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Antibiotic Sensitivity for Ready-to-Eat Fish-Based Food-Borne Bacterial Pathogens

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ABSTRACT

For the degradation of fish-based foods that consumed without any further processing, microbes play an important role. Proper knowledge of microbiological conditions can ensure quality food products that can save from foodborne diseases. The present study was carried out to investigate the microbiological quality of 30 ready-to-eat fish-based foods. The results exposed that the highest standard plate count (SPC) was 3.986×10^3 CFUg^{-1}. Hence, the analyzed samples were of acceptable microbial quality based on the total aerobic bacterial count. On the contrary, about 20% of food samples were identified as inappropriate because of containing fecal coliform. *Salmonella spp*. was not detected in any of the investigated samples. Six antibiotics namely tetracycline hydrochloride, erythromycin, amoxicillin, chlorpheniramine maleate, cephalexin, and ciprofloxacin were employed for susceptibility testing. Among the tested antibiotics, tetracycline hydrochloride and ciprofloxacin showed 87-100% susceptibility at the concentration of 240 µg/mL. In the same concentration cephalexin showed 78% but other three antibiotics showed very less susceptibility against ready-to-eat fish-based food born bacterial pathogens.

1. Introduction

From ancient times, fish and fish-based foods are the major food components because of their easy digestibility and high nutritional value [1]. Bangladesh is one of the world's leading fish producing countries with a total production of an average 3.68 million metric tons per year. Almost 60% of protein are consumed by the people of Bangladesh generally comes from fish [2]. This sector is contributing significantly to food security through providing safe and quality protein [3]. The nutritional value of fish-based foods is distinguishable because of availability of proteins, unsaturated fatty acids, as well as certain minerals and vitamins in fish. Mainly, fish-based foods are deliberated as a very important source of n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) [4,5]. The skin, gills, digestive tract, and light-emitting organs of fish contain bacterial populations [6,7]. Fecal coliform is a part of the total coliforms which is defined as Gram-negative, non-spore forming rods that ferments lactose at 44.5±0.2 °C within 24±2 hours with the production of acid and gas. Fecal coliform i.e. *E. coli* is a usual inhabitant of the intestinal tract of humans and other warm-blooded animals. On the other hand, nonfecal coliforms i.e. enterobacter aerogenes are widely distributed in nature and found in soil, water, grains in addition to the intestinal tract of humans and other warm-blooded animals [8]. Fish and fish-based food products can be spoiled by a variety of bacteria, viruses, and parasites including *Vibrio, Aeromonas, Flavobacterium, Yersinia, Edwardsiella, Streptococcus, lactococcus, Renibacterium, Mycobacterium*, etc. [9]. The potential sources for contamination of fish products are water, air, humans, microbes in fish, processing equipment of food and other ingredients, product to product and contamination of microbes from the poor manufacturing process, etc. [10,11]

In recent years' numerous attempts have been taken to assure safe food. Results showed that the quality of food depends on the quantity of the total and thermotolerant coliforms, *Staphylococcus aureus, Salmonella, Listeria monocytogenes*, mesophile aerobic microorganisms, etc. The presence of such pathogenic microorganisms decreases the lifetime of foods [12,13]. Most of the time consumers are not aware of the safety, quality, and hygiene of the ready-to-eat foods and face several uncontrolled food n food-borne infectious intestinal diseases including diarrhea (with or without blood), dehydration, vomiting, fever, abdominal cramping, headache, myalgia, and arthralgia [14-17].

Antibiotics kill bacteria or resist them from copying themselves or reproducing to stop bacterial infections. Inappropriate use and overuse of antibiotics are key points of fuelling the emergence and spread of anti-bacterial resistant pathogens which can contaminate the food products, reach the human body and cause different problems to health [18]. The antibiotic selectivity should be a household term acknowledged by policymakers, academia, industry, and the public. Because the appropriate use of antibiotics for targeted microbes is essential for healthcare systems, patients, and society [19].

The present investigation has been undertaken to assess the microbiological quality of fish-based foods served in different restaurants of Dhaka city, Bangladesh. Objectives of the present studies are to enumerate total microbial counts in different fish-based foods and to clarify the antibiotic sensitivity of that isolated microbes against different antibiotics.

2. Experimental Methods

2.1 Sampling

Foods from four different areas at Dhaka city of Bangladesh identified as DA, DB, DC, and DE were selected. Ilish (*Tenualosa ilisha*), Mola (*Amblypharyngodon microlepis*), Baim (*Mastacembelus armatus*), Prawn (*Penaeus monodon*), Kai (*Climbing perch*), Tengra (*Macrones vittalus*), Puti (*Puntius chola*), Chapila (*Gudusia chapra*), Pomfret (*Brama brama*) and Taki (*Channa punctata*) had been selected for this work. Five fish base foods as fish curry, fish-based fast food, vegetable mixed fish curry, chopped fish, and dry fish curry were nominated for analysis. Different parameters were analyzed such as standard plate count, total coliform count, fecal coliform count, presence of *Salmonella spp*. etc. The bacteriological conditions of quality and safety were as described by the International Commission on Microbiological Specifications for Foods (ICMSF).

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2.2 Microbiological Quality Assessment of the Samples

The standard plate count (SPC) was obtained by the standard pour plate method using plate count agar (PCA) as the culture medium. This is a nonselective media for bacterial counting. In this experiment, a serial dilution was made up to 10^{-3} of the original sample using a Ringer solution as a diluting agent.

2.3 Determination of Total Coliform Count

The most probable number method (MPN) was used for the determination of coliform count. The flow chart (Fig. 1) explains the procedure of the determination of the total coliform count.

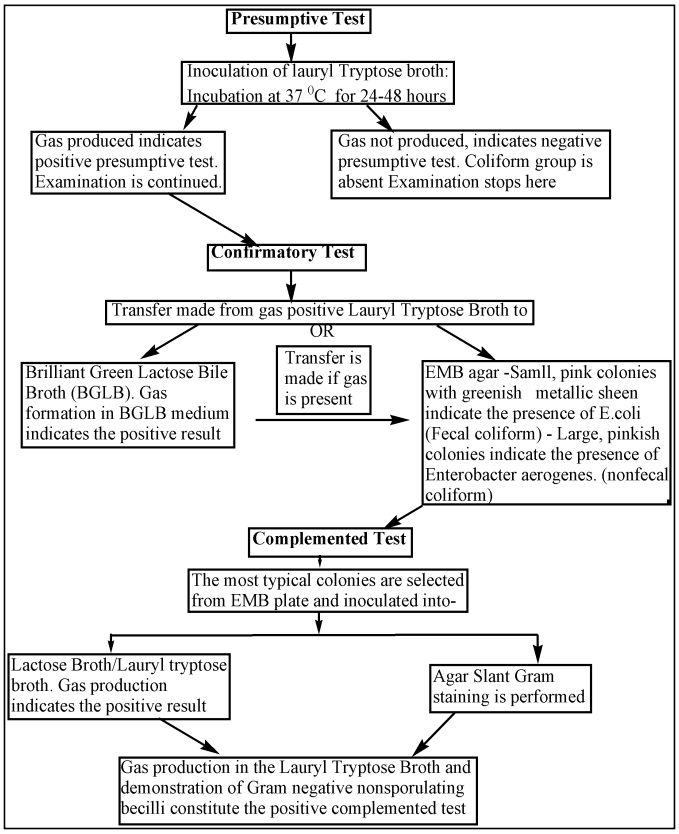

Fig. 1 Flowchart for determination of total coliform count

2.4 Count of Fecal Coliforms

Although the fecal coliform count can be determined primarily from EMB plate during the total coliform count, further confirmation was done by MPN method using selective media at a higher temperature. A loopful inoculum from each of the gas positive Lauryl Tryptose Broth was transferred to the fermentation tubes containing EC broth (5 mL of 10 mL/tube). Then inoculated tubes were incubated at 44.5±0.2 °C for 24±2 hours. The fecal coliform count was obtained by combining turbidity and gas production tubes with the statistical MPN-table.

2.5 Qualitative Detection of Salmonella spp.

Lactose broth was used as the pre-enrichment media and second step one milliliter of pre-enrichment culture was transferred to 10 mL of sterile selenite cystine broth medium, and then incubated at 37 °C for 24 hours. Finally, by streaking the loopful of enriched culture on to bismuth sulfite agar and incubated at 37 °C for 24-48 hours. But no growth occurred in samples.

2.6 Antibiotic Sensitivity of Different Isolates

Six antibiotics viz. tetracycline hydrochloride, erythromycin, amoxicillin, chlorpheniramine maleate, cephalexin, and ciprofloxacin were used for sensitivity testing on the bacterial species, isolated as contaminants of fish-based food products.

The antibiotics were dissolved at the concentration of 240 µg/mL, 120 µg/mL and 60 µg/mL. The isolated microbes were diluted to 10^{-2} cells/mL. One mL of diluted microbes is added to the malted Mueller Hinton Agar. The medium was plated and after solidification the porcelain beads were impregnated with antibiotic solution and placed up to the plates [20-22]. And incubated for 24 hrs. for producing zone of inhibition.

3. Results and Discussion

3.1 Microbiological Quality Analysis

Fish base foods play an important role to satisfy the demand of protein, lipids as well as essential micronutrients as Vitamin D, selenium, phosphorus, calcium, etc., [23]. Contaminated with foodborne pathogens can degrade the foods easily and become inedible. The presence of different bacteria species including human pathogenic bacteria in fish can be directly linked to human health. Thus, the detection of hazardous microbes is important to optimize product quality and ensure safe foods.

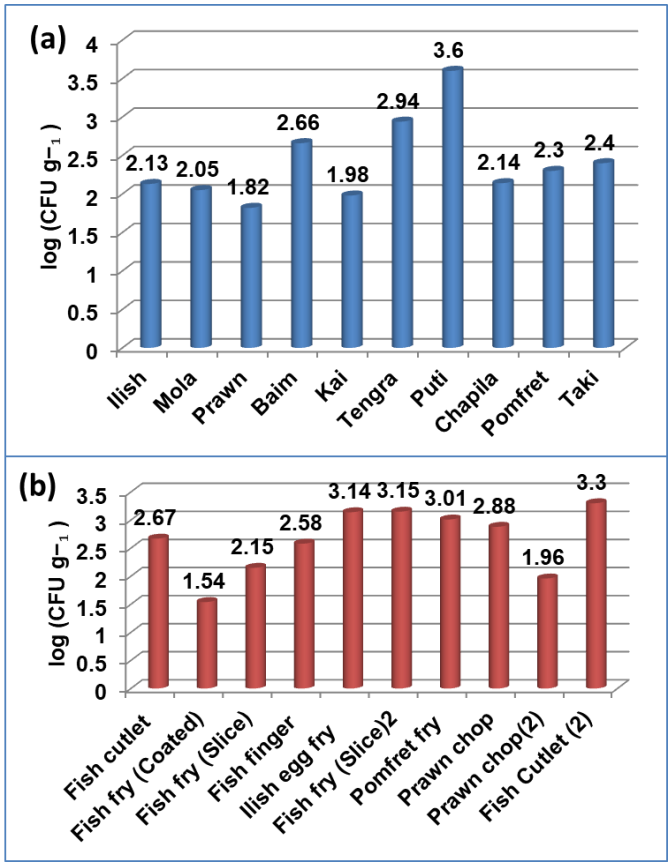

Fig. 2 Microbial populations of ready-to-eat fish-based food samples (a) fish curry samples and (b) fish-based fast food samples

Fig. 2 (a and b) and Table 1 represents the total aerobic bacterial count (TABC) by standard plate count (SPC). Fig. 2(a) shows that the fish curry samples have recorded a range of 0.67×10^2 to 3.986×10^3 $CFUg^{-1}$. The highest and lowest SPC were noted for puti fish curry and Prawn curry of DA and DE area respectively. Maximum of the curry samples contained around 2.5×10^2 $CFUg^{-1}$. Fig. 2(b) shows that the fish-based fast food samples have recorded a range 0.35×10^2 to 2.000×10^3 $CFUg^{-1}$. The highest SPC was found for the fish cutlet of DA area. Table 1 shows the standard plate count (SPC) of fish mixed vegetables, chopped fish, and sundried fish with vegetable samples. Result notifies that these samples contain a range 0.55×10^2 to 1.338×10^3 $CFUg^{-1}$. Only one sample (chopped prawn) reported over 10^3 $CFUg^{-1}$.

Table 1 Microbial populations of fish mixed vegetable, chopped fish and sundried fish with vegetable samples

	Name of the sample	Area	Log ($CFUg^{-1}$)
Vegetable	Fish vegetable	DB	2.58
	Prawn with vegetable	DC	1.40
	Prawn with vegetable	DE	2.94
	Prawn with potato	DB	2. 85
Chopped	Chopped Prawn	DB	3.13
	chopped Taki fish	DC	2.20
	Prawn with vegetable	DE	2.95
Dry fish curry	Dried laitka curry	DC	2.99
	Dried small fish curry	DC	2.50
	Dried Pomfret curry	DC	2.47

Table 2 represents the total coliform count and total fecal coliform count enumerated from 30 ready-to-eat fish-based food samples at four different phases of the processing line. This result shows that total

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coliform counts were identified by about 50% of samples. The highest 62.5% samples of DB and DC area were spotted the total coliform count. Only 33.33% samples of DE area were detected the total coliform count. About 20% of food samples were noticed with fecal coliform at a range of 0.05-0.2 MPN g^{-1}.

Table 2 The total coliform count and faecal coliform tallied from fish-based food samples

Sampling area (code)	Name of the sample	Total coliform count (MPNg^{-1})	Fecal coliform count (MPNg^{-1})
	Tangra fish curry	1.4	N.F
	Puti fish carry	N.F	N.F
DA	Ilish curry	0.75	N.F
	Prawn fry	N.F.	N.F.
	Fish fry (slice)	N.F.	N.F.
	Prawn chop	0.1	N.F.
	Fish cutlet	0.55	0.1
	Fish fry (Coated)	N.F.	N.F.
	Fish finger	1.05	N.F
DB	Pomfret fry	0.09	N.F
	Pomfret fry (2)	N.F.	N.F.
	Fish finger	N.F	N.F
	Fish vegetable	0.55	N.F
	Prawn with vegetable	0.6	N.F
	Ilish curry	0.18	0.2
	Mola curry	0.34	0.1
DC	Chapila fish curry	N.F.	N.F
	Prawn with vegetable	0.1	N.F
	Chopped taki fish	0.7	0.05
	Dried mola curry	0.7	0.05
	Dried Pomfret curry	N.F.	N.F.
	Prawn curry	N.F.	N.F.
	Prawn with Potato	N.F.	N.F.
	Baim fish curry	N.F.	N.F
	Kai fish curry	N.F.	N.F
	Kai fish curry(2)	0.5	N.F.
DE	Baim fish curry	N.F.	N.F
	Taki fish curry	N.F	N.F
	Prawn with vegetable	0.2	0.05
	Chopped Prawn	0.05	N.F
	Chopped mola fish	N.F.	N.F
	Total-30		

Four fish samples in raw, cooked, and frozen condition contain up to 10^6 CFU/mL of total viable bacteria count (TVC). The raw fish samples were mostly found to harbor a huge population of microorganisms up to 10^5 CFU/mL including the fecal coliforms [24]. Furthermore, different types of specific bacterial species, for example *E. coli*, *Staphylococcus spp.*, *Vibrio spp.*, *Shigella spp.*, *Salmonella spp.*, *Pseudomonas spp.* and *Klebsiella spp.* were also found in raw samples [25,26]. Salt dried marine fish samples were reported the total plate count (TPC) up to $5.3x10^6$ CFU g^{-1} with a different group of faecal coliforms and *Vibrio spp.* is an alarming situation [27]. Ready-to-eat food of Siu Mei and Lo Mei shops in Hong Kong was found SPC ranging from 1.97 to 6.84 log $CFUg^{-1}$, with a mean of 5.05 log $CFUg^{-1}$; *E. coli* counts ranging from none detected to 3.10 log $CFUg^{-1}$, with a mean of 1.78 log $CFUg^{-1}$; and *S. aureus* counts ranging from none detected to 1.42 log $CFUg^{-1}$, with a mean of 0.15 log $CFUg^{-1}$[28]. Ready-to-serve food samples were found as inappropriate according to Microbiological Criteria Regulation because of having *L. monocytogenes, B. cereus*, *E. coli*, etc. [29]. The maximum recommended bacterial counts for ready-to-eat good quality products is < 10^3 whereas 10^3 to < 10^4 $CFUg^{-1}$ is acceptable quality foods. But the more than 10^4 $CFUg^{-1}$ will be denoted as unsatisfactory class food [30,31]. The acceptable limit for the total coliform count and the total fecal coliform count is <1 MPNg^{-1} and not detected respectively [32]. In this research, 10% of curry samples and 40% fast food samples bring acceptable limits of aerobic colony counts. The rest of the samples contain satisfactory limits of aerobic colony counts. No sample was found the SPC value over 10^4 $CFUg^{-1}$. Hence, these ready-to-eat fish-based food samples taken for microbial quality assessment were of acceptable microbial quality based on the total aerobic bacterial count. On the contrary, about 20% of food samples inappropriate for taking because of containing Fecal coliform. No sample out of 30 was detected *Salmonella spp.*

3.2 Antibiotic Susceptibility Pattern

Cautious implementation of antibiotics can prevent the resistance of anti-bacterial pathogens which is very harmful to the human body and cause different problems to health. Table 3 represents the antibiotics susceptibility of ready-to-eat fish-based food born bacterial pathogens. Results showed that very negligible sensitivity was recorded for chlorpheniramine maleate group antibiotics.

Table 3 The zone of inhibition produced by the antibiotics

Trade name	The chemical name	Susceptibility (%)		
		240 μg/mL	120 μg/mL	60 μg/mL
Terax	Tetracycline Hydrochloride	86.67	80.00	74.50
Erocin	Erythromycin	8.00	0.00	-
Amoxicillin	Amoxicillin	33.34	20.00	0.00
Eramin	Cholorpheniramine maleate	6.66	-	-
Acelex	Cephalexin	78.25	40.00	25.00
Cipronil	Ciprofloxacin	100.00	100.00	96.40

The highest susceptibility was found for ciprofloxacin about 100% (Fig. 3). An experiment was conducted using three concentrations of ciprofloxacin antibiotic (60 μg/mL, 120 μg/mL, and 240 μg/mL) in the same dish with impregnated of porcelain beads. Results showed that all concentrations performed almost similarly.

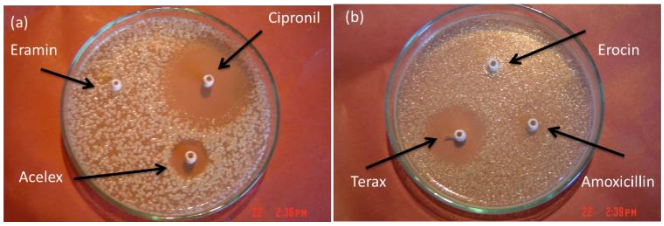

Fig. 3 Microbial populations and their antibiotic Sensitivity

It can be concluded that the 60 μg/mL dosage of ciprofloxacin is sufficient for ready-to-eat fish-based food born bacterial pathogens. Similar information was given by De Bruyn et al., [33]. They informed that a single dose of a fluoroquinolone (eg, ciprofloxacin, 500 mg) was effective in the treatment of traveler's diarrhea. Azage et al., reported that many types of bacterial isolates were resistant to tetracycline, cotriamoxazole, and erythromycin whereas many of them were sensitive to chloramphenicol, nalidixic acid, gentamicin and ciprofloxacin [34].

4. Conclusion

The presence of the pathogenic bacteria and biochemical parameters such as histamine risk might be a problem in fish-based food products. This paper introduces an assessment scheme for better evaluation of various microbiological qualities, hygiene, and safety parameters of ready-to-eat fish-based foods. Depending on total viable bacteria (TVC) 90% curry samples and 60% fast food samples bring satisfactory limits of aerobic colony counts. The rest of the samples contains acceptable limits of aerobic colony counts. About 20% of food samples unsuitable for taking because of having Fecal coliform. Among six antibiotics highest susceptibility was found for Ciprofloxacin (100%) and Tetracycline Hydrochloride (86.66%) for ready-to-eat fish-based food born bacterial pathogens.

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