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Phenotypic and Molecular Identification of Bacterial Species in Indian Major Carps and Exotic Carps from South 24 Parganas, West Bengal, India

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A B S T R A C T

The present study was done to find rapid and accurate identification of bacterial species which will help in prevention and cure of diseases in various aquatic animals. Phenotypic identification of bacterial isolates was done by conventional tests and commercial methods. Antibiotic sensitivity study revealed only gatifloxacin and chloramphenicol were found to be effective, both inhibited 83.33% sensitivity to the bacterial strains. Prevalence study revealed that aeromoniais were the dominant disease. The identities of selected bacterial strains were further confirmed by molecular characterization through 16S rDNA analysis. In 1.2% agarose gel electrophoresis, approximately 1.5 kbp bands were obtained by PCR amplification. In 2% agarose gel electrophoresis, approximately 675 bp and 1100 bp bands were obtained by PCR amplification for two *Flavobacterium columnare* specific primers set. Among the 7 isolates, 2 were identified as *Commonas aquatica* (accession number KT716080) and *Aeromonas hydrophila* (accession number KX455879) by using universal primers. Three isolates were identified as *Flavobacterium columnare* using specific primers by culture dependant and culture-independent methods. The sequence results of other bacterial isolates are awaited. The gene sequences of culture independent samples and culture dependent strains *Commonas aquatica* and *Aeromonas hydrophila* have been submitted to NCBI with accession numbers KX452118, KX452119, KT716080, KX455879, respectively.

Introduction

Carps are the major group of freshwater fish that have a global significance as a source of food and as experimental models for research. Modern fish farming with high stocking densities and intensive production units provide ideal conditions for the invasion and persistence of a range of pathogens (bacteria, viruses, protozoan and metazoan parasites). Infections by these disease-causing agents reduce the condition and survival of fish causing economical losses to farmers. However, treatments unavoidably cause problems related to environmental pollution, drug resistance and health issues, which is environmentally and ecologically sustainable

solutions are now being sought in fish farming. Reaching this long-term objective will depend on the detailed information and knowledge on the ecology of main disease threats (Karvonen *et al.,* 2005).

The development of aquaculture faces a number of problems, of which diseases particularly the emergence of new pathogens represent serious risk for the production of aquatic animals and also for the health of fish farmers and of the consumers of aquaculture products. Carps being the mostly cultured fish species in West Bengal and infectious diseases of cultured freshwater carps are one of the major problems to successful aquaculture, which cause economic losses in aquaculture industry. To study fish diseases, it is important to acquire knowledge on different pathogens, their biology and life cycle. Cultured carps are susceptible to various kinds of diseases, viz., bacterial, fungal, viral, parasitic, environmental and nutritional.

The most common bacteria isolated from carp culture systems are *Aeromonas* sp. and *Pseudomonas* sp. and *Enterobactericeae*. Among *Aeromonas* sp., the most common species are *Aeromonas hydrophila, A. sobria* and *A. caviae*. Other authors (Rehulka, 2002; Taylor, 2003) have depicted *A. hydrophila* and *A. sobria* as causative agents of motile aeromonas septicemia (MAS) in fish and other aquatic animals. Gopalakrishnan (1961) recorded epizootics of infectious dropsy due to *Aeromonas hydrophila* in the three major types of Indian major carps in fish stocking tanks in West Bengal. Ulcerative forms of infection in *Catla catla* (Hamilton) and septicemia in three species of carp were recorded by others (Karunasagar *et al.,* 1986; Karunasagar *et al.,* 1989). Since, carps are the most important freshwater species cultured in West Bengal and proper fish disease monitoring is still lacking as suitable surveillance system is far from reality, so, protection of these fish against diseases is vital to the aquaculture industry.

Therefore, the present study was undertaken to investigate the phenotypic and molecular identification of emerging fish pathogen (bacteria) in Indian Major Carps and Exotic carps from South 24 Parganas district, West Bengal.

Materials and Methods

Sampling area

The present study on the surveillance of diseases in Indian major and exotic carps was carried out for a period of 12 months from January 2016 to December 2016. The diseased fish samples were collected from different fish farms located in different areas of South 24 Parganas district of West Bengal, viz., Chakgaria, Haripota, Katipota, Sonarpur, Bonhooghly, Canning II, Uttardanga, Bamanghata, Bhangar, Gangasagar.

Experimental fish

The experimental fish for the present study include three species of Indian major and exotic carps, viz., *Labeo rohita, Catla catla, Cirrhinus mrigala* and *Hypophthalmichthys molitrix, Ctenopharyngodon idella, Cyprinus carpio* cultured in semi-intensive and intensive farms of the above mentioned areas.

Sampling

On each sampling day, a minimum of 60 Indian major and exotic carps were examined for diseases as per OIE guidelines (2013). Information on behavioural abnormalities, gross and clinical signs was recorded on the sampling sheet. Carps with typical disease symptoms were sampled for different experiments.

Bacteriological analyses

At the laboratory, inocula from each transport media or from the lesions or affected external and internal parts such as gills, intestine, cutaneous lesions and kidney of morbid fish were streaked on to GSPA, RSA, CA, SA and TSA/BHIA plates and incubated at 30±2^{0}C for 24-48 h.

Bacterial isolation and phenotypic characterization

Based on the dominance and definite colony morphology, representative colonies were picked from each plate and purified by repeated streaking on TSA and maintained on TSA slants.

A series of biochemical reactions as described by others (Lechevallier *et al.,* 1980; Collins *et al.,* 2004) were performed to identify bacteria up to genus level.

Taxonomic keys proposed by University of Idaho, USA as per Bergey's Manual of Determinative Bacteriology 9th Edition (Holt *et al.,* 1994) were also consulted for the identification bacterial species.

Taxonomic keys proposed by Arcos *et al.,*'1988 (Arcos *et al.,* 1988) and the current literatures on *Aeromonas* spp. were followed for *Aeromonas* identification (Minana-Galbis *et al.,* 2009; Alperi *et al.,* 2010; Figueras *et al.,* 2011; Austin *et al.,* 2012; Soto-Rodriguez *et al.,* 2013; Chen *et al.,* 2016; Shewan *et al.,* 1960), Bergey's manual (Collins *et al.,* 2004; Holt *et al.,* 1994) were consulted for *Pseudomonas* identification.

Identification of selected bacterial isolates on the basis of biochemical characterization was done by an automated bacterial identification system (VITEK 2 - compact, BioMerieux, France).

Molecular characterization

Bacterial DNA extraction and PCR amplification of 16S rDNA gene

Genotypic characterization of select bacterial isolates was done by 16S rDNA sequencing. The genomic DNA of bacterial isolates were extracted by using genomic DNA isolation kit (Macherey-Nagel, Germany) as per the manufacturer's protocol. The 16S rDNA gene was amplified through PCR reaction that was performed in a Master cycler Pro S system (Eppendorf, Germany). The universal primers (forward primer 8F and reverse primer 1492R) of amplification size 1400 bp were used.

The species specific primers (forward primer Col-72F and reverse primer Col-1260R) of amplification size 800-1000 bp were used. Another set of species specific primers (forward primer ColF and reverse primer ColR) of amplification size 675 bp were also used.

Agarose gel electrophoresis

The PCR products were analysed on 1.2% and 2% agarose (HiMedia, India) gels containing 0.5 µg/ml ethidium bromide in 1X Tris-acetate- EDTA (TAE) buffer.

DNA sequencing and analysis

Seven bacterial isolates were randomly selected for further characterization and identified through 16S rDNA analysis. This assay involved DNA isolation, amplification and sequencing of the gene coding for 16S rDNA, i.e., the 1.5 kbp 16S rDNA from bacterium and 675 bp and 1100 bp specific for *Flavobacterium columnare* bacterium.

The PCR amplified products were sequenced at the Genomics Division, Xcelris Labs Ltd, Ahmedabad, India.

Antibiogram

A total of 42 bacterial strains were screened for their sensitivity to 12 potential antibiotics by agar disc diffusion technique (Bauer *et al.*, 1966; CLSI, 2006) on MHA plates.

Statistical analysis

One-way analysis of variance (ANOVA) using Microsoft excel version 2007 was applied to test the significance of difference in antibiotic sensitivity assay among the antibiotic agents, bacterial species and fish species (Kholil *et al.,* 2015).

Results and Discussion

The phenotypic characteristics of selected Gram negative bacteria and Gram positive bacterial strains from diseased Indian major and exotic carps as determined by an automated bacterial identification system (VITEK 2 Compact, Biomerieux, France) and also conventional biochemical tests. The detailed information on the bacterial strains used, host species, site of infection, bionumbers with identified bacterial strains are listed in Table 1.

The selected bacterial strains were identified by automated bacterial identification system (VITEK 2 Compact, Biomerieux, France) as *Pseudomonas stutzeri*, *Citrobacter freundii*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex, *Pseudomonas fluorescens/ mendocina*, *Aeromonas sobria*, *Pseudomonas putida*, *Yersinia enterocolitica/frederikesenii*, *Klebsiella pneumoniae* sp. *pneumoniae*, *Enterobacter cloacae* complex, *Acinetobacter radioresistens* and *Kocuria rhizophila.* The Gram negative bacterial strains were identified through biochemical test as *Aeromonas schuberti*, *Aeromonas sobria*, *Aeromonas veronii biovar veronii*, *Aeromanas popoffii*, *Aeromonas fluvialis*, *Aeromonas jandaei*, *Aeromonas hydrophila* sub sp. *ranae*, *Aeromonas tecta*, *Aeromonas aquariorum*, *Aeromonas caviae*, *Aeromonas eucrenophila*, *Aeromonas bestiarum*, *Aeromonas veronii biovar sobria*, *Aeromonas hydrophila*, *Enterobacteriaceae*, *Pseudomonas* sp. and *Flavobacterium* sp.

Molecular characterization

16S rDNA gene analysis

Randomly selected 7 bacterial strains were further characterized and identified through 16S rDNA analysis. The detailed information on the bacterial strains used, host species, clinical signs, site of infection, NCBI GenBank accession numbers are listed in Table 2. In 1.2% agarose gel electrophoresis, approximately 1.5 kbp bands were obtained with 16S universal primers for bacterial isolates and in 2% agarose gel electrophoresis, approximately 675 bp and 1100 bp bands were obtained by PCR amplification for *F. columnare* bacterial isolates for two *F. columnare* specific primers having different amplification size by PCR amplification (Fig. 1, 2 and 3). Among the 7 isolates, 2 were identified as *Commonas aquatica* (BUBKT, accession number KT716080) and *Aeromonas hydrophila* (BTRCTR, accession number KX455879) by using universal primers. Three isolates were identified as *Flavobacterium columnare* using *F. columnare* specific primers by culture dependant and culture-independent methods. The sequence results of other bacterial isolates are awaited. The gene sequences of culture independent samples (RG1 and C1) and culture dependent strains *Commonas aquatica* BUBKT and *Aeromonas hydrophila* BTRCTR have been submitted to NCBI with accession numbers KX452118, KX452119, KT716080, KX455879, respectively.

Primers

Primers		Sequence (5'-3')	Amplification size	Reference
Universal primers for bacterial isolates	8F	AGAGTTTGATCCTGGCTCAG	1400 bp	Eden *et al.*, 1991
	1492R	TACGGYTACCTTGTTACGACTT		
Flavobacterium columnare specific primers	Col-72F	GAAGGAGCTT-GTTCCTTT	800-1000 bp	Triyanto *et al.*, 1999
	Col-1260R	GCCTACTTGCGT-AGTG		
	ColF	CAGTGGTGAAATCTGGT	675 bp	Darwish *et al.*, 2004
	ColR	GCTCCTACTTGCGTAGT		

Table.1 Identification of bacterial strains from diseased Indian major and exotic carps by Vitek 2 Compact System

Fish species	Site of infection	Strain	Bionumber	Identification
Labeo rohita	Kidney	R3SK-1	0003041203500000	*Pseudomonas aeruginosa*
Labeo rohita	Kidney	RGP-1	0201010103500302	*Acinetobacter baumannii* complex
Catla catla	Gill	CGP-1	0001010103500342	*Acinetobacter baumannii* complex
Catla catla	Gill	CBC-1	0001001103501252	*Pseudomonas fluorescens/mendocina*
Catla catla	Kidney	CG1-1	1425611151400271	*Aeromonas sobria*
Catla catla	Kidney	CRG-1	0003051101500352	*Pseudomonas putida*
Labeo rohita	Kidney	R3BK1-1	4231710450000210	*Yersinia enterocolitica/frederiksenii*
Labeo rohita	Kidney	R3BK2-1	6607735553561050	*Klebsiella pneumoniae* ssp. *Pneumonia*
Labeo rohita	Kidney	R2RGg-1	0001011101500352	*Acinetobacter baumannii* complex
Labeo rohita	Kidney	R2RGLg-1	0627634553532010	*Enterobacter cloacae* complex
Cirrhinus mrigala	Gill	MGG	001000000100202	Unidentified organism
Cirrhinus mrigala	Kidney	MBK	1465615151400271	*Aeromonas sobria*
Cirrhinus mrigala	Surface	MBH	010010302000000	*Kocuria rhizophila*
Cirrhinus mrigala	Gill	MBG	0000000102100000	*Acinetobacter radioresistens*
Cyprinus carpio	Kidney	CRK1.1	0000000102100000	*Acinetobacter radioresistens*
Cyprinus carpio	Kidney	CGK1	0041000000100202	Unidentified organism
Labeo rohita	Gill	R2BKc-1	0005201102500000	*Pseudomonas stutzeri*
Labeo rohita	Gill	R2GKp-1	0005201102500000	*Pseudomonas stutzeri*
Labeo rohita	Kidney	R3RKb-1	4617611747441210	*Citrobacter freundii*
Hypophthalmichthys molitrix	Gill	SRG-1	1465611351500271	*Aeromonas sobria*
Labeo rohita	Kidney	R2TK-1	1625703150540231	*Aeromonas hydrophila*
Labeo rohita	Kidney	R3SK1	423170551000210	Unidentified organism

Table.2 Molecular characterization of bacterial and parasitic strains isolated from diseased Indian major and exotic carps

Fish species	Disease/ Clinical sign	Site of infection	Strain code	Length (bp)	Gene Bank Accession number	Identification of Bacterial strains
Labeo rohita	Tail and fin rot	Fin	BUBKT	1332	**KT716080**	*Comamonas aquatica*
Catla catla	Deep ulceration	Kidney	BTRCTR	-	KX455879	*Aeromonas hydrophila*
Labeo rohita	Body haemorrhage	Kidney	R3BK1	-	-	Results awaited
Labeo rohita	Haemorrhage	Kidney	R3RKb	-	-	Results awaited
Labeo rohita	Focal gill necrosis	Gill	R3GS	-	Submitted to NCBI	*Flavobacterium columnare*
Labeo rohita	Gill rot and focal necrosis	Gill	RG1	-	KX452118	*Flavobacterium columnare*
Ctenopharyngodon idella	Saddle back	Dorsal region	C1	-	KX452119	*Flavobacterium columnare*

Table.3 ANOVA for antibiotics with respect to bacterial flora

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	122292.7	24	5095.529	2.620773	9.26E-05	1.557046
Within Groups	534678.2	275	1944.284			
Total	656970.9	299				

Table.4 ANOVA for antibiotics with respect to fish species

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4136.963	4	1034.241	0.923144	0.457215	2.539689
Within Groups	61619.01	55	1120.346			
Total	65755.97	59				

Fig.1 Agarose gel (1.2%) showing 16S rDNA gene amplicons of bacterial strains of diseased Indian major and exotic carps. M- 1Kb molecular weight DNA marker; 1.*Comamonas* aquatica (BUBKT), 2. *Aeromonas hydrophila* (BTRCTR), 3. *Citrobacter freundii* (R3BK1), 4. *Yersinia enterocolitica/ frederikesenii* (R3RKb)

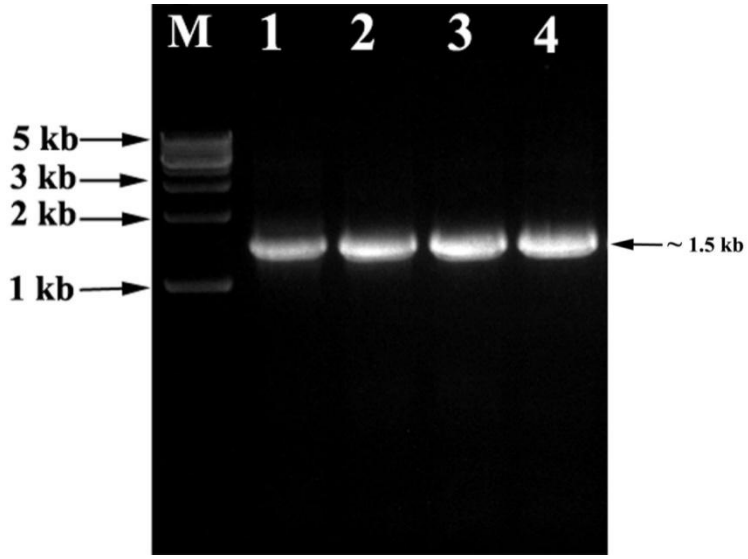


Fig.2 Agarose gel (2%) showing *Flavobacterium columnare* specific 16S rDNA gene mplicons. M – 100 bp molecular weight DNA marker; 1. *Flavobacterium columnare* (R3GS, culture dependent), 2. *Flavobacterium columnare* (RG1, culture independent), 3. *Flavobacterium columnare* (C1, culture independent)

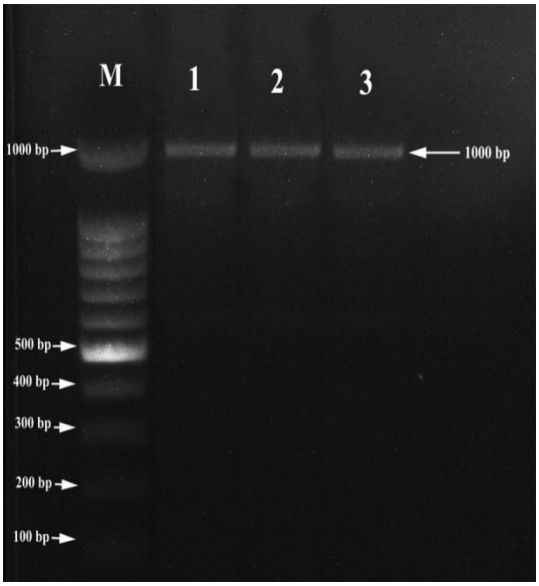


Fig.3 Agarose gel (2%) showing *Flavobacterium columnare* specific 16S rDNA gene amplicons. M – 100 bp molecular weight DNA marker; 1. *Flavobacterium columnare* (R3GS, culture dependent), 2. *Flavobacterium columnare* (RG1, culture independent), 3. *Flavobacterium columnare* (C1, culture independent)

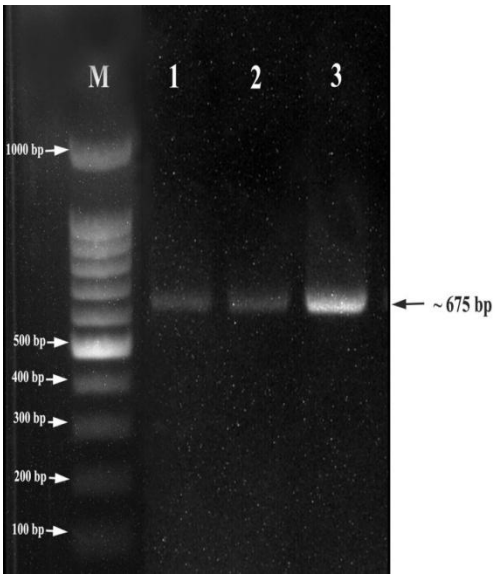


Fig.4 Prevalence of bacterial diseases in Indian major and exotic carps of South 24 Parganas district, West Bengal from January 2016 to December 2016

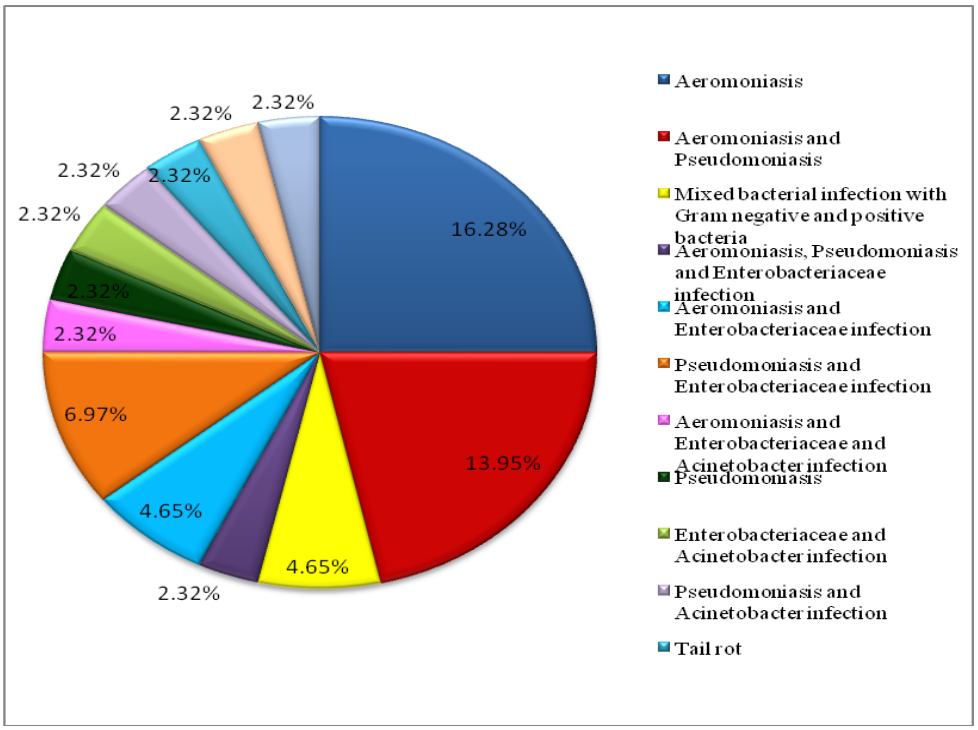
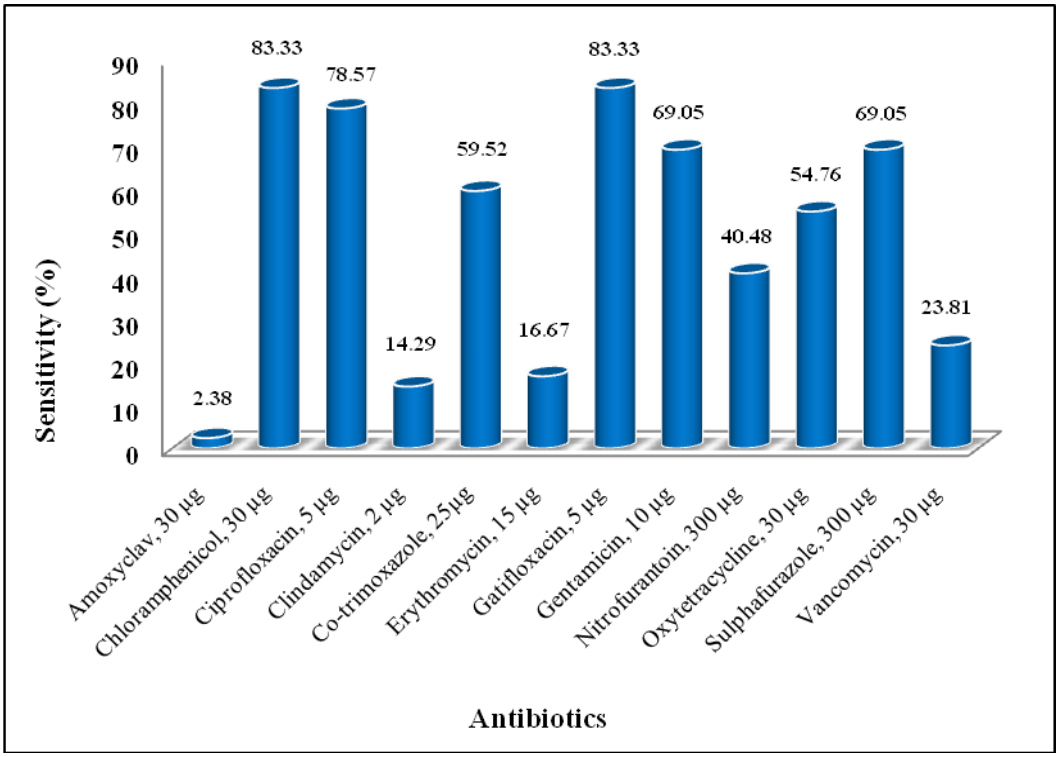


Fig.5 Antibiotic sensitivity (%) of bacterial flora (n=42) associated with diseased Indian major and exotic carps



Prevalence of diseases

The prevalence of bacterial diseases in Indian major and exotic carps in South 24 Parganas district, West Bengal is presented in Fig. 4. Aeromoniasis (16.28%) were the dominant disease among the bacterial diseases in the district, followed by aeromoniasis and pseudomoniasis (13.95%). Pseudomoniasis and *Enterobacteriaceae* infection (6.97%) were also common in the district. Mixed bacterial infection with Gram positive and negative bacteria and Aeromoniasis and *Enterobacteriaceae* infection were also observed with 4.65% prevalence.

Antibiotic sensitivity testing by agar-disc diffusion assay

A total of 42 bacterial strains from diseased Indian major and exotic carps were subjected to antibiogram against 12 antibiotics and the results are depicted in Fig. 5. About 83.33% of the bacterial flora of diseased Indian major and exotic carps from South 24 Parganas district, West Bengal were sensitive to chloramphenicol and gatifloxacin followed by ciprofloxacin (78.57%), gentamycin and sulphafurazole (69.05%), co-trimoxazole (59.52%), oxytetracycline (54.76%), nitrofurantoin (40.48%), vancomycin (23.8%), erythromycin (16.67%), clindamycin (14.29%), amoxyclav (2.38%).

Statistical analysis

Statistical analysis revealed that there was a significant difference among the 12 antibiotics ($F=2.62$, $P<0.05$) with respect to the bacterial flora (Table 3). Statistical analysis also revealed that there was no significant difference among the 12 antibiotics ($F=0.92$, $P<0.05$) with respect to the fish species (Table 4). Bacterial diseases are responsible for heavy mortality in both wild and cultured freshwater fish. The actual role of these microorganisms may vary from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other disease process (Richards *et al.,* 1978). The bad water quality, high organic load, contaminated feed and unhygienic conditions are some of pre-disposing factors for an outbreak of bacterial diseases in aquatic animals (Mastan, 2013). The aquaculture operation in most of the surveyed areas in the district was under sewage-fed aquaculture, increased conflicts of water for multipurpose use might have favoured the growth of these autochthonous bacteria and subsequently the onset of diseases. The other reasons might be mainly initiated due to environmental stress factors such as high water temperatures, pH disturbances and low dissolved oxygen levels, overcrowding, heavy parasite infestation, high organic loads in the water, spawning activity, rough handling and transport also may lead to outbreaks of disease. Serious episodes of stress such as oxygen depletion or cases of brown blood disease, caused by nitrite toxicity, often are followed by outbreaks of bacterial infection within a week (Kumar *et al.,* 2013; Jagoda *et al.,* 2014).

In the present study, among all the observed bacterial diseases, aeromoniasis alone was found to be the most dominant disease, occurring throughout the year in Indian major and exotic carps such as *H. molitrix, Labeo rohita, Catla catla, Cyprinus carpio*. Among all the six fish species screened, *H. molitrix* was most susceptible to aeromoniasis. Likewise, aeromonads infection in *H. molitrix* had been reported earlier (Sabur, 2006; Sarkar *et al.,* 2012; Rashid *et al.,* 2014; Ali *et al.,* 2014). *Aeromonas* infection accounted for 45.45% of diseases of exotic carps, followed by 6.25% of Indian major carps. Likewise, *Aeromonas* spp. were earlier reported in exotic carps, *H. molitrix* by Rashid *et al.,* (2014), in *Cyprinus carpio* by Yi *et al.,*

(2012), in *Ctenopharyngodon idella* by Zheng *et al.,* (2012). Motile *Aeromonas* species are widely distributed in aquatic environments (Beaz-Hidalgo *et al.,* 2013) and are isolated from water, healthy or diseased fish, food products, animal and human faeces and other clinical and environmental samples. When water quality fails, the fish suffer stress, thus making them more susceptible to infections by opportunistic pathogens such as *Aeromonas* sp. (Karvonen *et al.,* 2010; Tam *et al.,* 2011). The increased incidence of aeromoniasis in diseased Indian major and exotic carps population is an indication that it is emerging as a major pathogens with the intensification in carp culture system.

Besides aeromoniasis, aeromoniasis and pseudomoniasis (13.95%) was the dominant one, occurring throughout the year in diseased Indian major and exotic carps. Motile aeromonads and *Pseudomonas* sp. were documented in the cultured ponds of *Cirrhinus mrigala* in Bangladesh (Iqbal, 1995). Likewise, Darak and Barde (2014) reported *Aeromonas* spp. and *Pseudomonas* spp. as very common bacteria associated with major carps and live fish. Mixed bacterial infection with Gram positive and negative bacteria was accounted for a total of 4.65% disease incidence in diseased Indian major and exotic carps such as *Labeo rohita* and *Cirhhinus mrigala*. The associated bacterial flora identified were *Comamonas aquatica*, *Aeromonas veronii* biovar *veronii*, *Pseudomonas* sp. from *Labeo rohita* and *Aeromonas sobria*, *Acinetobacter radioresistens*, *Kocuria rhizophila*, *Pseudomonas* sp., *Aeromonas tecta*, *Aeromonas jandaei* from *Cirhhinus mrigala.* Likewise, other (Darak *et al.,* 2014) reported mixed bacterial infection in carps with the association of bacterial flora like *Aeromonas* sp., *Flavobacterium* sp., *Proteus* sp., *Staphylococcus* sp., *Enterobacterium* sp., *E. coli*, *Pseudomonas* sp. and *Vibrio* sp.

The antibiotic sensitivity test was done to understand the effectiveness of different antibiotics on 42 bacterial strains from diseased Indian major and exotic carps. Among the 42 bacterial strains tested, 25 strains were of the members of genus *Aeromonas*, which showed varying degrees of antibiotic resistance. All the species of *Aeromonas* were resistant to amoxyclav, except two strains of *Aeromonas veronnii* biovar *veronnii.* All the 25 strains of *Aeromonas* spp. were sensitive to chloramphenicol. In the present study, 96% of the *Aeromonas* spp. were sensitive to ciprofloxacin except, *Aeromonas diversa*, which, to some extent, corroborate the results of other (Hatha *et al.,* 2005). They reported that 63% of the 90 motile aeromonas strains isolated from freshwater fish were sensitive to ciprofloxacin. A wide range of antimicrobial compounds (oxytetracycline, ciprofloxacin, nitrofurantoin, furazolidone or chloramphenicol) are being used in the hatcheries and farms in India to control the bacterial population (Abraham *et al.,* 1997).

The present study was done to find rapid and accurate identification of bacterial species which will help in prevention and cure of diseases in various aquatic animals. The identities of selected bacterial strains were further confirmed by molecular characterization through 16S rDNA analysis (Fig. 1, 2 and 3). These days modern epidemiologists have been using a variety of tools which provide good molecular differentiation and which can be tailored to fit the needs of the both laboratory and clinical study.

The results of the present study can be concluded with a considerable population of Indian major and exotic carp species cultured in West Bengal are susceptible to diseases. Among the various diseases, bacterial diseases have been responsible for significant

economic problem. Aeromoniasis alone or with other infections were observed to be most dominant disease of Indian major and exotic carp species and evolved as major threat to freshwater aquaculture of West Bengal. Other bacterial pathogens such as *Pseudomonas* spp., *Enterobacteriaceae* group, *Acinetobacter* group, *Flavobacterium columnare* and Gram positive bacterium were also found to be associated with diseased Indian major and exotic carp but with lesser extent. Phenotypic identification of bacterial isolates were done by conventional tests and commercial methods (VITEK 2 Compact system (Biomerieux, France) and further 7 isolates were selected for genotypic identification, which was done by 16S rDNA method. 5 isolates were identified as *Comamonas aquatica* (n=1), *Aeromonas hydrophila* (n=1) and *Flavobacterium columnare* (n=3). Results of other two are still awaited. Antibiotic sensitivity study revealed only gatifloxacin and chloramphenicol were found to be effective, both inhibited 83.33% sensitivity to the bacterial strains.

Further concern is the increasing incidence of multiple antibiotic resistance. Thus, the results of the present study provide a foundation upon which we can understand the role of these opportunistic pathogens of diseases and their management in aquaculture systems South 24 Parganas district, West Bengal.

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