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Screening, Biochemical Characterization and Molecular Identification of Antibiotic Resistant Fish Pathogen *Pseudomonas plecoglossicida*

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Abstract

Two sample of bacterial *Pseudomonas plecoglossicida* were isolated from Bhojtal lake of Bhopal city. Strain one (HFgGr) was isolated from the fish gills (*Hetropnetus fossilis*) lesions and second strain (GH5Gf) was isolated from lake water sample. Bacterial identification were done on the basis of biochemical characteristics and 16S rRNA sequence similarity using BLAST similarity search against '16S rRNA Database' on NCBI sever. *Pseudomonas plecolgosicida* is one of the potential fish pathogen and also inhabit water belongs to the group of Pseudomonas bacteria. It is gram negative, non-fluorescent and motile belonging to division gama-proteobacteria. Earlier studiesreports that these bacteria a causative agent of bacterial haemorrhagic ascitis of Ayu (*Plecoglossus altivelis*) fish and belong to *Pseudomonas putida* group which are identified as main causative agent for the diseases in fish. In present study the isolated strains were tested for antibiotic sensitivity and MIC_{50} values for amoxicillin, ampicillin, chloramphenicol, tetracycline, streptomycin, norfloxacin, cloxacillin. The MIC_{50} were observed to be >70 µg ml^{-1} and decline of specific growth rate curve at the concentration >50 µg ml^{-1}. MIC_{50} values of erythromycin+amoxicilline, cefixime, ciprofloxacin and amoxicillin+clavualante were observed in the range as 43.48 µg ml^{-1} - 58.32 µg ml^{-1} and decline of specific growth rate curve were observed above the concentration as 25 µg ml^{-1}. The increased MIC_{50} values against the potential antibiotics indicated increased adaptability against the antibiotics in *Pseudomonas plecoglossicida* may be due to extensive use of antibiotics and concentration in environment. High frequency of *Pseudomonas* species in lake water is a serious concern for fish diversity and human, further extended to the fact of fish-human cross pathogenicity. The increased antibiotic resistance is an alarming condition due to spread of antibiotic resistance genetic material throughout the whole bacterial community.

Keywords: *Pseudomonas plecoglossicida*, Fish pathogen, Antibiotic resistance, 16S rRNA sequencing.

Introduction

Water is the wonderful gift, nature has bestowed mankind though freshwaterpresent less than 1% on the Earth (Nierzwicki-Bauer, *et al.*, 2008). The discharge from hospitals, containing pathogens such as bacteria, virus, protozoan can cause many disease that ranges from Typhoid, dysentery to minor respiratory and skin disease. The effluents may influence the normal microbial diversity of the lake. The increasing water pollution leads to increasing possibilities of infectious diseases (Chandra, 1999).Biodiversity comprises of three interrelated elements genetic, functional, and taxonomic diversity (Rolle *et al.*, 2012) as the consequence of genetic variability within the taxa, environmental effect of gene expression and ecological interaction among the taxa. The inland fish production in Madhya Pradesh has been dropped from

62.06 (‘000 tons) in 2004-05 to 56.45 (‘000 tons) during 2010-11 with the growth rate between -1 to -14.62 (Department of Fisheries Madhya Pradesh State Government www.mpfisheries.in). The major concern here is the health of fish that is dependent on the quality of the water (Ampofo and Clerk, 2010). Naturally occurring Gram negative pathogenic bacteria in aquatic environment causes the outbreak when the normal environmental conditions changed (Roberts, 1997). The *Pseudomonas fluorescens*, *P. angulliseptica*, *P. aeruginosa* and *P. putida* were identified as causative agents of *Pseudomonas* septicemia in various fish species (Altinok *et al*., 2006;El-Naggar, 2012). The important fish bacterial pathogens belong to the families Aeromonadaceae (Colwell *et al*., 1986), Pseudomonodaceae, Vibrionaceae, Enterobacteriaceae, Cytophagaceae and Corynebacteriaceae (Austin, 2011).

Human infections that may be caused by fish bacteria include food poisoning and gastroenteritis. Consumption of fish is responsible for 5-8 % of food-borne disease outbreaks (Silva *et al*., 2011).The risk to public health arises if toxigenic strains multiply to high number during improper handling and storage leading to auto enzymatic action and bacterial degradation. Fish becomes unfit for consumption on these grounds producing a strong odor and poses a health risk. The use of antibiotics as therapeutics agent in hospitals and in fish farming is increasing at alarming level and it is polluting all types of water sources. The increased antibiotics are pressurizing the increased cases development of antibiotics resistant bacteria and hence the bacterial infection in human as well as in fishes. The potential of lake as reservoir of multi drug resistance bacteria and potential risk is a serious concern discussed by many researchers (Garcia et al., 2007; Blank, 2012). Novais et al., (2008) and Czekalski et al., (2012) observed highest load of Multidrug Resistant Bacteria (MRB) in Hospital derived sewage during the study of lakes even after waste water treatment. The lake water gets heavily polluted by pathogenic bacteria, specifically Multi-Drug resistant bacteria, which are the major threats to human health (Midtvedt and Lingaas, 1992; Roberts, 2011). Antibiotics resistance is an adaptation which provide protection to bacteria from the antimicrobial substances. The horizontal gene transfer mechanisms results into the spread of antibiotic resistant genes among the bacterial populations (Roberts, 2011). The bacterial diversity and bacterial taxonomy can be studied by molecular and genetics techniques as 16S rRNA sequencing (Tindall et al., 2010).

The present study was mainly carried out to detect the population of bacterial strains *Pseudomonas plecoglossicida* and level antibiotic resistance among its population of upper lake in Bhopal city (Madhya Pradesh, India). The 700 year old upper lake (now Bhojtal) is a source of water to 40% resident of Bhopal city. It has the area of 34 sq. km and catchment area of 361 sq km. the watershed area of Bhojtal is mostly rural area besides some part of urban area. Upper Lake is surrounded by the densely, moderately and rarely populated area, which drop their sewage to upper lake. Gandhi medical (Hamidia hospital) site of upper lake receives heavy influent of domestic and urban swage besides hospital sewage containing pathogenic bacteria, antibiotics and drugs, bloods and tissue parts, discharges.

Materials and Methods

Sampling Sites and Sampling: The water sample from different stations of Bhojtal (Upper lake) of Bhopal, were collected in each month of three seasons (summer, monsoon and winter). The sampling were done according to method given by Speck, (1976) and samples were stored in sterilized bottles and brought to laboratory for further investigation.

Direct Plate Count (DPC) Technique: The samples were serially diluted by saline water and inoculated in of three different media plates(NAM, McConkey Agar Media, Blood Agar) by surface spread method (Speck, 1976). Total 10 typical colonies were selected from each triplicate set of plates and further purified, screened by morphological and biochemical properties to differentiated to the species level.

Examination of Diseased Fish and Bacterial Sampling:

The bacteriological examinations of infected fishes were carried out according to Austin and Austin, (1987) from the collected fishes in bulk by fisherman in living condition. Only Fishes with overt external lesions were brought to the laboratory and bacteria were isolated from the skin and gills of the fish (Austin, 2011) by taking swabs with the help of swab sticks. The swabs were diluted serially (10^1,10^2,10^3) and the inocula were poured on different selective agars media for growth, viz. NAM, McConkey and Blood Agar Media (Austin, 2011).

Incubation: The plates were incubated at temperature 37˚C for the period of 24 hrs to 48 hrs.Theplates were observed for the formation of visible colonies and the total numbers of unique colonies were measured.

Pure Culture of Bacterial Isolates: Pure culture was obtained by using the streak plate technique (Benson, 2005). Pure bacterial colonies were obtained by taking small inoculums from mixed colonies of bacteria and aseptically streaked on selective agar media with a sterile inoculating loop, incubated at 37˚C temperature for 24 hours. The colony which develops singly on a petri plate is further streaked on the agar plates till a pure colony is obtained.

Figure 1: Plate showing the pure culture of *P. plesioglycosida* obtained by streak plate method

Identification

Morphological, Functional and Biochemical Characterization of Bacterial isolates: Recovered bacterial colonies were screened for diversity studies on the basis of morphotypic traits as, shape, nature, pigmentation, Gram staining as described in *Bergey's Manual of Detereminative Bacteriology* (7th edition). Morphological characterization of colony was done with the help of compound microscope. For this purpose size, shape, color, margin, elevation and opacity of colony, were taken into account (Prescott *et al.,* 1996).

Biochemical Tests: Biochemical test were carried out by using Biochemical kit (HiMedia, Mumbai).

Drug Sensitivity (*in vitro*) Tests by Using Disc Plate Technique: The disk diffusion test (Kirby-Bauer method) was carried out using small paper disc (HiMedia, Mumbai) impregnated with known amount of chemotherapeutic agents and was placed upon the surface of inoculated plates. After incubation, the plates were observed for zones of inhibition surrounding the discs. The zone of inhibition was measured and compared to that of standard inhibition zone for specific therapeutic agents (Cappuccino and Sherman, 2002).

Drug Sensitivity (*in vitro*) Tests by using MIC (Minimum inhibitor concentration)

The Dilution susceptibility tests can be used to determine MIC values. A series of broth flask (Mueller-Hinton broth) containing antibiotic concentrations in the range of 10 - 100 µg ml^{-1} was prepared and inoculated with standard numbers of the test organism. The lowest concentration of the antibiotic resulting in no growth after 16 - 24 hours of incubation is the MIC (Cappuccino and Sherman, 2002). The antibiotics of analytical grade were obtained from Sigma-Aldrich Co. LLC. Stock solution of 250 mg of antibiotics $(10\ ml)^{-1}$ of solvent (DMSO) were prepared. A working antibiotics solution was prepared according to the graded concentration as, 10 µg ml^{-1}, 25 µg ml^{-1}, 50 µg ml^{-1}, 75 µg ml^{-1} and 100 µg ml^{-1}.

Molecular Characterization of Bacterial isolates:

The bacterial genomic DNA was isolated according to the method described by Abed *et al*., (1995), followed by 16S rDNA amplification using universal primer and purified using the PCR amplicon purification kit (SIGMA, Inc.).PCR amplified product were checked for quality by using Nano-Drop Photometer. The 16S rDNA was sequenced at nucleotide sequencing facility at IISER, Bhopal. The sequences were searched for homology by BLAST (Altschul *et al*., 1990) tools against bacterial 16S rDNA sequence database.

Results and Discussion

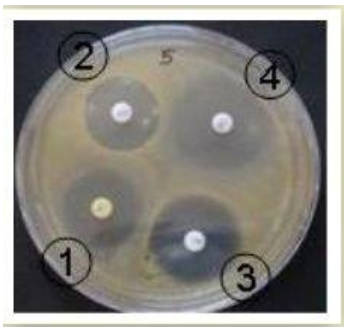
In water ecosystem fish is in direct contact with microflora of the ecosystem and opportunistic pathogens present in the system invade the host under stress (Trakroo and Agarwal, 2011). The economic losses in fish and other aquatic animals production is due to stressful conditions, infectious diseases and deterioration of environmental conditions (Balcazar *et al.*, 2006). Control of bacterial pathogens in fishes from aquatic system has been routinely achieved by the administration of antimicrobial agents. The excessive use of these antimicrobials has resulted into the development of antibiotic-resistant among the bacteria, which is transferred among the bacterial population through transferable genetic vectors, making the treatments less successful (Akinbowale *et al.*, 2007).

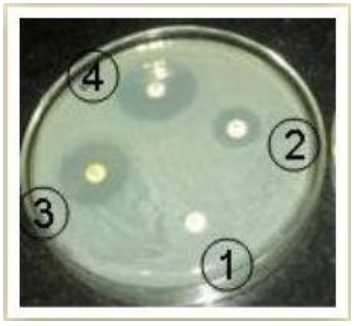
Antibiotic Sensitivity Screening

Table-1: Antibiotics sensitivity shown by different water pathogens from Bhoj wetland during the year 2010-12.

S. N.	Antibiotics	Conc/Disc (µg ml^{-1})	HFgGr	GH5Gf
1	Ampicillin	25	-	-
2	Amoxicillin	25	-	-
3	Tetracycline	30	+	+
4	Streptomycin	20	-	-
5	Cloramophenicol	25	-	-
6	Ciprofloxacine	5	+	+
7	Norfloxacin	15	+	+
8	Azithromycine	15	-	-
9	Cefixime	30	+	+
10	Cloxacillin	25	-	-
11	Erythromycin + Amoxicillin	15	++	++
12	Amoxicillin + Clavulanic Acid	15	++	++

***- = no inhibition (resistant); + = inhibitory zone between 5-15 mm; ++ = inhibitory zone between 16-25 mm; +++ = inhibitory zone between 26 – 35 mm**





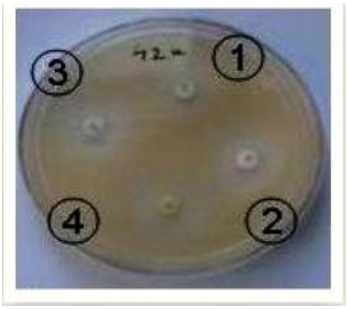


Fig : A **Fig B** **Fig C**

Fig A: Zone of inhibition of observed in the culture plate of bacteria, *P. plesioglycosida* for the antibiotics, (1) Cefixime,(2Ciprofloxacine, (3) Erythromycin + Amoxicillin, (4) Amoxicillin + Clavulanic Acid

Fig B: Zone of inhibition of observed in the culture plate of bacteria, *P. plesioglycosida* for the antibiotics, (1)Azithromycine,(2) Streptomycin, (3) Tetracycline, (4) **)** Norfloxacin

Fig C: Zone of inhibition of observed in the culture plate of bacteria, *P. plesioglycosida* for the antibiotics, (1) Ampicillin,(2) Amoxicillin, (3),Cloramophenicol, (4) Cloxacillin

Twelve different drugs namely ampicillin, amoxicillin, chloramphenicol, streptomycin, tetracycline, cloxacillin, azithromycin, cefixime, norfloxacin, ciprofloxacin, singly and two drugs erythromycin with amoxicillin and amoxicillin with clavulanic acids in combination were tested against 02 bacterial species isolated from the water and diseased fish. The antibioticsensitivity study were carried out by many researchers to keep monitoring of increased resistance in bacterial isolates of fish and water and observed varying level of resistance such as, increased susceptibility for ciprofloxacin (Gaudreau *et al.*, 2007; Thuy *et al.*, 2011), 60% - 100% resistance for tetracycline (Suzuki and Hoa, 2012; Sreedharan *et al.*, 2012) cause of this resistance is acquisition of 'tet' gene for degradation of tetracyclin, 50% - 70% resistance against erythromycin (Orozova *et al.*, 2008, Haznedaroglu *et al.*, 2011), 80% - 90% resistance azithromycin (Seppala *et al.*, 2005; Jacobs and Chenia, 2007), 80 % – 100 % resistance ampicillin (Mudryk, 2002) increased cause of resistance may be overuse of penicillin group antibiotics leading to development of extended-spectrum β-lactamase, increased MIC of cefixime (Capoor *et al.*, 2006) due to wide use of cephalosporin antibiotics leading to development of resistance strain, decreased susceptibility for norfloxacin (Sreedharan *et al.*, 2012), 100% resistance for streptomycin (Springer *et al.*, 2001; Roberts, 2011) due to presence of several copies of mutated ribosomal protein and 100% resistance for cloxacillin (Islam *et al.*, 2008). cefixime + clavulanate combination were studied for gram negative bacteria being effective (Rawat*et al.*, 2009) due to dual mode of action cefixime inhibit cell membrane formation and clavulanate inhibit cefixime degrading enzyme beta-lactamase.

It was observed that two isolates of bacteria were sensitive to ciprofloxacin, norfloxacin, amoxiciline+calvulanic acid, erythromycin + amoxicilin and tetracycline. Ciprofloxacin, amoxicilin+calvulanic acid, erythromycine +amoxicillin and norfloxacin were found to be most effective (+++) against all types of bacteria in accordance with the finding by Rawat *et al.*, (2009), while tetracycline, cefixime, and azithromycin showed moderate effect (++) which were also conveyed by Rajkumarbharati, *et al.*, (2011). Amoxicillin and ampicilin did not show any effect against 23 Rod shaped bacteria viz HFgGr, it well accepted fact of acquisition of penicillin resistance in most of the pathogens and reported by number of workers (Cebeci and Gurakan 2003; Kim *et al.*,, 2008; Allameh *et al.*, 2012). Approximate result were also observed by Akinijogunla *et al.*, (2010) against fish pathogens from aquaculture environment. The drug cefixime was effective against the isolates GH5Gf and HFgGr which in accordance with the results observed by Capoor *et al.*, (2006).

Determination of Minimum Inhibitory Concentration (MIC)

Table-2: MIC_{50} of 16 bacterial isolates against total 12 antibiotics, 10 in individual and 2 in combinations, water and fish of Bhoj wetland.

		Antibiotics (µg ml^{-1})											
S. No	Bacterial Isolates	Amoxicillin	Ampicillin	Chloramphenicol	Ciprofloxacin	Tetracycline	Streptomycin	Norfloxacin	Azithromycin	Cefixime	Cloxacillin	Erythromycin + Amoxicillin	Amoxicillin + Clavulanate
1	GH5Gf	83.33	90.91	71.43	43.48	83.33	76.92	71.43	66.67	50	83.33	58.82	47.62
2	HFgGr	100	83.33	71.43	52.63	83.33	66.67	76.92	76.92	47.62	83.33	66.67	43.48

The high MIC_{50} values were observed for four potential dominant pathogens as, *Pseudomonas plecoglossicida, P. aeruginosa, Serratia marcescens* and *Morganella morganii*. The MIC_{50} values of *Pseudomonas plecoglossicida* and *P. aeruginosa* were observed high and more than 75 µg ml^{-1} for amoxicillin, ampicillin, cloxacillin, chloramphenicol, tetracycline, streptomycin, norfloxacin, azithromycin. The moderate values of MIC_{50} were observed for the antibiotic ciprofloxacin (52.63 µg ml^{-1}), cefixime (47.62 µg ml^{-1}), erythromycin+amoxicillin (66.67 µg ml^{-1}) and amoxicillin+clavulanate (43.48 µg ml^{-1}). The moderate value of MIC_{50} in *P. aeruginosa* for the antibiotics, ciprofloxacin (58.82 µg ml^{-1}), cefixime (55.56 µg ml^{-1}), azithromycin (55.56 µg ml^{-1}), erythromycin+ amoxicillin (52.63 µg ml^{-1}), and amoxicillin+clavulanate (45.46 µg ml^{-1}). Czekalski*et al.,* (2012) identified streptomycin, tetracycline, ciprofloxacin and azithromycin resistant *Pseudomonas* species in waste water lake of Geneva (Switzerland) beside increased MIC for other antibiotics.

Effect of Graded Concentration of Antibiotics on Isolate HFgGr

MIC_{50} values of amoxicillin, ampicillin, tetracycline, cloxacillin are >75 µg ml^{-1} and decline in specific growth rate at 75 µg ml^{-1}. MIC_{50} values of chloramphenicol, streptomycin, norfloxacin, azithromycin, cefixime, ciprofloxacin are in the range between 55.56 µg ml^{-1} - 71.5 µg ml^{-1} and specific growth rate is declined at 50 µg ml^{-1} concentration. Ciprofloxacin and amoxicillin+clavulanate have the MIC_{50} values 52.63 µg ml^{-1} and 43.48 µg ml^{-1} and decline of growth rate at 25 µg ml^{-1} concentration.

Effect of Graded Concentration of Antibiotics on Isolate GH5Gf

MIC_{50} values of amoxicillin, ampicillin, chloramphenicol, tetracycline, streptomycin, norfloxacin, cloxacillin were observed to be >70 µg ml^{-1} and decline of specific growth rate curve at the concentration >50 µg ml^{-1} (fig. 20). MIC_{50} values of erythromycin+amoxicilline, cefixime, ciprofloxacin and amoxicillin+clavualante were observed in the range as 43.48 µg ml^{-1} - 58.32 µg ml^{-1} and decline of specific growth rate curve were observed above the concentration as 25 µg ml^{-1}.

Molecular Identification of Antibiotic Resistance Bacterial Isolates

Molecular identification of bacterial isolates based on similarity of conserved 16S rRNA sequences is full-proof method and widely used in characterization of bacteria and study of bacterial diversity (Sails *et al.*, 2002). It provides the accurate information for the identification of organisms with ambiguous phenotypic profiles. Universal primers Gm 3f and Gm 4r (Weisburg *et al.*, 1991) are widely used for identification. It is possible to find group, species, and even serotype with specific sequence patterns by analyzing the partial 16S rRNA sequence (Ravelo *et al*., 2003; Sharma *et al*., 2012). 16S rRNAsequences are assigned to phylum, class, order, family, subfamily, or species at sequence similarity cut-off values of 80, 85, 90, 92, 94, or 97%, respectively (DeSantis *et al*., 2007). For the present study 02 potential isolates (01 fish isolates and 01 water isolates) were assumed to be novel bacterial isolates identified by 16S rRNA sequences.

Fish isolate "HFgGr" and water isolate 'GH5Gf' were identified as *Pseudomonas plecoglossicida*with 98% similarity of 16SrRNA sequence through BLAST analysis and confirmed with the aid of biochemical characteristics. It is potential fish pathogens characterized and reported by many researchers (Austin and Austin, 1999). Nishimori*et al.,* (2000) reported this bacteria a causative agent of bacterial haemorrhagicascitis of Ayu (*Plecoglossusaltivelis*) fish and belong to *P. putida* group. Mao *et al.,* (2012) identified *Pseudomonas putida* group bacteria a causative agent for the diseases in fish yellow croaker in china. Isolate GH5GF and HFgGr are *Pseudomonas*sp (*Pseudomonas plecoglossicida*) as revealed from 16S rRNA sequences. Even this isolates are clustered together in RAPD dendogram with 100% similarity.

A) Isolate – HFgGr (*Pseudomonas plecoglossicida*) of fish *Hetropneustes fossilis*

>HFgGr

CTCGCAGTCTACCATGCCAGTCGAGCGGATGACAGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGA
GTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTC
CTACGGCAGAAAGCAGGGGACCTTCGAGGCCTTGCGCTATCACATGAGCCTAGGTCGGATTAGCTAGTT
GGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAGAGCCTGA
TCCAGCCATGCCGCGTGTGTGAGGAAGGTCTTCGGATTGTAAATCACTTTAAGTTGGGAGGAAGGGCAG
TAAGCTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCG
CGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGTCGTAAAGCGCGCGTAGGTGGTTCGTTAA
GTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCGAAACTGGTGAGCTAGAGTCAGGTAG
AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAATGGCGAAGGC
GACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCA
TTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACCTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCTAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCAGAGAAC
TTTCCAGATATGGATTGGTGCCTTCGGGAATCCTGACACAGGAGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCATAACGAGCGCAACCCTTGTCCTTAGTTACCAGCTCTATGTGGGGA
CCT

A) Isolate7 – GH5Gf (*Pseudomonas plecoglossicida*) of water

>GH5Gf

CATGCGCATGCGTACCGTGCAGTCGAGCGGATGACGGGAGCTTGCTGCTTGATTCAGCGAGCGGACGG
GTGAGTAAGCCTAGGAATCTGCCTGGTAGTGGGGGACAAGTTTCGAAAGGACGACTGTACCTGATACC
GTCCTACGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGT
TGGTGGGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGA
ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTG
ATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGC
AGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAAATAAGCACCGGCTAATCTCTGTGCCCCGCAG
CCGCGGTAATAACGAGGCGTGAGGAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTACGTGGTT
CGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCTGAAACTGGTGAGCTAGAGTAC
GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTATGATATAGGAAGGAACACCAGTGGC
GAAGGCGACCACCTGGACTGATACTGACACTGACGTGCGAAAGCGTAGGCGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGAATCTGAGATTTTAGTTGCGCAGCTA
ACGCAGTAAGTTGACCGCCTTGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCA
AGGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGC
TCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTAT
GGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGACCTAGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTAGCCAAGCCGCGAGGTG
GAGCTAATCTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATC
GCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACGTCACATGGAGAGCGCAACACAGACC

Conclusion

Necessary steps are required to minimize the overburden of resistance pathogenic bacteria and increased pollution level of Bhoj wetland. It is of great importance to investigate the health hazards prevailing in fish population and to devise prophylactic and therapeutic measures such as application of probiotic and vaccination, to save the fish from devastating bacterial diseases.

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