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# Experimental Analysis Of Generic Antibiotics Along With Honey Sensitivity Against Microbial Flora Obtained From Frozen Hilsa Fish (*Tenualosa ilisha*)

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**Abstract:** This study deals with the in-vitro experimental analysis of different generic antibiotics and season based honey, sensitivity against isolated bacteria. The characterization of bacteria isolated from the frozen hilsa is done through various biochemical reactions. The antibacterial sensitivity of honey and antibiotics are evaluated through zone of inhibition analysis of bacterial colony. So, it is obvious that this experiment provides providential values for future research including food value analysis.

Keywords: Tenualosa ilisha; Characterisation; Antibiotics; Honey; Sensitivity

### 1. Introduction

From the very past decade, the antibiotics are being used against bacterial disease. The antibiotics is one of the life saving drugs. If the all antibiotics are being resistant against bacterial, the bacterial infections will breakout. Then, life will be in a great threat.

Honey also has been used for a long time for its health benefits. Also, fish is a vital source of food for people and contributes about 60% of the world's supply of protein. Sixty percent (60%) of the developing countries derived 30% of their annual protein from fish [1].

Protein is an important component of our food stuffs. Fish meats contains about 16-12% protein [2]. It is also composed of water (66-81%), carbohydrate (0.5%), lipids (0.2-2.5%), Ash(1.2-1.5%) [3]. It also remains many minerals and vitamins in the fish meat. These are Calcium (Ca), Iron(Fe), Phosphorus (P), Magnesium (Mg), Manganese (Mn), Copper (Cu), Zinc (Zn), vitamin A, vitamin D, vitamin C. Hilsa (Tenualosa ilisha) is one of the well known fishes. The main ingredients of hilsa are vitamin A, vitamin C, omega-3 fatty acid, calcium (Ca), phosphorus (P), Zinc (Zn), magnesium (Mg). The component omega-3 fatty acid acts as a lipid lowering agent [4]. It is considered that one pound of hilsa meat contains average 3001100 calories energy. Hilsa is one of the most foreign exchange earing fishes. Hilsa is mainly found in revers and sea. The food ingredients (protein, carbohydrate, lipids) of frozen hilsa fish degrade for various invaded micro-organism including bacteria. These bacteria are susceptible for some antibiotics against isolated bacteria is observed, which is perquisite for study of fish food. This in-vitro analysis, the generic antibiotics which is resistant to isolated bacteria, provides intuitive information regarding the antibiotics used for bacterial

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infectious diseases. In addition, the antibacterial properties of honey is obliviously a providential evidence for future research in this perspective.

### 2. Materials and Methods

### 2.1. Sample collection

For the current research works, the 5-6 months frozen hilsa was collected from the local market and samples were obtained from different body parts including dermis, kidney, liver, gill, and intestine. The bacteria were isolated from the body parts (dermis, kidney, liver, gill, intestine) of the sample frozen hilsa with sterilized scalpel and from it a small portion was took in LB(Luria-Bertani broth) liquid medium and mixed bacterial cultures were grown.

### 2.2. Colony characteristics, Gram reaction and Cell morphology

The isolates were inoculated both on solid and liquid LB medium. Colony characteristics of the isolates on LB medium were noted after 24 hrs incubation at 28°C. **Gram's staining** [5](differentiation of Gram-positive and Gram-negative bacteria), **Oxidase test** [6](cytochrome c oxidase, an enzyme of the bacterial electron transport chain) test, **Catalase** [7](an enzyme present in most of the organisms and is involved in decomposition of the hydrogen peroxide in H<sub>2</sub>O and O<sub>2</sub>) test, **Sulfide-indole motility** (**SIM**) **test** [8](differentiation of bacteria based on three tests: sulfur reduction (cysteine desulfurase), indole production (tryptophanase), and motility, **Simmons citrate test** [9], **MacConkey agar test** [10](differentiation of lactose fermenting from lactose nonfermenting Gram-negative bacteria), **Kligler Iron agar** (**KIA**) [11](used in differentiating certain members of the Enterobacteriaceae by demonstrating hydrogen sulfide production and the fermentation of dextrose and lactose), **Urease** [12](an enzyme that hydrolyze urea providing a source of nitrogen for bacteria), **Antibiotic sensitivity test** [13](*in vitro* employing standard disc diffusion method), if inhibition zone was 5-10 mm it was considered as resistant (R), 11-15 mm inhibition zone was considered as intermediate (I), and ≥16mm was considered as sensitive (S).

Table 1. Antibiotic discs of different concentration used in the experiment

Antibiotic discs	Disc concentration
Oxytetracycline	30µg
Cefixime	5μg
Cefotaxime	30µg
Nalidixic Acid	30µg
Kanamycin	30µg
Vancomycin	30µg
Doxycycline	30µg
Neomycin	30µg
Gentamycin	10μg

Erythromycin	15µg
Sulfonamide	300 IU
Amoxicillin	30µg
Penicillin-G	10μg
Rifampicin	5µg

### 2.3. Antibacterial effect of honey on isolated bacterial strain

For evaluation of antibacterial efficacy of honey, we used different concentration of honey (10, 20 and 30  $\mu$ l). In our study we used 3 types of honey (Summer, Winter, Spring) and every type of honey consist of 3 concentration (10, 20 and 30  $\mu$ l). After that we measured zone of every of honey.

Table 2. of nutrient broth

Constituents	Amount (g/L)
Peptone	10
Yeast extract	5
NaCl	5

**Table 3.** of nutrient agar

Constituent	Amount (g/L)
Peptone	10
Yeast extract	5
NaCl	5
Agar	20

### 3. Observation and Results

# 3.1. Morphological and Biochemical Characterization of Isolate Bacterial Strains:

Various biochemical tests were carried out to characterize the isolated bacteria. The results of the biochemical tests are summarized in table 4.

Table 4. results of morphological and biochemical test

Name of tests	Results					
	Α	В	С	D	М	
Gram Staining	-	-	-	-	+	
Oxidase	+	+	+	-	+	
Catalase	+	-	-	+	+	
	-	-	-	-	-	
	_	_	_	_	_	

Sulfide Indole Motility	+	+	+	+	-
Simmon citrate utilization	+	+	+	+	-
Growth on MacConkey agar	+	+	+	+	-
Kligler iron agar	+	+	+	+	-
Urease	+	+	+	+	-

# **Gram Staining Test.**

Maintaining the suitable staining method, the following result was observed for isolated bacterial strain (Tab.5). Isolate M bacterial strains showed purple color indicating gram-positive rod-shaped bacteria but isolate G, D, Kw, Kc show pink color indicating gram negative. Here, G= Gill, D= Dermis, M= Muscle, Kw= Kidney White and Kc= Kidney creamy.



Figure 1. Gram Staining test of bacteria

### Antibacterial activity of antibiotics against isolated bacteria:.

In this observation, disk diffusion method was used to test antibacterial sensitivity of the five isolated bacteria. After incubation period the diameter of the inhibition zone was scrutinized.



Figure 2. staining result of isolated bacterial strains.

**Table 5.** sensitivity test used for the detection of the resistance pattern of the isolated bacteria.

Antibiotic discs	Disc	Zone of inhibition (mm) (Sensitivity)					
Antibiotic discs	concentration	A	В	С	D	E	
Oxytetracycline	30µg	11 (I)	10 (R)	15 (I)	14 (I)	30 (S)	

Cefixime	5µg	12 (I)	10 (R)	12 (I)	19 (S)	10 (R)
Cefotaxime	30µg	14 (I)	13 (I)	12 (I)	20 (S)	12 (I)
Nalidixic Acid	30µg	-	-	8 (R)	10 (R)	24 (S)
Vancomycin	30µg	-	-	7 (R)	-	22 (S)
Doxycycline	30µg	10 (R)	9 (R)	8 (R)	10 (R)	30 (S)
Neomycin	30µg	12 (I)	11 (I)	13 (I)	10 (R)	22 (S)
Gentamycin	10µg	14 (I)	18 (S)	17 (S)	19 (S)	25 (S)
Erythromycin	15µg	-	-	20 (S)	-	20 (S)
Sulfonamide	300 IU	10 (R)	15 (I)	15 (I)	10 (R)	10 (R)
Amoxicillin	30µg	-	-	-	-	10 (R)
Penicillin-G	10µg	-	-	10(R)	10 (R)	23 (S)
Rifampicin	5μg	-	-	8 (R)	9 (R)	20 (S)
Azithromycin	15µg	15 (I)	18 (S)	13 (I)	11 (I)	13 (I)

Note: Resistant=<10 mm; Intermediate =10-15 mm; Susceptible=>15 mm

# Antibacterial activity of honey sample.

In this study, honey disk was used to test antibacterial sensitivity of the five isolated bacteria. The patterns of sensitivity and resistance of isolated bacterial cultures honey was tested by the disk diffusion method using nutrient agar medium. After overnight incubation at 37°C the diameter of the inhibition zone was measured.

Table 6. sensitivity against bacteria.

	Concentration	n Honey (Zone of Inhibition at mm)						
Isolate	(μ1)	Summer	Response	Winter	Response	Spring	Response	
A	10 μ1	8 mm	Resistant	6 mm	Resistant	8 mm	Resistant	
	20 μ1	10 mm	Resistant	9 mm	Resistant	10 mm	Resistant	
	30 μ1	14 mm	Intermediate	12 mm	Intermediate	12 mm	Intermediate	
В	10 μ1	15 mm	Intermediate	12 mm	Intermediate	6 mm	Resistant	
	20 μ1	18 mm	Sensitive	17 mm	Sensitive	6 mm	Resistant	
	30 μ1	22 mm	Sensitive	18 mm	Sensitive	8 mm	Resistant	
$\mathbf{c}$	10 μ1	6 mm	Resistant	10 mm	Resistant	8 mm	Resistant	
	20 μ1	10 mm	Resistant	22 mm	Sensitive	10 mm	Resistant	
	30 μ1	12 mm	Intermediate	23 mm	Sensitive	14 mm	Intermediate	
D	10 μ1	08 mm	Resistant	08 mm	Resistant	8 mm	Resistant	
	20 μ1	10 mm	Resistant	12 mm	Intermediate	10 mm	Resistant	
	30 μ1	10 mm	Resistant	15 mm	Intermediate	10 mm	Resistant	

E	10 μ1	20 mm	Sensitive	24 mm	Sensitive	20 mm	Sensitive	
	20 μ1	25 mm	Sensitive	25 mm	Sensitive	24 mm	Sensitive	
	30 µl	29 mm	Sensitive	27 mm	Sensitive	28 mm	Sensitive	

#### 4. Discussion

The present studies were carried out to identify and characterize bacteria isolated from frozen hilsa (*Tenualosa ilisha*). It was found that among the five isolated bacteria (isolate – D, G, Kc, Kw, and M); isolate- D, G, Kc, Kw were gram-negative and isolate – M was gram-positive. Colonies of the first four isolates were fairly round shaped and later one was irregular in shape. Biochemical tests indicate that isolates G, D, Kc and Kw were catalase, KIA, SIM test, MacConkey agar test Urease test positive. Other one isolates M showed negative result for all these biochemical tests. All the five isolates were positive to catalase test. In SIM test all the isolates showed negative result for Hydrogen Sulphide and Indole. In case of isolates G, D, Kc, Kw, simmon citrate, urease was positive.

In antibiotic test, the result showed that all of the five isolate were susceptible to Oxytetracycline, Cefotaxime and cefixime while they were resistant to Penicillin-G and Rifampicin. However, the results revealed that the different antibiotics as well. Where isolate D, G, Kc, Kw exhibited highest sensitivity to Cefotaxime, Cefixime and Erythromycine and M showed highest sensitivity to Oxytetracyline and Doxycycline. In this study, the highest inhibition was 30.0 mm for isolate M created by Oxytetracyline. The lowest 8.0 mm zone was found by Rifampicin. In a study it was reported that Oxytetracycline showed highest 30.0 mm diameter of zone of inhibition against isolated bacteria.

In antibacterial test, the use of honey as a traditional remedy for microbial infections dates back to ancient times. The difference in antibacterial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source as well as through harvesting, processing and storage condition. The antibacterial activity of honey is attributed largely to osmolarity, hydrogen peroxide production. Honey contains glucose and glucose oxidase to produce hydrogen peroxide.

In this study, the honey of summer, winter, and spring were collected from a local trader and used for experiment to which amount was 10, 20,  $30\,\mu l$  respectively. In the in vitro experimental methods, it was used agar diffusion disc method to where honey was used as an antimicrobial agent to observe the zone of inhibition of bacterial growth. The highest zone of inhibition of summer, winter, spring season collected honey was  $20\,mm$ ,  $18\,mm$ ,  $22\,mm$ ,  $24\,mm$ ,  $25\,mm$ ,  $18\,mm$ ;  $20\,mm$ ,  $24\,mm$ ,  $8\,mm$ 

### 5. Conclusion

Most of the isolated bacteria show positive results for most biochemical tests, reveals the negative strain of the isolates. Antibiotic sensitivity test shows highest inhibition for Muscle with Oxytetracycline and Doxycycline. Where Kideny white shows highest inhibition with Cefixime and Cefotaxime. Gill and Kideny Creamy show highest inhibition with Gentamycin, whereas Dermis does not show any susceptible results at all. Antibacterial activity test in case of honey Muscle showed highest inhibition during summer and winter. Where Kideny Creamy shows higher inhibition during winter.

### 6. Acknowledgements

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## 7. Data Availability Statement

No data availablity statement.

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