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Drug Resistance Profiles of Coliforms from Sewage Exposed Fish

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Abstract: This study aimed at characterization of bacteriological pollution of water and fish of a domestic sewage supplied pond followed by determination of resistance for antibiotics. Bacteriological examination of pond water and fish comprised of quantitative analysis of aerobic plate counts (APC) at 37°C, total coliforms (TC), faecal coliforms (FC), *E.coli*, faecal streptococci (FS) and also the presence and absence test of pathogenic bacteria like *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. Muscles and digestive tract contents of fish contained high APC, TC, FC, *E.coli* and FS. Pathogenic bacteria like *Salmonella* spp. and *Shigella* spp. were almost completely absent in pond water and fish samples whereas *Vibrio* spp. were found very frequently. Patterns of resistance to Ampicillin, Amikacin, Chloramphenicol, Cotrimoxazole, Gentamycin, Kanamycin, Streptomycin and Tetracycline were determined in thermotolerent coliform isolates from water and fish by the disc diffusion method. Most of the isolates were predominantly ampicillin-resistant. 26.66% of the total isolates (16 out of 60) were resistant to three to four antibiotics. High rate recovery of antibiotic resistant bacteria from fish flesh may pose risk to public health specially if the resistance is plasmid mediated.

Key words: Domestic sewage · Thermotolerent coliforms · Water quality · Antibiotic Resistance · Public health

INTRODUCTION

Wastewater fed fish pond system is a constructed aquatic ecosystem, consisting of one or several water bodies with an integrated food web, charged with nutrient rich wastewater. The central aim of the system is the assimilation of dissolved nutrients into fish biomass. Simultaneously organic compounds are either consumed or mineralized and in consequence the wastewater gets purified.

Fish being cultivated in wastewater is living with the danger of contaminating fish flesh because of the possible pollution by toxic chemicals, heavy metals and pathogenic micro organisms. Besides, indiscriminate use of different antibiotics for the treatment of public and veterinary diseases has caused development of antibiotic resistant bacteria as well as resistance plasmids entering in waste fed pond through wastewater and contaminate fish.

One of the important concern of wastewater fisheries is the contamination of fishes by faecal bacteria [1]. Their presence in fish intended for human consumption may constitute a potential danger not only by causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human-infecting bacteria from nonaquatic sources [2]. Therefore, periodic and comprehensive sanitary survey of wastewater fishery is required.

In the present study, an attempt has been made to evaluate the level of indicator as well as some pathogenic bacteria in the muscles and digestive tract contents of *Oreochromis* sp. and *Labeo* sp. reared in wastewater fed pond. Random thermotolerent coliform isolates from fish and their aqueous environment were examined for their antibiotic resistance patterns to some commonly used antibiotics.

MATERIALS AND METHODS

Study Site: A sewage fed pond of Bandipur waste fed wetland, Rahara, North 24 Parganas, (22°44'N Latitude and 88°24'E Longitude) was taken into consideration for this study and to examine bacterial load of water and fish. Raw sewage was entirely of domestic origin, coming from Titagarh town of North 24 Parganas, West Bengal.

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Sampling and Dissection: Fish samples were caught with a net and were immediately transferred to the laboratory in containers with pond water. Five live fish of both the species were randomly selected from the catch at each sampling time (bimonthly from March 2010 to November 2010). Fish were dissected according to Buras *et al.* [3]. Muscles and digestive tract contents were isolated and placed in sterile glass vessels. The tissues were weighed under sterile conditions, ground in a mortar and suspended in sodium chloride (NaCl) physiological solution (10 ml of the solution for each 1 g of the muscle or digestive tract content). The suspensions were homogenized using Universal Laboratory Aid Type MPW-309 homogenizer, at 1000 rpm, for 10 minutes. The homogenates were then serially diluted (10^{-1} to 10^{-8} for muscles and 10^{-1} to 10^{-10} for digestive tract contents) and inoculated into culture media. Time lag from fish collection to the analyses did not exceed 6 hours. Water from sewage-supplied pond was sampled and analysed simultaneously with fish sampling.

Microbiological Analyses

Total Coliforms: Lauryl Tryptose (LT) Broth at 35±0.5°C for 48±3 hours was used for three-tube most-probable-number (MPN) presumptive determinations of coliforms. From all positive presumptive tubes, total coliforms were confirmed by the formation of gas in any amount in the Durham fermentation tubes of brilliant green lactose bile broth (BGLB) for 48±3 hours at 35±0.5°C. Growth from positive confirmed BGLB tubes was streaked on Endo agar and incubated at 35±0.5°C for 24±2 hours. Typical coliform colonies from Endo agar were transferred to secondary LT tubes for 48±3 hours at 35±0.5°C and to Nutrient agar slants for 24±2 hours at 35±0.5°C. Production of gas in secondary LT tubes and presence of non spore forming gram negative rod shaped bacteria from gram stained preparations of Nutrient agar slants constitute a positive result of the coliform group [4].

Faecal Coliforms: All positive Lauryl Tryptose (LT) MPN tubes to tubes of *Escherichia coli* (EC) Broth followed by incubation at 44.5±0.2°C for 24±2 hours constitute a positive faecal coliform test [4].

E. coli: The growth from positive EC tubes was then streaked onto Levine Eosin Methylene Blue (EMB) Agar plates and incubated at 35±0.5°C for 18 to 24 hours. Colonies from EMB Agar plates typical of *E. coli* were transferred to Nutrient agar (NA) slants from which GIMViC tests were performed where "G"-is the Gas and Acid production from secondary EC broth, "I" – Indole production from Tryptone broth, "M"- and "V"- Methyl red and Voges Poskauer test from Buffered Glucose broth and "C"-is Citrate utilization test from Simmon's Citrate agar. MPN of *E. coli* was then computed based on the number of tubes found to contain isolates that produce GIMViC reaction patterns characteristic of *E. coli* [5].

Faecal Streptococci: Azide dextrose Broth at 35±0.5°C for 48±3 hours was used for three-tube most-probable-number (MPN) presumptive determinations of faecal streptococci. Growth from positive Azide dextrose tubes was streaked on Pfizer Selective Enterococcus agar (PSE) and incubated at 35±0.5°C for 24±2 hours. Brownish black colonies with brown halos confirmed as faecal streptococci [4].

APC: Standard plate count methods at 37°C on Tryptone glucose yeast extract agar were used for the determination of aerobic plate counts of bacteria [4].

***salmonella* spp., *shigella* spp. *and vibrio* spp:** Presence / lack of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. on Enrichment media at 37°C for 24 Hrs. followed by a selective media under same incubation condition were used [4].

Typical colonies of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. from selective agar were streaked aseptically several times onto freshly prepared nutrient agar plates to obtain pure culture of bacteria. Pure isolates of *Salmonella* spp., *Shigella* spp. were subjected to IPViC tests (I= indole production from Tryptophan, P= Phenylalanine deaminase activity, Vi= Voges-Proskauer test and C= Citrate utilization test), Methyl red test, Lysine decarboxylation test, Ornithine decarboxylation test, Motility test, Urease activity test, Hydrogen sulphide production test and Sugar fermentation test (Sucrose and Lactose).

Pure isolates of *Vibrio* spp. were subjected to Gram stain reaction, Oxidase test and Oxidative -fermentative test. The genus *Vibrio* is gram negative, oxidase reaction and oxidative-fermentative test positive. Besides these characteristics, they were also subjected to Lysine decarboxylation test, Ornithine decarboxylation test, Hydrogen sulphide production test, Motility test, Urease activity test, Sugar fermentation test (Sucrose and Lactose), Hydrolysis test (Starch and Gelatin), IViC tests and NaCl tolerance test (Tolerance to NaCl was determined by the addition of NaCl to 1 % peptone medium with percentage of 0, 3, 6, 8 and 10 % and cultures were examined for growth after 3 days at 30°C).

Tests were carried out on each isolate following the procedures described by Prescott *et al.* [6] to enable identification to the generic levels with the aid of the Bergey's Manual of Determinative Bacteriology [7].

Antibiotic Resistance Tests: Representatives of typical thermotolerent coliform isolates from water and fish samples were selected randomly by colony morphology from different plates of Eosine methylene blue agar and were streaked aseptically several times on freshly prepared Nutrient agar plates to obtain pure isolates, for antibiotic resistance test. Pure faecal isolates were subjected to several biochemical tests which include indole production, methyl red, Voges proskauer, Citrate utilization and by reaction in motility and urea medium.

Purified faecal bacterial isolates (60 in Numbers) were inoculated in Tryptone Soya Broth medium for 24 hours. The turbidity of the bacterial suspension was then compared with 0.5 MacFarland's Barium Sulphate standard solution (corresponding to $1.5x10^8$ CFU [colony forming unit] ml^{-1}). If the bacterial suspension was more turbid, autoclaved normal saline (containing 0.89% sodium chloride) was added drop by drop; if less turbid the inoculated tube was further incubated to reach the desired cell concentration. 0.1 ml of this culture was inoculated over Tryptone Soya Agar plate as per spread plate technique for determination of antibiotic resistance by a disc diffusion method [8] for the antibiotics (µg /mL) ampicillin (amp, 10), amikacin (ami, 10), chloramphenicol (ch, 30), cotrimoxazole (cotri, 25), gentamycin (genta, 30), kanamycin (kana, 30), streptomycin (strep, 10) and tetracycline (tet, 25). Antibiotic discs were placed on inoculated agar plates. After overnight incubation at 37°C, resistance was estimated by measuring the inhibition zone as per standards [9].

Statistical Analyses: Logarithmic transformation of bacterial counts was used to normalize data before statistical analysis. Results were expressed as mean±standard errors (SE). Bacterial levels in the digestive tract contents and the muscles of the two species of fish investigated were compared statistically using pair data *t*-tests, where $P<0.05$ was judged indicative of a significant difference. To determine the differences in the bacterial accumulation in *Oreochromis* sp. and *Labeo* sp., values of different bacterial parameters found in the digestive tract contents and muscles of the fish were compared by a two –way Analysis of Variance (ANOVA.) The data were analyzed in Excel 2003 (Microsoft Corp., Seattle, WA).

RESULTS

Water: APC at 37°C in pond water were 5.83±0.47 log cfu ml^{-1}. Numbers of total coliforms, faecal coliforms, *E.coli* and faecal streptococci were 4.88±0.48, 4.04±0.44, 2.87±0.40 and 3.46±0.56 log MPN 100 ml^{-1}, respectively (Table 1). *Salmonella* spp. and *Shigella* spp. were never found in pond water but *Vibrio* spp. were found very frequently.

Fish: Oreochromis muscles contained APC was 5.77±0.42 log cfu/gram. Number of total coliforms, faecal coliforms, *E.coli* and faecal streptococci were 4.74±0.70, 3.63±0.78, 2.15±0.84 and 3.02±0.74 log MPN $100g^{-1}$ (Table 2). Muscle samples of *Oreochromis* sp. were frequently positive for *Vibrio* spp., whereas only 1 sample was positive for *Salmonella* spp. and *Shigella* spp.

APC determined in the digestive tract contents of *Oreochromis* sp. was 8.07±0.348 log cfu/gram, respectively. Concentrations of total coliforms, faecal coliforms, *E.coli* and faecal streptococci were 7.38±0.42, 6.88±0.37, 5.62±0.68 and 4.25±0.61 log MPN $100g^{-1}$ (Table 2). All the intestinal samples were negative for *Shigella* spp., only 1 sample was positive for *Salmonella* spp., except few most of the intestinal samples were positive for *Vibrio* spp.

Labeo muscles contained APC was 6.09±0.45 log cfu/gram. Number of total coliforms, faecal coliforms, *E.coli* and faecal streptococci were 4.46±0.65, 3.57±0.77, 2.21±0.77 and 2.76±0.67 log MPN $100g^{-1}$ (Table 3). Most of the muscle samples from *Labeo* sp. examined for *Vibrio* spp. were found frequently positive, whereas all the muscle samples were negative for *Salmonella* spp. and *Shigella* spp.

APC determined in the digestive tract contents of *Labeo* sp. was 7.66±0.60 log cfu/g respectively. Concentrations of total coliforms, faecal coliforms, *E.coli* and faecal streptococci were 7.10±0.38, 6.12±0.49, 5.37±0.58 and 5.01±0.68 log MPN $100g^{-1}$ (Table 3). All the intestinal samples tested were negative for *Salmonella* spp. and *Shigella* spp. whereas most of them were positive for *Vibrio* spp.

Table 1: Bacterial load (mean±SE, N =18) in water of waste fed fish pond N: Number of samplings

Total coliform (log MPN 100 ml^{-1})	Faecal coliform (log MPN 100 ml^{-1})	*E.coli* (log MPN100 ml^{-1})	Faecal streptococci (log MPN 100 ml^{-1})	Aerobic plate count log cfu/ml	*Vibrio* sp.	*Salmonella* sp.	*Shigella* spp.
4.88±0.48	4.04±0.44	2.87±0.40	3.46±0.56	5.83±0.47	Present	Absent	Absent

N: Number of samplings

Table 2: Bacterial load (mean±SE, N =90, values having same superscript are significantly different at 5% level) in muscles and digestive tract contents of *Oreochromis* sp. collected immediately after harvest from wastewater-fed fish pond

Type of Tissue	Fish species	Coliforms log MPN100g-1	Faecal streptococci log MPN100g-1	Aerobic plate count log cfu/g	Pathogenic bacteria
Muscles	*Oreochromis* sp.	TC:4.74±0.70^a			*Vibrio* spp.
		FC: 3.63±0.78^b			++
		E.coli: 2.15±0.84^c	3.02±0.74^d	5.77±0.42^f	*Salmonella* spp. +(1)
					Shigella spp.+(1)
Digestive tract contents	*Oreochromis* sp.	TC: 7.38±0.42^a			*Vibrio* spp. ++
		FC: 6.88±0.37^b			*Salmonella* spp. +(1)
		E.coli: 5.62±0.68^c	4.25±0.61^e	8.07±3.84^f	*Shigella* spp. NF

* ++ - Positive for most of the tested samples

NF – Not found

+ (1) – Found in one fish

Table 3: Bacterial load (mean ± SE, N =90 values having same superscript are significantly different at 5% level)) in muscles and digestive tract contents of *Labeo* sp. collected immediately after harvest from wastewater-fed fish pond

Type of Tissue	Fish species	Coliforms log MPN100g^{-1}	Faecal streptococci log MPN100g^{-1}	Aerobic plate count log cfu/g	Pathogenic bacteria
Muscles	*Labeo sp.*	TC: 4.46±0.65^a			*Vibrio* spp. ++
		FC: 3.57±0.77^b	2.76±0.67^d	6.09±0.45^e	*Salmonella* spp. NF
		E.coli: 2.21±0.77^c			*Shigella* spp.- NF
		TC: 7.10±0.38^a			*Vibrio* spp. ++
Digestive tract contents	*Labeo* sp.	FC: 6.12±0.49^b	5.01±0.68^d	7.66±0.60^e	*Salmonella* spp. NF
		E.coli: 5.37±0.58^c			*Shigellas* pp. NF

* ++ — Positive for most of the tested samples

NF - Not found

Table 4: Antibiotic resistance (%) among bacterial isolates from water and fish

Source	Ampicillin µgml^{-1}	Amikacin µgml^{-1}	Chloramphenicol µgml^{-1}	Cotrimoxazole µgml^{-1}	Gentamycin µgml^{-1}	Kanamycin µgml^{-1}	Streptomycin µgml^{-1}	Tetracycline µgml^{-1}
Water	83.30 %	SENSATIVE	8.33 %	41.66 %	SENSATIVE	8.33 %	8.33 %	66.66 %
Labeo sp.	62.50 %	SENSATIVE	25.00 %	4.16 %	SENSATIVE	8.33 %	4.16 %	16.66 %
Oreochromis sp.	75.00 %	SENSATIVE	33.33 %	8.33 %	SENSATIVE	20.83 %	4.16 %	37.50 %

Significant differences were found between different bacterial concentrations in digestive tract contents and muscles of both the species ($p < .05$). The two way ANOVA showed no significant differences in bacterial recovery from digestive tract contents and muscles between *Oreochromis* sp and *Labeo* sp.

Antibiotic Resistance Bacteria from Water and Fish: Of the 60 thermotolerent coliform isolates examined for antibiotic sensitivity, 17 (28.33%) were resistant to one active compound, 13 (21.66%) showed a double resistance phenotype and 16 (23.33%) presented a multiple resistance one. Comparison of the prevalence of resistance revealed that thermotolerent coliforms isolated from pond water as well as from fish were predominantly ampicillin-resistant.

The incidence of resistance exhibited predominance to ampicillin (83.3%), followed by tetracycline (66.66%) and co-trimoxazole (41.66%) by the isolates from pond water (Table 4). Approximately 25% of the isolates were observed with double antibiotic resistance and 41.66% with multiple antibiotic resistances (MAR) from the same pond water.

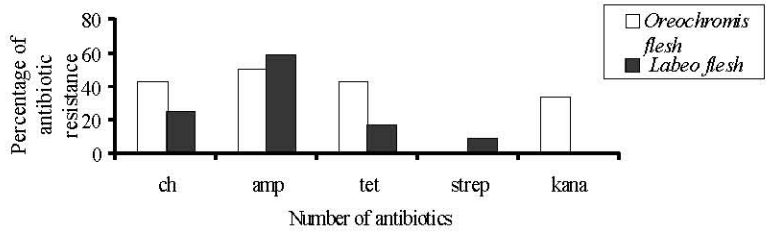

Fig. 1: Antibacterial multiresistance of bacteria isolated from flesh of *Oreochromis* spp. and *Labeo* spp.

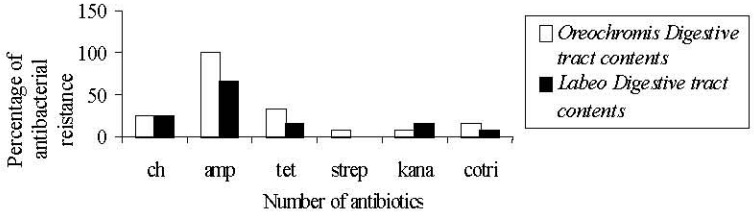

Fig. 2: Antibacterial multiresistance of bacteria isolated from digestive tract contents of *Oreochromis* spp. and *Labeo* spp.

Thermotolerent coliform isolates obtained from *Oreochromis* sp. displayed resistance to ampicillin (75%), tetracycline (37.5%), chloramphenicol (33.33%) and kanamycin (20.83%). Resistance was also observed, but to a lesser extent, to co-trimoxazole (8.33%) and streptomycin (4.16%). No resistance was found for amikacin and gentamicin (Table 4).

Thermotolerent coliform isolates obtained from *Labeo* sp. displayed resistance to ampicillin (62.5%), chloramphenicol (25%) and tetracycline (16.66%). Resistance was also observed, but to a lesser extent, to kanamycin (8.33), co-trimoxazole (4.16%) and streptomycin (4.16%). No resistance was found for amikacin and gentamicin (Table 4).

An important portion of bacteria isolated from fish were resistant to ampicillin, chloramphenicol tetracycline and kanamycin whereas streptomycin and co-trimoxazole resistant bacteria were infrequent (Figures 1 and 2). 33.33% bacterial strains from flesh and digestive tract contents of *Oreochromis* sp. exhibited simultaneous resistance against three to four anti bacterials, whereas 8.33% and 16.66% isolates from flesh and digestive tract contents of *Labeo* sp. were predominantly resistant to three to four anti bacterials, respectively.

DISCUSSION

Comparison of Faecal coliform counts in the wastewater-fed pond with the WHO [10] guideline value of <1000 per 100 ml, suggests considerable contamination of the pond water with human and animal faeces. Prevalence of faecal streptococci in the pond water might have resulted from pollution by poultry and cattle. The data on the concentrations of indicator bacteria in the muscles and digestive tract contents of *Oreochromis* sp. and *Labeo* sp. reared in wastewater-supplied pond revealed considerable bacteriological contamination of the fish. This resulted from particularly high concentrations of the APC, total coliforms, faecal coliforms, *E.coli* and faecal streptococci in water. Strong correlation between bacterial microflora in different tissues of fish and the concentration of bacteria in water was reported by Geldreich and Clarke [11]; Buras *et al.* [3]; Ogbondeminu [12]; Apun, Yusofand and Jugang [13]. Bacteria in muscles of *Oreochromis* sp. and *Labeo* sp. reared in sewage supplied pond were found when value of APC at 37°C in water was 5.83±0.47 log cfu ml^{-1}. According to Buras *et al.* [3], if concentration of bacteria in water exceeded 1.0×10^4 CFU ml^{-1} (4 log cfu ml^{-1}) Standard Plate Count level, they were found in the muscles of silver carp, common carp and tilapia reared in the ponds for 90 to 120 days. In the present study, fish muscles contained not only numerous total coliforms faecal coliforms, *E.coli* and faecal streptococci, but most of the individuals contained *Vibrio* spp and in very few *Salmonella* spp. and *Shigella* spp. were also detected.

In this study significant greater contamination of bacteria ($p<0.05$) were found in digestive tract contents than in muscles which may cross contaminate fish fillet. High bacterial count in the gut contents are due to the nature and degree of contamination of food and water ingested, to the activity of gut as a bioreactor and also to the occurrence of a natural flora in the gut.

Faecal bacteria in fish reflect the level of pollution of their environment, as the normal floras of fish do not include them [14]. Fish living in the natural environment are known to harbour Enterobacteriaceae that may cause diseases for humans and other warm-blooded animals [15]. At low numbers, microorganisms will be present on the surface and gut of the fish but not in muscle tissue. Above a certain threshold level, which represents the limit of the natural defense mechanisms of fish, pathogens are capable of penetrating muscle. The poor water quality of the waste fed pond may have induced weakness in the fish, resulting in a greater susceptibility to bacterial infection [16]. Contrasting results were reported by other investigators who showed that various stress factors (high turbidity, low oxygen levels) did not result in increased accumulation of bacteria in different fish tissues [17]. In our research high rate recovery of both indicator as well as pathogenic bacteria in fish muscles could indicate a decrease in the immune response by the fishes inhabiting waters with a severe quality environmental degradation.

Pathogenic bacteria like *Salmonella* spp. and *Shigella* spp. were almost completely absent in pond water and fish samples. Pheleps [18] isolated *Salmonella* from 2 of 63 wastewater fish samples. This may be due to either low excreted load or low survival time of them in environment.

Vibrio spp. were very frequently found in pond water and fish samples. *Vibrio* species have been isolated and described from normal healthy *Penaeus vannamei* juveniles [19]. Senderovich *et al.* [20] reported fish as possible reservoirs of *Vibrio cholerae*. *Vibrio* spp. is ubiquitous in aqueous environments; thus its presence does not necessarily imply a health risk [21]. Although in the natural environment fish usually harbour bacteria only in the digestive tract but not in muscles [22]. In our experiment *Vibrio* spp. were detected in muscles of both the fish. Thus in this context species level detection of *Vibrio* spp is required for specifying the presence of food borne diseases causing as well as cholera causing 01 strains.

The results of the present study revealed that 31.25% and 16.66% of bacterial isolates from fish and their aqueous environment respectively were resistant to a single antibiotic. The random bacterial isolates showed resistance in decreasing order for ampicillin (71.66%), tetracyline (35%), chloramphenicol(25%), kanamycin (13.33%), co-trimoxazole (13.33%) and streptomycin (5%). Similar findings with highest resistance to ampicillin were reported in the samples of untreated sewage by Niemi *et al.* [23]. Occurrence of thermotolerent coliforms with high resistance to ampicillin and tetracycline reflect human influence in the environment [24].

An important proportion of bacteria resistant to ampicillin, tetracycline, chloramphenicol, streptomycin, kanamycin and co-trimoxazole were observed among thermotolerent coliforms isolated from fish and water. The simultaneous resistance to β- lactem, chloramphenicol and aminoglycoside may be due to dissemination of antibiotic resistance plasmids in the aquatic environment. The β-lactam groups of antibiotics such as ampicillin and carbenicillin have a more pronounced effect on the antibiotic-resistant bacterial profile in the primary water source than those antibiotics used as feed additive [25].

In the present study high rate recovery of thermotolerent coliforms from intestines of *Oreochromis* and *Labeo* with simultaneous resistance to three to four antibiotics suggest changes in the nutritionally beneficial intestinal microflora with unexpected consequences on fish health.

High rate recovery of antibiotic resistant bacteria from flesh of both the fish have immense ecological and public health implications specially if the resistance is plasmid mediated then there could be a problem associated with the transfer of resistance determinants to human pathogenic bacteria which may enter in human population through fish consumption. According to Walia *et al.* [26] antibiotic resistance genes against ampicillin, streptomycin and tetracycline are known to be transferable to other bacteria. It may possible that these antibiotic resistant bacteria from wastewater may transfer their antibiotic resistant determinants to indigenous flora of fish, provoking their spread and prevalence in aquatic environment.

It has been claimed that the risk to public health from antimicrobials in aquaculture probably is very low [27]. This is likely true in North American and European aquaculture where the use of antimicrobials is generally decreasing. In contrast, legislation and enforcement of antimicrobial use in developing countries may be less strict. Furthermore, environmental bacteria from warmer climates may be acclimatized to temperatures near human body temperature and may upon ingestion survive and transfer antimicrobial resistance genes to human gut bacteria [27].

Thus, we can say that uncontrolled use of antibiotics and common practice of self medication typical of the Indian setting would pose a selection pressure in wastewater and fishes reside there in favour of organisms possessing genes that code for resistance. Observations

by other investigators indicate that resistance to antimicrobials may persist for a considerable number of years after antibiotic usage has been discontinued [28]. Several studies indicate that the environmental conditions in wastewater may enhance the likelihood of gene transfer [29]. Mach and Grimes [30] demonstrated the high transfer frequencies of enteric bacteria in a wastewater. Additionally resistant bacteria may pose a risk of therapeutic problems to public health and fish population. So the study demands an elaborate investigation on the plasmids profile of the members of predominant multidrug resistant bacterial microflora associated with sewage fed fishery as an evidence of conjugal transfer of antibiotic resistance genes in human and animal food chain through fish consumption.

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