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Short Communication

Comparative Analysis of Antibiotic Resistance Pattern of Bacteria Isolated from Fish of Cultured and Natural Ponds: A Study based on Noakhali Region of Bangladesh

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ABSTRACT: The indiscriminate use of antibiotics in fish farming emerges of antimicrobial resistant bacteria which is one of the most important current threats to public health. This study aimed to observe the pattern of multidrug resistant bacteria isolated from fish samples at cultured (antibiotic used) and natural ponds (no antibiotic) in Noakhali region. A total 58 Bacteria (nine bacterial genera) isolated from ten fishes (five fishes from cultured ponds and five from natural ponds) were identified presumptively by cultural, microscopic and biochemical test. After comparative analysis in both ponds samples, the predominant bacteria were *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli*, *Vibrio spp.* and *Staphylococcus* spp. which showed higher multidrug resistant pattern in antibiotic-used pond fish than non-antibiotic one. Therefore, safe human consumption of fish needs to consider continuous monitoring with avoidance misuse of antibiotics.

KEYWORDS: Fish, Bacterial disease, Antibiotics, Multidrug resistance

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INTRODUCTION

Fish, a nutritious food with safe, high quality protein and low fat content, contributes about 60% of our daily supply of animal protein intake[1].The fisheries sector, in Bangladesh, the third largest contributor to Bangladesh's export earnings with growing annually by 5-8 %, plays a particularly crucial role among poor as a main or additional source of livelihood and income in the overall economy of the country[2].

However, fish are prone to diseases caused by a wide variety of bacterial pathogens[3]. Bacterial agents are implicating as pathogens of fresh water and marine fish such as *Vibrio* spp., *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* spp., *Klebsiella* spp., *Streptococcus* spp., *Salmonella* spp., *Micrococcus* spp., *Serratia* spp. and *Escherichia* coli. These bacteria are found in fish and fish products harvesting in feral and cultured ponds of some aquatic environments which have been identified as the most commonly causing of fish diseases[4,5,6].

Antibiotics are used to effectively prevent and treat bacterial infections[7]. Farmers, in the greater Noakhali region, use antibiotic (beta lactam group, oxytetracycline, erythromycin etc.) and others chemicals to control the diseases of fish along with pond preparation[8]. The overwhelming use of antibiotics has developed antibiotics resistance in fish pathogens with the emergence of antibiotics resistant bacteria in aquatic environments. It has been reported

that after administration many antibiotics persist in the sediment and in the aquatic environment for long time which affect the sedimentary microbial community[9, 10]. This study attempted to investigate the presence of bacteria in fish samples and to analyze multidrug resistance pattern of the bacteria of fish samples collected from different drug treated cultured and non-drug used natural ponds in Noakhali region whether bacteria of natural pond fish acquire drug resistance or not. The fish contamination with antibiotic-resistant bacteria and their spread would be one of the most serious threats to public health in this century.

MATERIALS AND METHODS

Sample collection

About ten fish samples were collected aseptically in which five fishes from five different drugs used cultured ponds and five fishes from five different non-drug used natural ponds in Noakhali region. In case of cultured ponds, the fish samples were collected within one-month period after the antibiotic treatment in those ponds. Samples were transported immediately (approximately within 1 hour) to the laboratory, the department of Microbiology, Noakhali Science and Technology University, Sonapur; Noakhali for microbiological analysis.

Processing of fish samples

About 5g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The cut samples (such as intestine, gill, and muscle) were crushed into small pieces in a sterile mortar with about 10 ml sterile water. About one ml aliquot from the crushed sample was taken and homogenized in a sterile beaker with 9 ml of distilled water which gave 1:10 dilution.

Isolation and identification of bacterial isolates

Diluted samples were inoculated onto some selective media for bacterial isolation such as, Coliform organisms and Gram negative enteric bacteria were isolated using pour plate method with MacConkey agar, EMB (Eosin Methylene Blue) agar, then Citrimide agar for *Pseudomonas* spp., XLD (Xylose Lysine Deoxycholate) agar for *Salmonella* spp., *Shigella* spp. and TCBS (Thiosulphate Citrate Bile Salt Sucrose) agar for pathogenic *Vibrio* spp., MSA (Manitol salt agar) for *Staphylococcus aureus*. To identify the bacteria presumptively, microscopic and a series of biochemical tests were performed. Biochemical characteristics of the isolates were determined by employing the following tests on a fresh culture; Oxidase test, Coagulase test, Citrate utilization test, Urease test, Indole formation test, motility test and Triple sugar ion test.[11]

Antibiotic Susceptibility assay

The bacterial isolates were selected for antimicrobial susceptibility testing according to Kirby-Bauer disc diffusion techniques[12] on Mueller Hinton agar using the following antibiotic discs (Oxoid): gentamycin (GM) 10 µg, tetracyclin (TE) 30 µg, oxacillin (OX) 1 µg, sulphadiazine and trimetoprime (SXT) 25 µg, ciprofloxacin (CIP) 5 µg, erythromycin (E) 15 µg, cefalexin (CL) 30 µg, ceftriaxone (CRO) 30 µg, ampicillin (AMP) 10 µg, ceftazidime (CAZ) 30 µg, doxycycline (D) 30 µg, imepenem (IPM) 10 µg, cefepime (FEP) 30 µg, amikacin (AN) 30 µg, amoxycillin (AMX) 30 µg and clindamycin (CC) 2 µg. The zone of inhibition was interpreted according to Clinical Laboratory Standard Institute[13].

RESULTS AND DISCUSSION

A total of 58 bacterial isolates were isolated from 10 fish samples, among these five fishes were from drug used cultured ponds and five were from non drug used natural ponds then identified presumptively as 9 different genera of bacteria by cultural characteristics, Gram's staining and biochemical tests (Table 1).

Table 1. Biochemical tests of bacteria isolated from fish of cultured and natural ponds

Types of Pond	Types of Fish	Isolates ID	TSI test	Coagulase test	Citrate test	Oxidase test	Motility test	Indole test	Urease test	Presumptive Isolates
Antibiotic used cultured pond	Rui (*Labeo rohita*)	R1	+	-	-	-	+	+	+	*Proteus* spp.
		R2	+	-	+	-	-	-	+	*Klebsiella* spp.
		R3	-	-	+	+	-	-	-	*Pseudomonas* spp.
		R4	+	-	-	-	+	+	-	*E. coli*
		R5	+	-	+	-	+	-	-	*Cirobacter* spp.
		R6	+	-	+	-	-	-	+	*Klebsiella* spp.
		R7	+	-	+	-	-	-	+	*Klebsiella* spp.
	Mrigel (*Cirrhinus cirrhosis*)	M1	+	-	+	+	+	+	-	*Vibrio* spp.
		M2	+	-	+	-	-	-	+	*Klebsiella* spp.
		M3	+	-	+	-	-	-	+	*Klebsiella* spp.
		M4	+	-	+	-	+	-	-	*Enterbacter* spp.
		M5	+	-	+	-	+	-	-	*Enterobacter* spp.
		M6	-	-	+	+	-	-	-	*Pseudomonas* spp.
		M7	+	-	+	-	-	-	+	*Klebsiella* spp.
		M8	+	-	-	-	+	-	-	*Salmonella* spp.
		M9	+	+	+	-	-	-	+	*S. aureus*
		M10	+	+	+	-	-	-	+	*S. aureus*
	Catla (*Catla catla*)	C1	-	-	+	+	-	-	-	*Pseudomonas* spp.
		C2	+	-	+	-	-	-	+	*Klebsiella* spp.

table continued...

		C3	-	-	+	+	-	-	-	*Pseudomonas* spp.
		C4	-	-	+	+	-	-	-	*Pseudomonas* spp.
		C5	+	-	-	-	+	+	-	*E. coli*
		C6	+	-	+	-	-	-	+	*Klebsiella* spp.
		C7	+	-	+	+	+	+	-	*Vibrio* spp.
		C8	+	+	+	-	-	-	+	*S. aureus*
		C9	+	+	+	-	-	-	+	*S. aureus*
	Tengra (*Macrones vittalus*)	TG1	+	+	+	-	-	-	+	*S. aureus*
		TG2	+	+	+	-	-	-	+	*S. aureus*
		TG3	-	-	+	+	-	-	-	*Pseudomonas* spp.
		TG4	+	-	+	-	-	-	+	*Klebsiella* spp.
		TG5	+	-	+	-	-	-	+	*Klebsiella* spp.
	Telapia(*Oreochromis niloticus*)	TE1	+	-	+	-	+	-	-	*Enterobacter* spp.
		TE2	+	-	-	-	+	+	-	*E. coli*
		TE3	-	-	+	+	-	-	-	*Pseudomonas* spp.
		TE4	+	-	-	-	+	+	-	*E. coli*
		TE5	+	+	+	-	-	-	+	*S. aureus*
Non antibiotic used natural pond	Calbaus (*Labeo calbasu*)	CB1	-	-	+	+	-	-	-	*Pseudomonas* spp.
		CB2	+	-	+	-	-	-	+	*Klebsiella* spp.
		CB3	+	-	+	+	+	+	-	*Vibrio* spp.
		CB4	+	-	+	+	+	+	-	*Vibrio* spp.
		CB5	+	-	+	-	-	-	+	*Klebsiella* spp.
	Taki (*Channa punctate*)	TK1	+	-	-	-	+	+	-	*E. coli*
		TK2	+	-	-	-	+	+	-	*E. coli*
		TK3	+	-	+	-	-	-	+	*Klebsiella* spp.
		TK4	+	-	+	-	-	-	+	*Klebsiella* spp.
		TK5	+	-	+	-	-	-	+	*Klebsiella* spp.
	Puti(*Puntius chola*)	PU1	-	-	+	+	-	-	-	*Pseudomonas* spp.
		PU2	+	+	+	-	-	-	+	*S. aureus*
		PU3	+	-	+	-	-	-	+	*E. coli*
		PU4	+	-	-	-	+	+	-	*Klebsiella* spp.
		PU5	+	-	-	-	+	+	-	*E. coli*
	Koi (*Anabas Testudinus*)	KO1	-	-	+	+	-	-	-	*Pseudomonas* spp.
		KO2	+	-	+	-	-	-	+	*Klebsiella* spp.
		KO3	+	-	-	-	+	+	-	*E. coli*
		KO4	+	-	-	-	+	+	-	*E. coli*
	Sorputi (*Puntius sarana)*	SP1	+	-	+	-	-	-	+	*Klebsiella* spp.
		SP2	+	-	+	+	+	+	-	*Vibrio* spp.
		SP3	-	-	+	+	-	-	-	*Pseudomonas* spp.

Aeromonas spp., *Pseudomonas* spp, *Serratia* spp., *Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli*, *Enterobacter* spp. and *Salmonella* spp. are periodically implicated as fish pathogens [6]. From drug used pond fish, it was found that ten (28%) were *Klebsiella* spp., seven (19.4%) were identified as *Pseudomonas* spp., four (11.11%) were *E. coli*, seven (19.4%) were *Staphylococcus aureus*, two (5.55%) were *Vibrio* spp. From non-drug used natural pond fish, *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *Staphylococcus aureus* and *Vibrio* spp. were isolated by (8) 36.4%, (4) 18.2%, (6) 27.3%, (1) 4.54% and (3)13.6% respectively (Table 2). The isolation rate of *Klebsiella* spp. was higher than other bacterial isolates in both types of ponds (Table 2).

Table 2: Rate (%) of presumptive bacterial isolates

Presumptive Isolates	Rate of Isolation	
	bacteria (%) of cultured pond fish samples	bacteria (%) of natural pond fish samples
Klebsiella spp.	10 (27.77)	8 (36.36)
Pseudomonas spp.	7 (19.4)	4 (18.2)
E. coli	4 (11.11)	6 (27.3)
S. aureus	7 (19.4)	1 (4.54)
Salmonella spp.	1 (2.7)	0
Vibrio spp.	2 (5.55)	3 (13.6)
Citrobacter spp.	1 (2.7)	0
Proteus spp.	1 (2.7)	0
Enterobacter spp.	3 (8.33)	0
Total	36	22

These are the common pathogenic bacteria which are associated with fish diseases. These bacteria are belonging to enterobacteriaceae family that means their presence could be attributed to the contamination of the ponds by human and animal wastes[14, 15].

E. coli, *Klebsiella* spp. and other enterobacteriaceae have been found to survive and multiply in the gut of fish which could be a potential source of human disease over long periods of time[16].

Uses of antibiotic indiscriminately to control fish pathogens evolve multidrug resistant bacteria[17]. Antibiotic resistance gene in bacteria isolated from fish has been reported worldwide[18]. From soil and aquatic environment, spreading of antibiotic resistance genes to fish bacteria along with the passage of this resistant gene could be transmitted among human, animal and environment[19, 20].

The antibiogram profiles of isolated bacteria were investigated against sixteen commonly used antibiotics of eight classes. Most of the isolates were highly resistant to antibiotics classes like tetracyclines, penicillins, cephalosporins, aminoglycosides, sulfonilamides and macrolides. The antibiotic resistance pattern of predominant bacteria (*Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *Staphylococcus* spp. and *Vibrio* spp.) in both types pond fish sample were analyzed. The predominant bacteria of fish in drug used ponds were completely resistant (100%) to tetracyclines, penicillins, cephalosporins, aminoglycosides and macrolides classes; highly resistant (80%) to sulfonilamides class, moderately resistant (80%) to fluroquinolones class, and completely sensitive (100%) to carbapenems class (Figure 1a). On the other hand, in case of non-drug used ponds fish, these predominant bacteria were completely resistant (100%) to penicillins, cephalosporins and aminoglycosides classes, highly resistant to tetracyclines (80%), macrolides (60%) and sulfonilamides (60%) classes, moderately resistant to fluroquinolones (40%) class, completely sensitive (100%) to carbapenems class (Figure 1b).

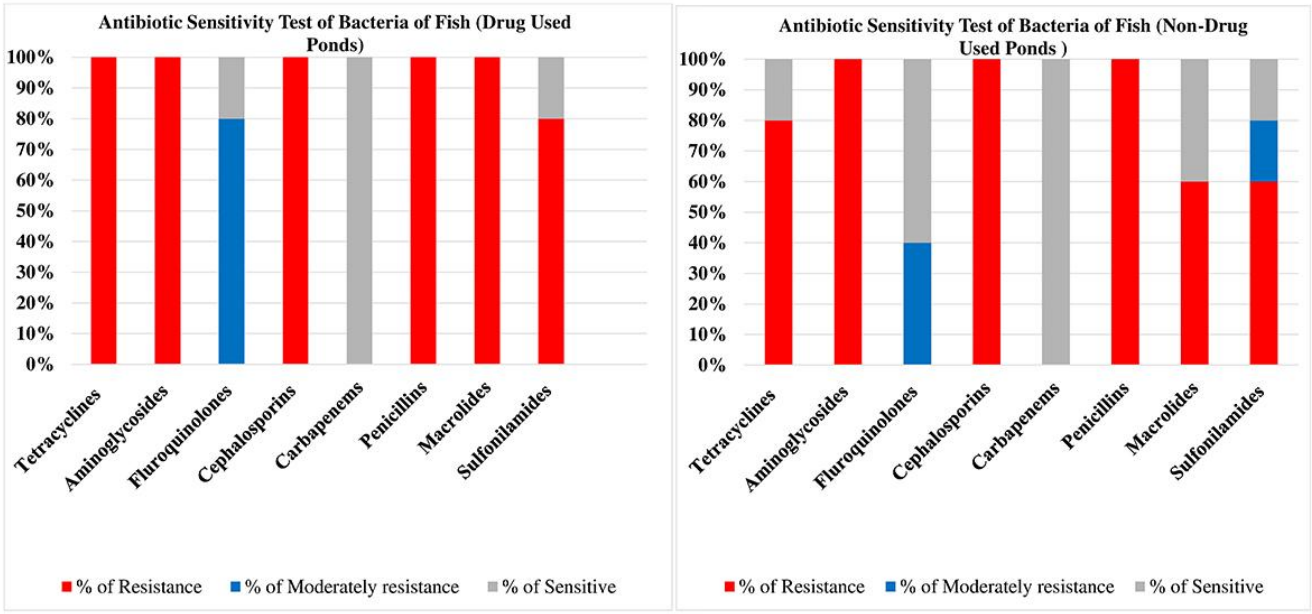

Figure 1: Antibiotic sensitivity profile of the predominant bacteria isolated from fish samples of a. cultured ponds (antibiotic used) and b. natural ponds (no antibiotic)

It was found that higher resistant pattern of bacterial isolates in drug used cultured pond fish than non- drug used natural pond fish by comparative analysis (Figure 2) of the predominant bacteria. For example, *Klebsiella* spp. isolated in cultured pond was highly resistance (80%) to oxacillin, clindamycin, cephalexin, ampicillin, tetracycline, erythromycin, sulfamethoxazole trimethoprim whereas in non-drug used pond fish sample, *Klebsiella* spp. was highly resistance (75%) to oxacillin, clindamycin, tetracycline, ceftazidime, sulfamethoxazole trimethoprim. Another examples of *E. coli* which was highly resistance (80%) to clindamycin, oxacillin, ampicillin, erythromycin, ceftazidime isolated from cultured pond, also *E. coli* from natural pond showed resistance (67%) against clindamycin, oxacillin, amoxycillin, ceftriaxone, sulfamethoxazole trimethoprim (Figure 2).

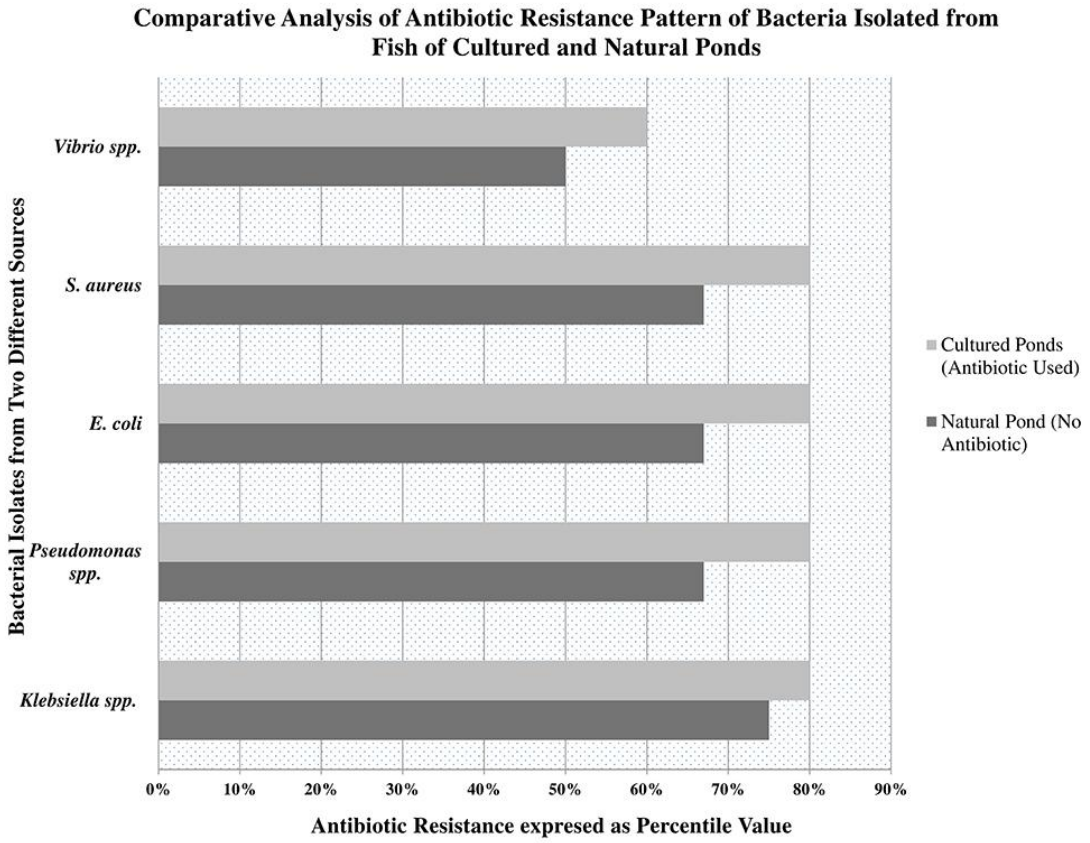

Figure 2: Comparative analysis of antibiotic resistant pattern of bacteria (*Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *S. aureus* and *Vibrio* spp.) isolated from both cultured ponds (antibiotic used) and natural ponds (no antibiotic) fish samples.

Remaining most of the isolates were resistant to about four or more different classes of antibiotic. Bacteria which are resistant to four[21] or even two[22] different classes of antibiotics called multi-drug resistant bacteria.

The result of this study revealed the presence of multidrug resistant bacteria from fish sample obtained from nondrug used natural pond which indicating indiscriminately apply of drug in cultured pond might have resulted into development of resistance due to spread of drug resistant gene into surrounding natural environment.

The antibiotic resistant bacteria persisting in soil and aquatic environments may provide a threat to fish farms; sequentially it could be a reservoir of antibiotic-resistance genes for fish pathogens in the farms surrounding environments[23].

It could be the potential danger of antibiotic resistance transfer from aquatic bacteria to human.

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