobacterium sp. KG3	0.000
Flavobacterium sp. M2AH4	0.250
Flavobacterium sp. MG1	0.583
Flavobacterium sp. MMU4	0.667
Flavobacterium sp. CBH1	0.000
Shewanella putrefaciens CWG5	0.250
Shewanella putrefaciens MSG2	0.333
Spingomonas paucimobilis MMU1	0.417
Spingomonas paucimobilis MMU3	0.417
Spingomonas paucimobilis MSG1	0.833

**MAR Index: Number of antibiotics to which the bacterium is resistant ÷ Total number of antibiotics tested; MAR: Resistant to at least 3 antibiotics.*

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abraham TJ, Sarker S, Dash G, Patra A and Adikesavalu H. (2017) *Chryseobacterium* sp. PLI2 and *Aeromonas hydrophila* co-infection in pacu, *Piaractus brachypomus* (Cuvier, 1817) fries cultured in West Bengal, India. Aquaculture 473: 223–227.

Austin B and Austin DA (2012) Bacterial fish pathogens. Disease of farmed and wild fish, 5th edition. Springer, Heidelberg. 652 pp.

Bauer AW Kirby WMM, Sherris JC and Turck M (1966) Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 45(4): 493–496.

Bernardet JF, Hugo C and Bruun B (2006) The genera *Chryseobacterium* and *Elizabethkingia*. In: The prokaryotes pp. 638–676, Springer, New York.

Bertolini JM and Rohovec JS (1992) Electrophoretic detection of proteases from different *Flexibacter columnaris* strains and assessment of their variability. Diseases of Aquatic Organisms 12: 121–128.

Bloch KC, Nadarajah R and Jacobs R (1997) *Chryseobacterium meningosepticum*: an emerging pathogen among immunocompromised adults. Medicine (Baltimore) 76: 30–41.

Collins CH, Lyne PM, Grange JM and Falkinham III JO (2004) Collins and Lyne's Microbiological methods, 8th edition. Arnold, London. 456 pp.

Das MK (2018) Fish diseases in wastewater aquaculture and remedial measures. In: Jana B, Mandal R and Jayasankar P (eds), Wastewater management through aquaculture (pp. 139–159) Springer, Singapore.

Declercq AM, Boyen F, Van den, Broeck W, Bossier P, Karsi A, Haesebrouck F and Decostere A (2013) Antimicrobial susceptibility pattern of *Flavobacterium columnare* isolates collected worldwide from 17 fish species. Journal of Fish Diseases 36: 45–55.

Decostere A, Haesebrouck F, Devriese L and Ducatelle R (1996) Identification and pathogenic significance of *Shewanella* sp. from pond fish. Vlaams Diergeneeskd Tijdschr 65: 82–85.

Eden PA, Schmidt TM, Blakemore RP and Pace NR (1991) Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16SrRNA-specific DNA. International Journal of Systematic Bacteriology 41(2): 324–325.

FAO (2018) The state of world fisheries and aquaculture 2018 - meeting the sustainable development goals. Food and Agriculture Organization of the United Nations, Rome.

FAO/OIE/WHO (2006) Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance: Seoul, Republic of Korea, 13-16 June 2006, WHO, Geneva. 97 pp.

Farmer BD (2004) Improved methods for the isolation and characterization of *Flavobacterium columnare*. Master's thesis, Louisiana State University and Agricultural and Mechanical College.

Griffin BR (1992) A simple procedure for the identification of *Cytophaga columnaris*. Journal of Aquatic Animal Health 4: 63–66.

Hawke JP and Thune RL (1992). Systemic isolation and antimicrobial susceptibility of *Cytophaga columnaris* from commercially reared channel catfish. Journal of Aquatic Animal Health 4: 109–113.

Heil N (Ed) (2009) National wild fish health survey. Laboratory procedures manual, 5th edition. U.S. Fish and Wildlife Service, Warm Springs, GA.

Ibrahim MK, Galal A-MoneimM, Idriss M Al-Turk and Khalid D Al-Zhrany (2010) Antibiotic resistance in gram-negative pathogenic bacteria in hospitals' drain in Al-Madina Al-Munnawara. Journal of Taibah University for Science 3: 14–22.

Inglis V, Roberts RJ and Bromage NR (1993) Bacterial diseases of fish. Blackwell Scientific Publications, Oxford, England.

Loch TP and Faisal M (2015) Emerging flavobacterial infections in fish: a review. Journal of Advanced Research 6: 283–300.

Marks JE, Lewis DH and Trevino GS (1980) Mixed infection in the columnaris disease of fish. Journal of the American Veterinary Medical Association 177(9): 811–814.

Michel C, Matte-Tailliez O, Kerouault B and Bernardet JF (2005). Resistance pattern and assessment of phenicol agents' minimum inhibitory concentration in multiple drug resistant *Chryseobacterium* isolates from fish and aquatic habitats. Journal of Applied Microbiology 99(2): 323–332.

Mohammed HH and Arias CR (2015) Potassium permanganate elicits a shift of the external fish microbiome and increases host susceptibility to columnaris disease. Veterinary Research 46: 82.

Peix A, Rivas R, Mateos PF, Martinez-Molina E, Rodriguez-Barrueco C and Velazquez E (2003) *Pseudomonas rhizosphaerae* sp. nov., a novel species that actively solubilizes phosphate in vitro. International Journal of Systematic and Evolutionary Microbiology 53: 2067–2072.

Rajpakshe ADWR, Prasad KP, Mukherjee SC, Kundan K, Brahmachari RK, Meena CT and Kumar N (2012) In vitro sensitivity of three bacterial pathogens of koi carp (*Cyprinus carpio* L.) to certain antibiotics. Journal of Agricultural Science and Technology 2: 93–98.

Ray-Chaudhuri SI, Mukherjee I, Ghosh D, and Thakur AR (2012) East Kolkata wetland: a multifunctional niche of international importance. Online Journal of Biological Sciences 12(2): 80–88.

Ronald N, Jones MAI, Croco MA, Saller M and Beach KCK (1999) The SENTRY Antimicrobial Surveillance Program Participants (The Americas). Antimicrobial activity evaluations of gatifloxacin, a new fluoroquinolone: contemporary pathogen results from a global antimicrobial resistance surveillance program (SENTRY, 1997). Clinical Microbiology and Infection 5: 540–546

Sarker S, Abraham TJ, Banerjee S, Adikesavalu H, Patra A (2017) Characterization, virulence and pathology of *Flavobacterium* sp. KG3 associated with gill rot in carp, *Catla catla* (Ham.). Aquaculture 468: 579–584.

Sarker S, Patra A, Adikesavalu A, Abraham TJ (2016) Flavobacteriosis in cultured freshwater ornamental telescopic eye goldfish *Carassius auratus*. International Journal of Current Microbiology and Applied Sciences 5(4): 39–46.

CONTRIBUTION OF THE AUTHORS

SS generation of field and laboratory data and analysis of samples; **TJA** conception, experimental design, data analysis, interpretation and manuscript editing; **AP** molecular characterization of bacterial isolates

TJ Abraham https://orcid.org/0000-0003-0581-1307