Antibiotic Resistant Indicator Bacteria from Organs of Sewage Fed Fish

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Abstract

Bacteriological contamination of *Oreochromis* spp. reared in a pond supplied with domestic sewage was enumerated followed by determination of resistance for antibiotics. The evaluation system comprised of quantitative analysis of thermotolerent faecal coliforms (Th FC) and *Escherichia coli* (*E.coli*). Muscles and digestive tract contents of fish were found to be frequently infected by bacteria. Levels of resistance to eight different antibiotics were determined in Th FC isolates by the disc diffusion method. Most of the isolates were predominantly ampicillin-resistant. 36.1% of the total isolates were resistant to three to four antibiotics. High rate recovery of drug resistant bacteria from fish definitely imposes public health risk.

Keywords: Domestic sewage; Thermotolerent faecal coliforms; Antibiotic resistance; Health risk

Introduction

The central aim of the Wastewater fed fish pond 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 resistant bacteria entering in waste fed pond through wastewater and contaminate fish flesh.

One of the important concerns of wastewater fisheries is the contamination of fishes by faecal bacteria (Fapohunda, MacMillan, Marshall and Waites, 1994). 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 (Olayemi, Adedayo and Ojo, 1991). 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 bacteria in the muscles and digestive tract contents of *Oreochromis* spp. reared in wastewater fed pond. Random thermotolerent coliform isolates from fish and their aqueous environments were examined for their antibiotic resistance patterns to some commonly used antibiotics.

Materials and Methods

Study site

A sewage fed pond of Bandipur, Rahara, North 24 Parganas, (22°44'N Latitude and 88º24'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.

Sampling and Dissection

Fish samples (*Oreochromis* spp.) were caught with a net and were immediately transferred to the laboratory in containers with pond water. They were dissected according to Buras et al. 1987. Water from sewage-supplied pond was sampled and analyzed simultaneously with fish sampling.

Bacteriological analysis

Standard three-tube most-probable-number (MPN) methods were used for the determination Th FC and *E.coli*. (APHA, 1998). Representatives of typical thermotolerent coliform isolates from water and fish samples were selected randomly by colony morphology and were subjected to antibiotic sensitivity test (Ogbonna, Sokari and Amaku 2008). Antibiotic

sensitivity of the isolates was tested in vitro against a wide variety of antibiotics using octodisks (Hi media, 0D053R) containing following antibiotics Ampicillin (Amp,10 µg), Amikacin (Ami, 10µg), Chloramphenicol (Ch, 30µg), Cotrimoxazole (Cotri, 25µg), Gentamycin (Genta, 30µg), Kanamycin (Kana, 30µg), Streptomycin (Strep, 10µg) and Tetracycline(Tet, 25µg). Agar disc diffusion method (Bauer et al., 1966) was employed for the purpose.

Statistical analysis

Logarithmic transformation of bacterial counts was used to normalize data before statistical analysis. Results were expressed as mean ± standard errors. Bacterial levels in the digestive tract contents and the muscles of the fish species investigated were compared statistically using pair data *t*-tests, where $P<0.05$ was judged indicative of a significant difference. The data were analyzed in Excel 2003 (Microsoft Seattle, WA, USA) with the add-in software Statcel 2 (Yanai, 2004).

Results

Thermotolerant faecal coliforms were commonly found in all analyzed fish tissues. Additionally, however, *Escherichia coli* were present both in digestive tract contents and muscles of the fish (Table 2). Bacterial loads in the fish were significantly higher ($p<0.05$) in the digestive tract contents than in edible muscles (Table 2).

The incidence of resistance exhibited predominance to ampicillin followed by tetracycline and co-trimoxazole by the isolates from pond water (Table 3). Thermotolerent coliform isolates obtained from *Oreochromis* spp. displayed resistance to ampicillin, tetracycline, chloramphenicol and kanamycin (Table 3). Resistance was also observed, but to a lesser extent, to co-trimoxazole and streptomycin. No resistance was found for amikacin and gentamicin. 33.33% bacterial strains from flesh and digestive tract contents of *Oreochromis* spp. exhibited simultaneous resistance against three to four anti bacterials (Figure 1).

Discussion

Faecal coliform counts in the wastewater-fed pond were higher than the WHO (1989) guideline value of <1000 per 100 ml, suggests considerable contamination of the pond water with human and animal faeces. The data on the concentrations of indicatory bacteria in the muscles and digestive tract contents of *Oreochromis* spp. reared in wastewater-supplied pond reveal considerable bacteriological contamination of the fish. Faecal bacteria in fish reflect the level of pollution of their environment, as the normal floras of fish do not include them (Cohen and Shuval 1973). Fish living in the natural environment are known to harbour Enterobacteriacea that may cause diseases for humans and other warm-blooded animals (Pillay, 1992). At low numbers, microorganisms will be present on the surface of fish and gut 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. Poor quality of water induces stress, which is manifested in elevated cortisone levels, a hormone known to be a very potent immunosuppressant (Escher et al., 1999).

The random bacterial isolates showed resistance in decreasing order for ampicillin, tetracyline, chloramphenicol, co-trimoxazole, kanamycin and streptomycin. Similar findings with highest resistance to ampicillin were reported in the samples of untreated sewage by Niemi et al., 1983. Occurrence of thermotolerent coliforms with high resistance to ampicillin and tetracycline reflect human influence in the environment (Andersen and Sandaa 1994).

The simultaneous resistance to â lactem, chloramphenicol and aminoglycoside in Th FC from fish and water may be due to dissemination of antibiotic resistance plasmids in the aquatic environment. The â - lactam groups of antibiotics such as ampicillin and carbenicillin have a more pronounced effect on the antiobiotic-resistant bacterial profile in the primary water source than those antibiotics used as feed additive (Mulamattathil et al., 2000).

In the present study high rate recovery of thermotolerent coliforms from intestines of *Oreochromis* spp. 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 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.* (2004) antibiotic resistance genes against ampicillin, streptomycin, and tetracycline are known to be transferable to other bacteria.

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 (Hinton et al., 1986). Several studies indicate that the environmental conditions in wastewater may enhance the likelihood of gene transfer (Pote *et al.* 2003). Mach and Grimes (1982) 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.

Parameters	
Th FC log MPN $100ml^{-1}$	*E.coli* log MPN $100ml^{-1}$
3.72±0.73	2.84±0.70

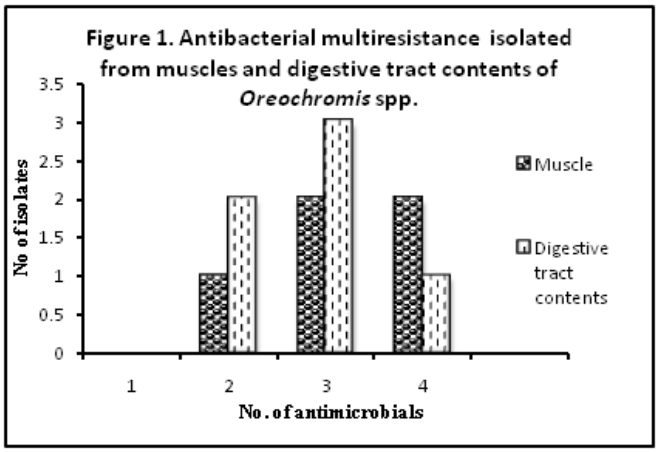
Table 1: Faecal indicator bacterial load (mean ± SE, n =3) in water of waste fed fish pond

Fish species	**Tissue Type**	Parameters	
		Th FC log MPN $100ml^{-1}$	*E.coli* log MPN $100ml^{-1}$
Oreochromis spp.	Muscles	3.11 ± 1.06^{a}	1.67 ± 0.77^{c}
	Digestive tract contents	5.61 ± 1.00^{b}	3.97 ± 0.94^{d}

Table 2: Faecal indicator bacterial load (mean ± SE, n =3, values having different superscript are significantly different at 5% level) in muscles and digestive tract contents of fish collected immediately after harvest from wastewater-fed fish pond

Source	Amp µg/ disc	Ami µg/ disc	Ch µg/ disc	Cotr µg/ disc	Genta µg/ disc	Kana µg/ disc	Strep µg/ disc	Tet µg/ disc
Water	83.3%	S	8.33%	41.66%	S	8.33%	8.33%	66.66%
Oreochromis spp.	75%	S	33.33%	8.33%	S	20.83%	4.16%	37.5%

Table 3: Antibiotic resistance (%) among bacterial isolates from water and fish



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