

Study of Antibiotic Resistivity of Some Bacteria from Selected Fish Species of Patna

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Abstract

Infection caused by pathogens transmitted to humans by various means and fish is one of them. Fish are susceptible to various microorganisms (bacterial pathogens) which is the part of the micro environment. The purpose of this study was for isolation of bacteria from fish organ and impact of generic antibiotics upon isolated bacteria for potential checking. The study conducted between October to December, 2019 in the Department of Biotechnology, by selecting three species of fish *Eutropiichthys vacha* (Batchwa), *Ctenopharyngodon idella* (Grass Carp) and *Labeo rohita* (Rohu) from local fish market of Patna district. Possible bacteria isolated from fish are: *Proteus vulgaris*, *Bacillus subtilis*/*P. aeruginosa*, *Vibrio cholera*, *Klebsiella aerogenes*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Salmonella typhi* from the selected organ are gills, intestine, heart, liver and muscle. The isolated bacteria identified by using biochemical test (iMVIC) and antibiotics susceptibility test was conducted for testing impact of antibiotics on it by using 13 antibiotics were used in this work. The result of the study showing that rohu fish sold in the market has highly bacterial contamination and antibiotics resistance. We are strongly suggested the urgent need to improve the quality of fish supplied in the fish market Patna.

Keywords: Antibiotics; Bacteria; Fish; Pathogen; Resistivity.

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1. Introduction

Fishes are the most important and basic source of protein and other nutrients and it for estimated more than 30% of fish is used for human consumption from aquaculture [1]. The health of the fish is needed to be good and hygiene, because it is the part of food chain. Due to some reasons the fish becomes polluted, start deteriorated and decrease in the quality. Microorganisms are found almost everywhere in nature and occupying an important place in the life of human. Various studies conducted out on microbial flora associated with marine and aquaculture fish [2,3,4]. The two types of classified bacterial pathogens associated with fish are indigenous and non-indigenous [5]. The bacteria exist in the fish are normally associated with those found in natural environment with its condition like season and harvesting process [6]. The microorganisms are not entering easily into the fish when they alive, due to defense mechanism were activated. After death of the fish microorganisms are present in internal organs of fish may indicate that bridge of immunological defense mechanisms.

Antibiotics resistant bacteria or other pathogenic bacteria transmitted to the human by using as food or may be in contact [7]. The use and misuse of antibiotics in pond or fish farm may disturb the natural micro flora of fish intestine and give rise of antibiotics resistant bacteria [8].

2. Materials and methods

2.1 Sample and sampling site

Patna is situated between 24° 97' - 25° 27' North latitude and 84° 44' - 86° 57' East longitude at an elevation of about 129 meter above mean sea level. It is about 20 km long (from east to west) and 4 km width (from north to south) [9]. The total three species of fish Batchwa, Grass Carp and Rohu were selected, one fish from each different site Rajiv Nagar, Rajapur, and Mainpura respectively. The fishes collected from sites was live, aseptically and transported in the thermal regulating box with ample amount of water and aeration to the laboratory of Department of Biotechnology, within 2 hours of sampling and processed immediately for dissection and testing. Samples were preserved in refrigerator at 4° C in the sterile condition.



Figure 1: Images of selected fish species based on the literature

2.2 Preparation of the samples

Preparation of sample by using the methodology described previously by [10], according to these 10 grams of organ part dissected from fish with sterile cutter. After that the sample were crushed in mortar pestle into small pieces with 10 ml of distilled water. The sample was diluted by using serial dilution with distilled water and making 1:10 dilution, and this process was conducted for all further next sample.

2.3 Isolation and identification of Bacteria

The examination was conducted to isolate, identify and confirm bacterial isolates. Conventional methods of bacterial isolation such as growth on selective media, and biochemical tests were used for the identification of the different bacterial isolates. The media used include MacConkey agar/Broth, Nutrient agar, peptone water and EMB agar. The samples were plated within 2-4 hours of specimen collection the plates were incubated aerobically at 37°C for 24 hours. After incubation, pure isolates based on cultural and biochemical characteristics were identified [11].

2.4 Antibiotics analysis

Antibiotic susceptibility test was carried out using Nutrient agar prepared according to manufacturer's specification and sterilized at 121°C for 15 minutes. The medium was then poured into appropriate Petri dishes aseptically. Isolates were selected for antimicrobial susceptibility testing according to Kirby-Bauer disc diffusion techniques on Nutrient agar using the following antibiotic discs listed in Table.

Table 1: Antibiotics used for the study

S. No.	Antibiotics	Code	Dose (mcg)
1.	Amikacin	Ak	30
2.	Ampicillin	A	10
3.	Azithromycin	Azm	15
4.	Cefadroxil	CD	30
5.	Ceftriaxone	Ctr	30
6.	Ciprofloxacin	CIP	5
7.	Doxycycline	Dc	30
8.	Erythromycin	E	15
9.	Gentamycin	G	10
10.	Levofloxacin	Lv	5
11.	Nalidixic Acid	Na	5
12.	Norfloxacin	Nf	10
13.	Ofloxacin	Of	5

The diameter of complete inhibition zone formed around the antibiotic discs was measured to the nearest whole millimeter using standardized ruler. The results obtained were analyzed as resistant or susceptible according to standard interpretative table by CLSI [12] and Bonnet [13]. Multiple antibiotic resistances (MAR) index was then determined for each isolate by dividing the number of antibiotics to which an isolate is resistant with the total number of antibiotics tested [14]. The MAR indexes an indicator to identify the risk contamination that is potentially hazardous to human. Calculated value of more than 0.2 indicates that the isolates were isolated from high-risk sources [15].



Figure 2: Some results of the Antibiotic Resistance test on an agar plates of fish samples

3. Results

3.1 Sampling and sampling site

The samples are collected from three different site of district Patna namely Rajiv Nagar, Rajapur and Mainpura from where following fish species are collected as shown in the table 1. The size and weight of the fish are also noted and observed.

3.2 Isolation and identification of bacteria

The isolated bacterial colonies are biochemically tested whose results for fish samples of Batchwa, Grass Carp and Rohu collected from Rajiv Nagar, Rajapur and Mainpura are presented in the table 2, 3, and 4 respectively.

3.3 Antibiotics analysis

The antibiotic resistivity test conducted for the isolated bacterial colonies for fish samples of Batchwa, Grass Carp and Rohu results are presented in the table 5, 6, and 7 respectively. The figure 3 and 4 represent the MAR of the bacteria from all fish samples and the antibiotic resistance/susceptibility of the bacterial isolates respectively.

Table 2: Three species of fish selected from Patna fish market

S. No.	Common Name	Scientific name	Size (cm)	Weigh (kg)	Area
1	Batchwa	<i>Eutropiichthys vacha</i>	30	0.35	Rajiv Nagar
2	Grass Carp	<i>Ctenopharyngodon idella</i>	155	1.5	Rajapur
3	Rohu	<i>Labeo rohita</i>	143	1.5	Mainpura

Table 3: Possible bacteria isolated from the fish *Eutropiichthys vacha* (Batchwa)

Organ type	Isolates code	Indole	MR	VP	Citrate	Possible bacterium
Gills	B/G/1A	+VE	+VE	-VE	+VE	<i>Citrobacter spp.</i>
	B/G/1B	+VE	-VE	-VE	+VE	<i>Enterobacter spp.</i>
Liver	B/L/2	-VE	-VE	+VE	+VE	<i>Klebsiella pneumoniae</i>
Intestine	B/I/3	+VE	-VE	+VE	+VE	<i>Citrobacter freundii</i>
Heart	B/H/4A	-VE	+VE	-VE	+VE	<i>Citrobacter freundii</i>
	B/H/4B	+VE	-VE	-VE	+VE	<i>Klebsiella oxytoca</i>
	B/H/4C	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>

Table 4: Possible bacteria isolated from the fish *Ctenopharyngodon idella* (Grass carp)

Organ type	Isolates code	Indole	MR	VP	Citrate	Possible bacterium
Gills	GC/G/1A	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>
	GC/G/1B	+VE	-VE	-VE	+VE	<i>Providencia rettgeri</i>
	GC/G/1C	+VE	-VE	+VE	+VE	<i>Klebsiella oxytoca</i>
Intestine	GC/I/2A	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>
	GC/I/2B	+VE	-VE	-VE	+VE	<i>Providencia rettgeri</i>
Liver	GC/L/3A	-VE	-VE	+VE	+VE	<i>Enterobacter aerogenes</i>
	GC/L/3B	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>
	GC/L/3C	+VE	-VE	+VE	+VE	<i>Klebsiella oxytoca</i>
	GC/L/3D	+VE	+VE	-VE	+VE	<i>Escherichia coli</i>
Heart	GC/H/4	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>
Muscle	GC/M/5A	-VE	-VE	+VE	+VE	<i>Klebsiella spp.</i>
	GC/M/5B	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>

Table 5: Possible bacteria isolated from the fish *Labeo rohita* (Rohu)

Organ type	Isolates code	Indole	MR	VP	Citrate	Possible bacterium
Gills	R/G/1	+VE	-VE	-VE	+VE	<i>Providencia rettgeri</i>
Intestine	R/I/2A	+VE	-VE	-VE	+VE	<i>Providencia rettgeri</i>
	R/I/2B	+VE	+VE	-VE	+VE	<i>Citrobacter amalonaticus</i>
Liver	R/L/3A	-VE	-VE	-VE	+VE	<i>Serratia marcescens</i>
	R/L/3B	+VE	-VE	+VE	+VE(D)	<i>Citrobacter amalonaticus</i>
Heart	R/H/4	-VE	+VE	-VE	-VE	<i>Shigella spp.</i>
Muscle	R/M/5A	-VE	-VE	+VE	+VE	<i>Citrobacter freundii</i>
	R/M/5B	-VE	+VE	-VE	-VE	<i>Shigella spp.</i>

Table 6: Antibiotic analysis of bacteria isolated from the fish *Eutropiichthys vacha* (Batchwa)

	Ak	A	Azm	CD	Ctr	CIP	Dc	E	G	Lv	Na	Nf	Of
B/G/1A	27	6	31	20	6	24	5	15	24	25	30	23	29
B/G/1B	34	6	35	17	6	14	5	6	33	38	24	30	28
B/L/2	25	6	26	23	6	21	10	6	22	26	22	28	18
B/I/3	35	6	23	10	17	30	6	20	23	30	17	6	26
B/H/4A	31	5	36	25	6	44	11	19	21	37	21	40	36
B/H/4B	32	6	25	29	11	6	6	6	28	41	20	42	18
B/H/4C	25	6	27	32	6	11	6	6	27	40	27	42	35

Table 7: Antibiotic analysis of bacteria isolated from the fish *Ctenopharyngodon idella* (Grass carp)

	Ak	A	Azm	CD	Ctr	CIP	Dc	E	G	Lv	Na	Nf	Of
GC/G/1A	26	14	25	23	6	35	18	6	23	30	28	30	31
GC/G/1B	28	6	3	25	10	35	10	13	25	35	25	25	30
GC/G/1C	32	6	25	21	6	37	17	20	18	37	30	35	32
GC/I/2A	28	6	27	15	6	36	6	20	22	39	33	26	30
GC/I/2B	25	6	28	21	6	27	15	15	21	34	30	31	26
GC/L/3A	25	6	24	21	25	35	10	15	24	36	27	24	30
GC/L/3B	26	6	26	21	15	38	18	22	23	35	30	37	32
GC/L/3C	28	6	25	33	23	30	16	23	25	40	30	28	34
GC/L/3D	30	6	6	10	6	35	6	18	20	35	27	35	28

Table 8: Antibiotic analysis of bacteria isolated from the fish *Labeo rohita* (Rohu)

	Ak	A	Azm	CD	Ctr	CIP	Dc	E	G	Lv	Na	Nf	Of
R/G/1	20	6	25	17	14	30	10	15	20	30	16	25	22
R/I/2A	23	6	24	26	6	30	13	15	15	35	26	27	30
R/I/2B	25	6	25	27	6	30	15	16	23	32	25	30	27
R/L/3A	25	6	21	20	6	23	10	6	20	29	11	30	26
R/L/3B	23	6	25	15	6	26	6	20	6	24	30	28	28
R/H/4	30	6	30	29	15	18	20	16	25	34	25	24	29
R/M/5A	24	18	25	30	22	25	21	25	20	40	30	38	28
R/M/5B	25	6	25	33	28	35	17	25	20	39	29	39	28

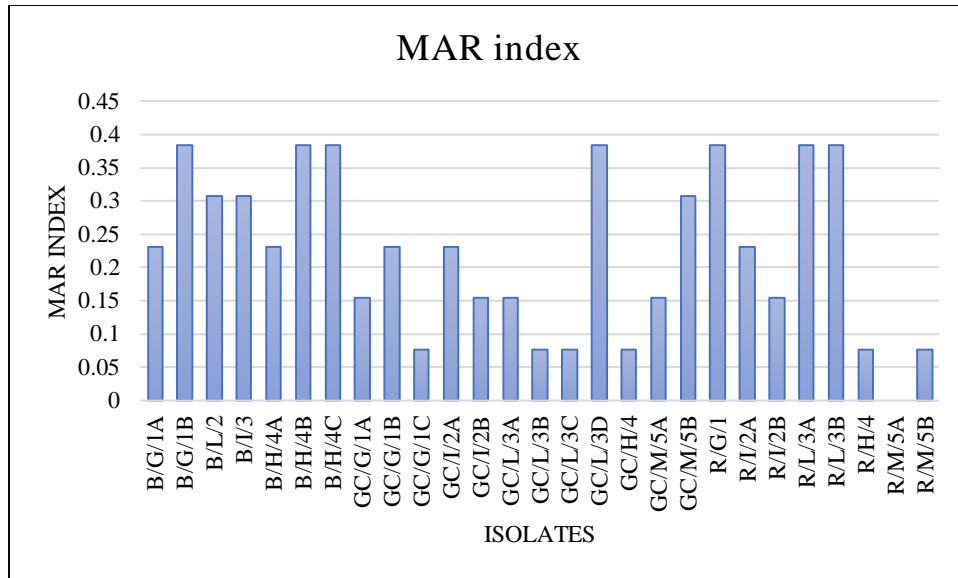


Figure 3: The graphical representation of the MAR of the bacteria from all 3 fish samples

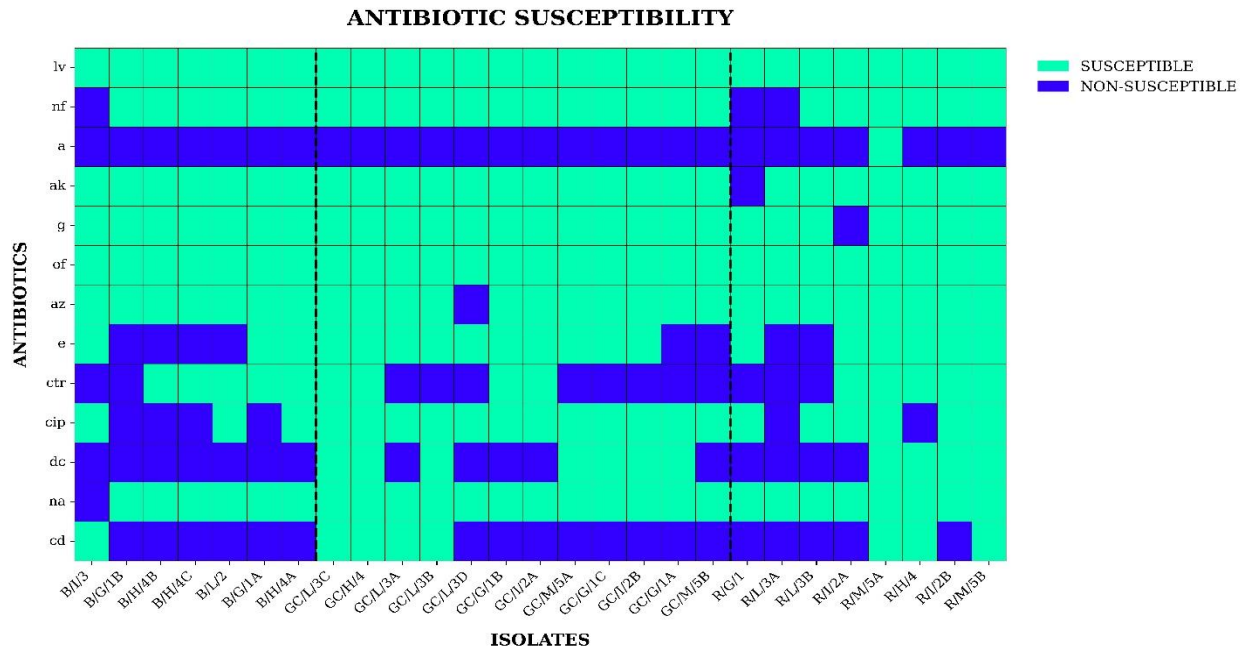


Figure 4: The heat map representing the antibiotic resistance or susceptibility of the bacterial isolates from all three fish samples

4. Conclusion

This study aims to check the AMR status of the fish in different fish markets of the district of Patna. The fish collected from different fish markets of Patna have high microbial loads indicative of coliform contamination from

environmental factors. These environmental factors have severely affected the gills, intestine, liver, heart, and muscle organs of the fish's body. This poses a health concern to the fish-consuming population of the society, as 11 out of 27 isolates have a MAR index of more than 0.2, showing heavily detreating antibiotic effectiveness. This also indicates the environmental hazard, which also influences the health of a large population of society that corresponds to the ill condition of the water bodies where they were cultivated, as well as the lack of healthy transportation facilities in comparison to the well-developed countries. These types of studies are also important in the south-eastern developing nations to keep a check on the food quality index of these countries.

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