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Food Safety Hazards Related to Emerging Antibiotic Resistant Bacteria in Cultured Freshwater Fishes of Kolkata, India

T. Jawahar Abraham
Department of Fishery Pathology and Microbiology, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata - 700 094, India

Abstract: Association of opportunistic human bacterial pathogens in cultured freshwater fishes of Kolkata, India and their sensitivity to broad spectrum antibiotics was investigated. Both indigenous and non-indigenous human bacterial pathogens such as *Aeromonas hydrophila*, *A. caviae*, *Edwardsiella tarda*, *Escherichia coli*, *Pseudomonas* spp. and *Vibrio parahaemolyticus* were isolated from freshwater fishes of Kolkata. These strains were highly resistant to oxytetracycline (62%) and nitrofurantoin (46%), and sensitive to ciprofloxacin (91%) and chloramphenicol (89%). Multiple Antibiotic Resistance (MAR) was high in catfishes (76%) followed by miscellaneous fishes (66%) and sewage-fed farm grown carps (55%). Among the bacterial species, the MAR was high in *Ed. tarda* (86%). More than 50% of the strains of *A. hydrophila*, *A. caviae*, *E. coli*, *Pseudomonas* spp., *V. parahaemolyticus* and unidentified Gram positive rods exhibited MAR. The results suggested that there is added risk of antibacterial resistance developing in the emerging human bacterial pathogens from freshwater aquaculture and of such antibiotic resistant bacterial pathogens entering the food chain.

Key words: Antibiotic resistance, food safety hazards, fish-borne pathogens, freshwater aquaculture

INTRODUCTION

Fishery products constitute an important part of international trade, currently worth more than US$ 50 billion, indicating increasing consumer interest in the commodity. Fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture. More than 80% of global aquaculture products are produced in freshwater (Håstein *et al*., 2006; FAO, 2009). Aquaculture production includes the selection of breeding stock, the rearing of fry and fingerlings and the growth of adult fish. There are wide variations in methods and practices for the production of different species (FAO/NACA/WHO, 1997). Microbiological quality evaluation of fish aims to quantify the hygienic quality of fish, including temperature abuse and the possible presence of pathogenic microorganisms in the fish. In food business, assurance of quality has become a central part of all activities focusing on safety. The huge interest in freshwater fish culture and consumption in West Bengal, as well as the current tendency for consumption of various forms of fresh fish products, has made public health safety of cultured fish a more pressing issue. The aim of the present study was to determine the frequency of antibiotic-resistance in emerging human bacterial pathogens associated with cultured freshwater fishes of commercial importance in Kolkata, West Bengal.

MATERIALS AND METHODS

A total of 489 strains of opportunistic bacterial pathogens comprising *Aeromonas hydrophila* (n = 179), *Aeromonas caviae* (n = 151), *Edwardsiella tarda* (n = 35), *Escherichia coli* (n = 44), *Pseudomonas* spp. (n = 55), *Vibrio parahaemolyticus* (n = 6), unidentified Gram negative, oxidase positive, fermentative rods (n = 16) and unidentified Gram positive rods (n = 3) isolated from various cultured freshwater fishes of commercial importance in and around Kolkata, West Bengal, India between 2005 and 2010 were used in this study. These bacterial strains were isolated following standard methodology (APHA, 1992), maintained on tryptic soy agar slants and identified as per the Bergeys' manual (Holt *et al*., 1994). Sensitivity of these strains to six broad-spectrum antibiotics namely chloramphenicol (30 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), gentamycin (10 μg), nitrofurantoin (300 μg) and oxytetracycline (30 μg) was tested by agar-disc diffusion method (Bauer *et al*., 1966) on Mueller Hinton agar (Hi-Media, India). Multiple Antibiotic Resistance (MAR) was derived from the antibiogram data.

RESULTS AND DISCUSSION

Fish and fish products have long been considered a vehicle of food-borne bacterial and parasitic infections leading to human illnesses (Huss *et al*., 2003; Novotny *et al*., 2004; Håstein *et al*., 2006). The hazards associated with human pathogenic bacteria in the cultured fish can arise from the bacteria naturally present in the aquatic environment, referred to as indigenous bacteria, and those present as a result of contamination with human or animal faeces, or otherwise introduced to the aquatic environment. Hazards may also arise through the introduction of bacteria during post-harvest handling and

Table 1: Bacterial pathogens transmissible to human beings through contact with fish and/or associated with fish and fish products

Bacterial species	Disease
Bacterial pathogens transmissible to human beings through contact with fish	
Erysipelothrix rhusiopathiae	Endocarditis, fish handler's disease, fish rose
Leptospira interrogans	Leptospirosis
Mycobacterium marinum and *M. fortuitum*	Mycobacteriosis, fish tank granuloma
*Photobacterium damselae**	*Photobacterium damselae* sepsis
Pseudomonas aeruginosa# and *P. fluorescens#*	Wound infections
Streptococcus iniae	Bacteraemia, cellulitis, endocarditis, meningitis, septic arthritis, mad fish disease
Vibrio alginolyticus#*	Otitis media
Vibrio vulnificus#*	Septicaemia
Bacterial pathogens associated with fish and fish products	
Aeromonas hydrophila#*	Enteritis and septicaemia
Bacillus cereus	*Bacillus cereus* gastroenteritis
Campylobacter jejuni	Campylobacteriosis, gastroenteritis
Clostridium botulinum Type E*	Botulism
Clostridium perfringens	*C. perfringens* food poisoning
Delftia acidovorans (*Pseudomonas acidovorans*)	Endocarditis, bacteraemia
Edwardsiella tarda#	Gastroenteritis, septicaemia, meningitis, cholecystitis, cellulitis
Escherichia coli#	Gastroenteritis
Hafnia alvei	Septicaemia, gastroenteritis, meningitis, pneumonia, wound infections
Legionella pneumophila	Legionnaire's disease
*Listeria monocytogenes**	Listeriosis
Plesiomonas shigelloides#*	Waterborne diseases, diarrhea
Salmonella spp.#	Salmonellosis
Shigella spp.#	Shigellosis
Staphylococcus aureus#	Gastroenteritis
Vibrio cholerae#*	Cholera
Vibrio parahaemolyticus#*	Acute gastroenteritis
Yersinia enterocolitica	Yersiniosis

*: Indigenous bacterial pathogen; #: Reportedly present in cultured fishes of West Bengal

Table 2: Antibiotic resistance (%) in bacterial flora of freshwater fishes from different sources of West Bengal (N = 489)

Antibiotic	Carps (n = 90)	Sewage farm grown carps (n = 154)	Ornamental fishes (n = 75)	Catfishes (n = 85)	Miscell-aneous fishes* (n = 85)	Pooled (N = 489)
Chloramphenicol (30 mg)	11.11	11.69	10.67	16.47	8.23	11.66
Ciprofloxacin (5 mg)	05.56	10.39	01.33	15.29	10.59	09.00
Co-trimoxazole (25 mg)	34.44	25.32	34.67	52.94	23.53	32.92
Gentamycin (10 mg)	01.11	20.78	05.33	12.94	29.41	14.93
Nitrofurantoin (300 mg)	30.00	64.29	29.33	44.71	49.41	46.63
Oxytetracycline (30 mg)	31.11	85.71	37.33	63.53	70.59	61.76

*: Miscellaneous varities other than Indian major carps and catfishes

processing (Huss *et al*., 2003). The bacterial pathogens transmissible to human beings through contact with fish and/or associated with fish and fish products are listed in Table 1.

The present study (Table 1 and 3) isolated both indigenous and non-indigenous human bacterial pathogens from freshwater fishes of Kolkata. *Aeromonas hydrophila* and *V. parahaemolyticus* are indigenous to freshwater fish and marine fish, respectively; while the others are extraneous contaminants (Huss *et al*., 2003). Aeromonad bacteria are ubiquitous in the aquatic environment and several *Aeromonas* sp. have been reported to cause disease in fish, as well as being potential food-borne pathogens that may cause disease in humans (Novotny *et al*., 2004). Incidence of *V. parahaemolyticus*, which cause acute gastroenteritis, has been reported earlier from a variety of freshwater fishes in Kolkata (Sarkar *et al*., 1985), due to cross-contamination or mishandling of freshwater fishes along with marine and brackishwater fishes at the fishmongers stalls. The

Table 3: Multiple Antibiotic Resistance (MAR) in bacterial flora (N = 489) of freshwater fish and fish farm environment of West Bengal

Source	MAR (%)
Carps (n = 90)	32.22
Sewage-fed farm grown carps (n = 154)	54.55
Ornamental fishes (n = 75)	47.62
Catfishes (n = 85)	76.46
Miscellaneous fishes (n = 85)	65.88
Bacterial species	
Aeromonas hydrophila (n = 179)	69.27
Aeromonas caviae (n = 151)	54.30
Edwardsiella tarda (n = 35)	85.71
Escherichia coli (n = 44)	52.27
Pseudomonas spp. (n = 55)	72.73
Vibrio parahaemolyticus (n = 6)	50.00
Unidentified Gram positive rods (n = 3)	66.67
Unidentified Gram negative, oxidase positive, fermentative rods (n = 16)	12.50

demographic character and local custom in West Bengal are quite different from those in other States of India. Consumption of true marine fishes in comparison to those of freshwater origin is infrequent. The association of *V. parahaemolyticus* with freshwater fishes and their ability to survive in the freshwater environment is, therefore, of public health significance Human infections caused by enteric flora, *Ed. tarda* are considered a serious problem in tropical or subtropical areas. Infections associated with this species include gastroenteritis, wound infections, and systemic diseases such as septicaemia, meningitis, cholecystitis, and osteomyelitis (Janda and Abbott, 1993). Incidence of *Ed. tarda* in fishes from freshwater aquaculture environment and retail market (Pankajkumar, 2009) and human liver abscess caused by *Ed. tarda* biogroup 1 in India (Manchanda *et al*., 2006) have been reported. The isolation of *E. coli* in fishes grown in sewage-fed farms and also in retail market fishes of Kolkata indicated contamination of fishes with faecal matter of animal and human origin so also the earlier study (Manna *et al*., 2008). Food products that show evidence of faecal contamination are generally regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens. Some strains of *E. coli* are capable of causing food-borne disease, ranging from mild enteritis to serious illness and death (FAO/NACA/WHO, 1997). In developing and densely populated countries like India, the faecal contamination of natural water bodies has emerged as a major challenge. The water bodies are often contaminated by the activities of adjoining populations and partially treated or untreated sewage from the townships is released into these water bodies. The fishes harvested from such areas often contain enteric pathogens as has been observed in this study. In addition, poor sanitation in market centers and the open fish markets exacerbates the situation.

High levels of individual - and multiple antibiotic resistant bacteria were detected in freshwater fishes including ornamental fishes. The antibiotic resistance in the bacterial flora of freshwater fishes also varied greatly among different sources (Table 2 and 3). The bacterial strains were highly resistant to oxytetracycline (62%). Resistance to nitrofurantoin (47%) and co-trimoxazole (33%) was also seen. However, they were highly sensitive to ciprofloxacin (91%), chloramphenicol (88%) and gentamycin (85%). The MAR was high in catfishes (76%) followed by miscellaneous fishes (66%), sewage-fed farm grown carps (55%) and ornamental fishes (48%). However, the sampling events were not correlated with antibiotic therapy except catfish culture systems, wherein the use of antibiotics was quite common. The MAR was low (32%) in bacterial flora of carps grown in non-sewage-fed aquaculture system. Among the bacterial species, MAR was high in *Ed. tarda* (86%) probably due to the intrinsic resistance capabilities, largely been attributed to outer membranes, of these strains to the tested antibiotics. Majority of the strains of *Pseudomonas* spp. (73%), *A. hydrophila* (69%), unidentified Gram positive rods (67%), *A. caviae* (54%), *E. coli* (52%) and *V. parahaemolyticus* (50%) also exhibited MAR.

The results suggested that the fishes grown in certain types of fish farming systems, which use livestock manures or raw and/or diluted sewage, may pose a risk of development of antimicrobial resistance in bacterial flora. The SVARM (2001) report indicated that prevalence of acquired resistance to antimicrobials among bacteria of the normal enteric flora can serve as indicator of the selective pressure exerted by the use of antibiotics. Although the present study has not established whether the emergence of antibiotic resistance is due to aquaculture practices or other means, it is probable that these resistant bacteria might reach the public through the cultured fish, i.e., the food chain. Also, the resistance can be transferred both to fish and human pathogens, causing eventual difficulties in chemotherapy. There are reports on the association of food-borne infections due to antibiotic resistant enteric bacteria (Temelli, 2002) and on the use of antimicrobials in aquaculture with risks for the therapy of human infections and treatment failure (Blondeau, 2004), which is a serious cause for concern.

CONCLUSION

The risk of transfer of antibiotic resistance to human bacterial flora is probably low in countries where the use of antimicrobials is limited. However, in countries with less restrictive legislation as in India, the risk of contaminating fish and aquaculture products with resistant bacteria is greater. Regarding hazards due to antibacterial drugs, since there is no harmonized regulatory view around the world, it is difficult to extract universal approaches. However, consumers and regulatory bodies are becoming more and more aware of the different hazards, regardless of whether they could be listed as regulatory hazards or not.

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