Research Article

Detection and Antibiogram Study of Bacteria Isolated from Dried and Cooked Fish

Mst. Deloara Begum[1], Md. Muniruzzaman[1], Md. Salauddin[2]* and Md. Mostafizer Rahman[1]

[1]Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh; [2]Department of Microbiology and Public Health, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh.

Mst. Deloara Begum and Md. Muniruzzaman contributed equally to this work as first authors.

Abstract | Fishes are very rich source of animal proteins. The soft tissues of fish and aquatic environment are extremely susceptible to microbial contamination. In this research a total of 79 samples were collected from different local market. In which 54 samples were from dried fish and 25 from cooked fish samples. In this research there were 18 different types of dried fish and 6 types of cooked fish were used as a sample. Laboratory work was done by different bacteriological laboratory methods and purified isolates were identified according to gram's staining reaction, colony morphology, cultural characteristics, biochemical and antibiotic susceptibility test. All most 100% dried and 20% cooked fish sample were contaminated. In this study seven different species and 168 isolates were identified from dried fish and these were *Escherichia coli* 21.43% (36), *Vibrio* spp. 18.45% (31), *Staphylococcus* spp.17.86% (30), *Pseudomonas* spp.17.86% (30), *Salmonella* spp.12.5% (21), *Shigella* spp. 8.93% (15) and *Klebsiella* spp. 2.97% (5). In cooked fish 9 isolates were identified and species were *Escherichia coli* 66.66% (6) and *Shigella* spp. 33.34% (3). Total viable count varies from 1.28×10^7CFU/g to 3.74×10^9CFU/g. The highest concentration was found in Loitta fish and lowest in Ruhi from dried fish. Most species of bacteria isolated were resistant to amoxicillin, penicillin-g, kanamycin, azithromycin, cefuroxime sodium, cephalexin, nalidixic acid, cephradine and erythromycin. The isolates were found to be of medical importance. Hence it is considered that a variety of bacterial species can be associated with dried fish related pathogen to animal and humans.

Editor | Muhammad Abubakar, National Veterinary Laboratories, Park Road, Islamabad, Pakistan.
Received | October 09, 2021; **Accepted** | November 29, 2021; **Published** | December 17, 2021
***Correspondence** | Md. Salauddin, Department of Microbiology and Public Health, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh; **Email:** salauddin@kau.edu.bd
Citation | Begum, M.D., M. Muniruzzaman, M. Salauddin and M.M. Rahman. 2021. Detection and antibiogram study of bacteria isolated from dried and cooked fish. *Veterinary Sciences: Research and Reviews*, 7(2): 134-142.
DOI | https://dx.doi.org/10.17582/journal.vsrr/2021.7.2.134.142
Keywords | Fish borne diseases, Dried fish, Cooked fish, Public health hazard, Microbial quality of dried and cooked fish

Introduction

Fish has become an increasingly important source of protein, necessary for the maintenance of good health. The quality of fish is of major concern to the food processors, consumers, and public health authorities (Pal *et al.*, 2016). Fish and Fish products are not only nutritional importance but also importance in global market as foreign currency earner for a number of countries in the world (Sohana and Karim, 2016). Human food habit dramatically changes day by day on fish in the last three decades, especially in East Asia (mainly in China) and in the Near East/ North African region. Recent report shows that global

per capita fish consumption is 20 kg per year which provides 6.7% of all protein consumed by human all over the world (FAO, 2016). Fish revolution also occurs in Bangladesh about 25-fold growth in farmed fish market over the last three decades (Ahmad, 2017). Bangladesh is considered one of the most suitable regions for fisheries in the world, with the world's largest flooded wetland and the third largest aquatic biodiversity in Asia after China and India (Mozumder *et al.*, 2018). Bangladesh mainly exports ten categories of fishery products (Frozen freshwater fish, frozen marine water fish, frozen shrimp, chilled fish, live fish, dry fish, salted dehydrate, live kusia, live crab, and fish scale/shrimp scull) to more than 55 countries. Only in 2016-17, Bangladesh earns BDT 42876.40 million by exporting almost 68.31 Thousand MT of fish and fisheries products. This success is due to export of quality shrimp introducing HACCP procedure and traceability regulation according to the requirement of European Union (EU) and USA (Shamsuzzaman *et al.*, 2017).

According to the Department of Fisheries (DoF, 2017), fisheries contribute 3.69 percent of Bangladesh's GDP and over 23 percent of agricultural GDP. With an average fish intake of 53 grams per person a day, fish now account for 60 percent of protein supply for the entire population (DoF, 2016). In Seventh Five Year Plan (7FYP) Bangladesh Government has taken 5 major goal, and the 5th is Improved food safety (a) Good Aquaculture Practices (GAP) and Good Manufacturing Practices (GMP) at all stages of fish/shrimp supply chain to comply international market. (b) Food safety measures for domestic markets. From the food safety point of view provision of safe sound and wholesome fish and fish products are more essential for control the contamination of fish. Quality of fish deteriorates due to a complex process of physical chemical and microbial changes in the content of fish. Fish of good quality should have bacterial count less than 105 per gram. The greatest risk to human health occurs due to the consumption of raw, inadequately cooked or insufficiently processed fish, and fish products (Pal *et al.*, 2016).

Bacteria are the main fish-borne zoonotic agents (diseases transmitted from fish to human vice versa), infection is typically acquired through abrasions, cuts, or penetrating wounds in the skin when handling infected fish or fomites (Boylan, 2011). Human infections caused by pathogens transmitted from fish or the aquatic environment are mostly depending on the season, patients contact with fish and surrounding environment, dietary habits and the immune system status of the exposed individual. The infection source may be fish kept for food or as a hobby (aquarium fish). Some bacterial species are facultatively pathogenic for both fish and humans. They may be isolated from fish without apparent symptoms of the disease. Human infections and intoxications with the following bacteria have been recorded: *Mycobacterium* spp., *Streptococcus iniae*, *Photobacterium damselae*, *Vibrio alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *V. cholerae*, *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Aeromonas* spp., *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum*, *C. perfringens*, *Campylobacter jejuni*, *Delftia acidovorans*, *Edwardsiella tarda*, *Legionella pneumophila*, and *Plesiomonas shigelloides* (Novotny *et al.*, 2017). The results of numerous studies indicate that fish possess bacterial populations on or in their skin, gills, digestive tract, and light-emitting organs (Austin, 2006).

Fish borne zoonotic diseases can be transmitted directly by consuming raw and improperly cooked fish meat or indirectly via contaminated water from the surroundings of infected fish. Due to lack of available data about microbiological quality of fish it is difficult to achieve this goal. We are also facing problem to meet up the requirement HACCP protocol in the processing of frozen fishes for export. Therefore, the purpose of the present study was to investigate the microbiological quality of dried and cooked fish. The study also investigates the significant effect of antibiotic sensitivity pattern of the isolated bacteria. Bearing in mind the above facts the present study was undertaken with following specific objectives: (a) To isolate and identify the bacterial pathogens with the evaluation of microbial load of cooked and dried fish samples; (b) To create awareness among local beneficiaries and consumers.

Materials and Methods

Study area, collection and processing of samples

The present research work was carried out on dried and cooked fish available in the local market in Dinajpur city. All the samples were brought to the laboratory for detection and antibiogram study of bacterial pathogens from these samples. The laboratory works was conducted in the Bacteriology laboratory of the Department of Microbiology, Faculty of Veterinary

and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. A total 79 samples were collected from local market and different restaurant in Dinajpur city. Out of 79 samples 54 samples were dried fish and 25 were cooked fish samples. There were 18 different types of dried fish and 6 different types of cooked fish product samples. Firstly, Samples were rinsed thoroughly with sterile distilled water. Then samples were homogenized through blending with 90 ml peptone water (Cappuccino and Sherman, 1996). Then 1-10-fold dilutions were performed (Figure 1).

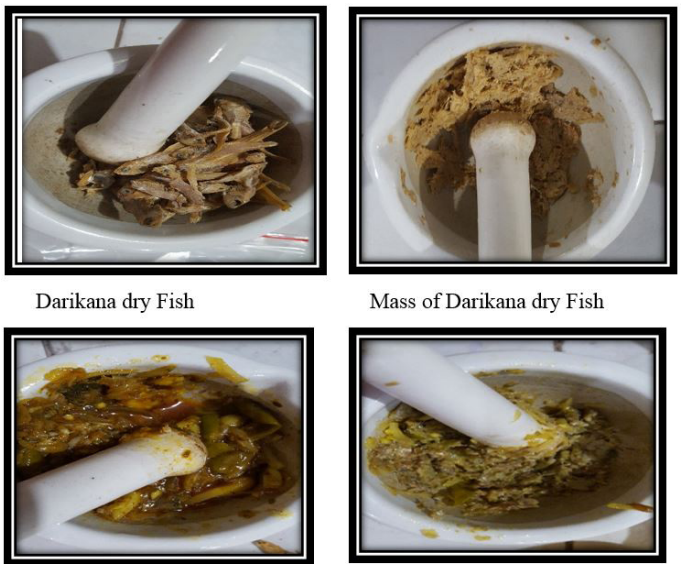

Figure 1: *Preparation of dried and Cooked fish sample.*

Serial dilution of samples

10g of each fish sample were weighed aseptically and homogenized in 90ml sterile PBS water. Then, serial dilutions were made by mixing 1.0ml of the suspension in 9.0ml sterile PBS water to obtain 10^{-1} dilution. The dilution was then made to 10^{-2}, and 10^{-6} diluents. At first, for each of the processed samples 10 sterile test tubes were placed on a test tube holder rack containing 9 ml of 2% buffered peptone water. 1 ml processed sample was mixed with 9 ml of Phosphate buffer solution in the 1st test tube in order to make 10^{-1} dilution. Then 1ml solution from 1st test tube mixed with 2nd test tube, then from 2nd test tube to 3rd test tube and finally 5th to 6th test tube and 1ml discard from 7th test tube by the help of pipette and in every step, mixing was done properly.

Total viable count (TVC) of samples

50µl of each fivefold dilution was transferred and spread onto Plate Count Agar using a micropipette for each dilution for the determination of total bacterial count. The diluted samples were spread as quickly as possible on the surface of the plate. The plates were kept in an incubator at 37 °C for 24 hrs. After incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of colonies forming units (CFU) per ml of food samples. The formula is given bellow.

Number of cells per ml=number of colonies × Dilution factor

Antimicrobial sensitivity discs and susceptibility test

To determine the drug sensitivity pattern of different bacterial isolate with different types of commercially available antimicrobial discs (Oxoid Ltd., UK) were used. The antibiotics that were tested against the selected organisms with their disc concentration are Gentamicin (GEN) 10 µg/disc, Amoxicillin (AMX) 30 µg/disc, Cefuroxime Sodium (CXM) 30 µg/disc, Cephalexin (CN) 30 µg/disc, Cefixime (CEF) 5 µg/disc, Azithromycin (AZM) 30 µg/disc, Erythromycin (E) 15 µg/disc, Penicillin G (P) 10 µg/disc, Nalidixic Acid (NA) 30 µg/disc, Colistin (CL) 10 µg/disc, Streptomycin (S) 10 µg/disc, Kanamycin (K) 30µg/disc, Cefradine (CH) 25 µg/disc. Antibiotic sensitivity assay of isolated bacteria used to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds was determined in vitro by using the standardized agar disc-diffusion method known as the Kirby Bauer (K-B) (Hudzicki, 2009).

Reading plates and interpreting results

After 24 hours of incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones oh inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies were apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, nonreflecting background and zones are measured in millimeter (mm) from the upper surface of the agar illuminated with reflected

light, with the cover removed (EUCAST, 2015).

Results and Discussion

The results of microbial loads, staining, cultural, biochemical, antibiotic sensitivity pattern and percentage of incidence of isolated bacteria are presented in different table and described below under the following headings.

Results of cultural examinations

Cultural characteristics of each type of bacteria isolated from different dried and cooked fish sample were studied for the determination of size, shape and colony characteristics in various bacteriological media. The staining property of primary culture of each of the different samples indicated the presence of more than one type of bacteria in the same smear. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different simple and selective solid media for study. The individual cultural characteristics of bacterial isolates are considered to pick the best one. Based the cultural characteristics of *Klebsiella* spp., *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp., *Pseudomonas* spp., *Vibrio* spp. and *E. coli* on the media matched with (Merchant and Packer *et al*., 1967). All the organisms identified by microscopic examination as well as biochemical test result confirmed with (Buxton and Fraser; 1977).

Result of total viable count (TVC)

After preparation of tenfold dilution all 50µl sample were spread Plate Count Agar (PCA) media up to dilution 10^{-6}. Then the number of colonies was observed and recorded (in the form of CFU/gm) of samples are presented in Table 1 for dried fish sample and Table 2 for cooked fish.

Result of isolation and identification of bacteria

Isolation and identification of bacteria was made by microscopic characteristic examination and different biochemical examination from the pure culture of the organisms. Seven species of bacteria such as *Escherichia coli, Salmonella* spp., *Pseudomonas* spp., *Vibrio* spp., *Staphylococcus* spp., *Shigella* spp. and *Klebsiella* spp. were isolated from dried fish but two species of bacteria such as *Escherichia coli* and *Shigella* spp. isolated from cooked fish respectively. Following Table 3 represent summary of bacteria isolates from dried fish samples and Table 4 of cooked fish samples.

Table 1: *TVC result of dried fish sample.*

Local name	Dried fish name (Scientific Name)	Dilution	Number of colonies	Total viable count cfu/gm
Boal	Wallago Catfish *Wallago attu*	10^{-4}	Over 300	TNTC
		10^{-5}	204	4×10^{8}
		10^{-6}	160	3.2×10^{9}
Shol/ Shoal	Snauchead Murrel/ Snakehead fish *Channa striata*	10^{-4}	212	4.24×10^{7}
		10^{-5}	180	3.6×10^{7}
		10^{-6}	153	3.06×10^{9}
Darikana/ Dariki	Slender rasbora *Rasbora daniconius*	10^{-4}	240	4.8×10^{7}
		10^{-5}	80	1.6×10^{8}
		10^{-6}	32	6.4×10^{8}
Churi	Churi Fish	10^{-4}	88	1.76×10^{7}
		10^{-5}	60	1.2×10^{8}
		10^{-6}	38	7.6×10^{8}
Ilish	Hilsa Shad/Hilsa fish *Tenualosa ilisha*	10^{-4}	76	1.52×10^{7}
		10^{-5}	40	8.0×10^{7}
		10^{-6}	32	6.4×10^{8}
Baspata	Sind Danio *Devario devario*	10^{-4}	80	1.6×10^{7}
		10^{-5}	44	8.8×10^{7}
		10^{-6}	Less 30	TFTC
Ruhi	Rohu Carp/ Rohu *Labeo rohita*	10^{-4}	64	1.28×10^{7}
		10^{-5}	Less 30	TFTC
		10^{-6}	Less 30	TFTC
Rupchada	Elongate glassy perchlat *Pampus chunesis*	10^{-4}	187	3.74×10^{7}
		10^{-5}	87	1.74×10^{8}
		10^{-6}	63	1.26×10^{9}
Bhetki	Barramund/Koral *Lates calarifer*	10^{-4}	222	4.44×10^{7}
		10^{-5}	166	3.32×10^{8}
		10^{-6}	123	2.46×10^{9}
Mola/ Ulfa	Mola craplet *Amblypharyngodon mola*	10^{-4}	203	4.06×10^{7}
		10^{-5}	76	1.52×10^{8}
		10^{-6}	47	9.4×10^{8}
Cheli	Silver razorbe belly minnow *Salmostoma acinaces*	10^{-4}	141	2.82×10^{7}
		10^{-5}	120	2.4×10^{8}
		10^{-6}	105	2.1×10^{9}
Baim	Tixe track spiny ell *Mastacebelus armatus*	10^{-4}	179	3.58×10^{7}
		10^{-5}	121	2.42×10^{8}
		10^{-6}	Less 30	TFTC
Lakhoa/ lokkha	Indian threadfin *Polynemus indicus*	10^{-4}	Over 300	TNTC
		10^{-5}	105	2.1×10^{8}
		10^{-6}	Less 30	TFTC
Loitta/ Lotey	Bombay Duck *Harpadon nehereus*	10^{-4}	Over 300	TNTC
		10^{-5}	290	5.8×10^{8}
		10^{-6}	212	4.24×10^{8}

Local name	Dried fish name (Scientific Name)	Dilution	Number of colonies	Total viable count cfu/gm
Kechhki	Ganges river sprat *Corica soborna*	10^{-4}	Over 300	TNTC
		10^{-5}	256	5.12×10^{8}
		10^{-6}	98	1.96×10^{9}
Taki	Spotted Snakehead *Channa punctatus*	10^{-4}	Over 300	TNTC
		10^{-5}	284	5.68×10^{8}
		10^{-6}	138	2.76×10^{9}
Chingri	Shrimp/ Prawn *Penaneus monodon*	10^{-4}	167	3.34×10^{7}
		10^{-5}	54	1.08×10^{8}
		10^{-6}	Less 30	TFTC
Chapila	Ganges river/ Indian river shad *Gadusia chapra*	10^{-4}	Over 300	TNTC
		10^{-5}	269	5.38×10^{8}
		10^{-6}	187	3.74×10^{9}

TNTC: Too numerous to count; TFTC: Too Few to Count.

Frequency of isolated bacterial organism

From 54 dried fish samples of 18 types fish showed 100% bacterial contamination. Total isolated organism was 168 from dried fish. In case 25 samples of cooked fish 20% were found contaminated, total isolated organisms were nine (9). Frequency of occurrence of Bacteria isolated from cooked fish samples were *Escherichia coli* (n=6) 66.66% and *Shigella* spp. (n=3) 33.34%. The Figures 2 and 3 showed the frequency of organisms isolated from dried and cooked fish respectably.

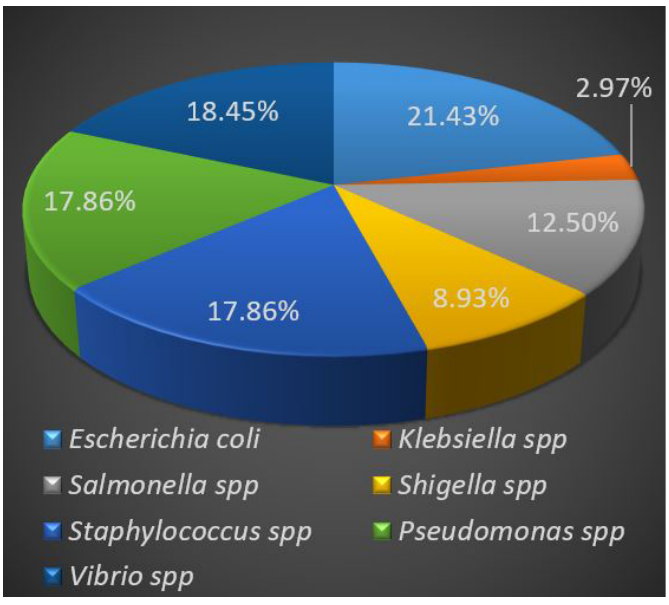

Figure 2: *Frequency of isolated bacteria from dried fish.*

Results of antibiotics sensitivity tests

Antimicrobial susceptibility testing was performed using Muller-Hinton agar (Mumbai, India) plates as recommended by the Clinical and Laboratory Standards Institute. Seven (7) isolates of *E. coli*, *Shigella* spp., *Klebsiella* spp., *Salmonella* spp., *Staphylococcus* spp., *Vibrio* spp. and *Pseudomonas* spp. were subjected to antibiotic sensitivity tests for dried fish sample. Two isolates *E. coli* and *Shigella* spp. from cooked fish samples. The results of antibiotics sensitivity tests are presented in Tables 5, 6, 7.

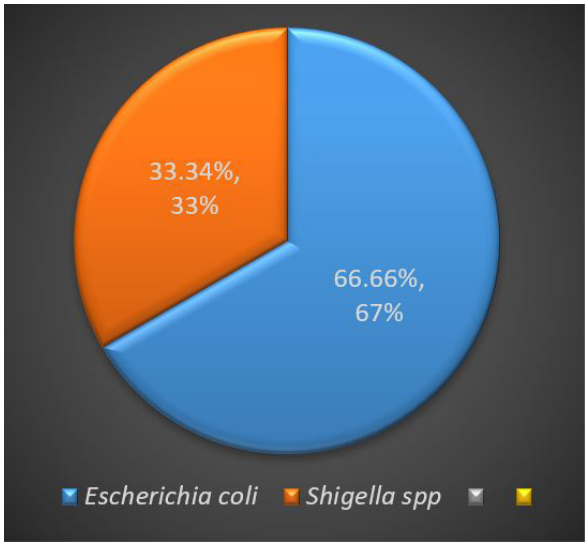

Figure 3: *Frequency of isolated bacteria from cooked fish.*

The experiment was carried out for detection and antibiogram study of bacterial pathogens isolated from 79 fish samples. Out of the 79 fish samples 54 samples were collected from dried fish and 25 samples were collected from cooked fish. There were 18 different types dried fish sample collected from the local fish markets and 6 different types cooked fish product from 15 different restaurants of Dinajpur city. For present study a series of test ware conducted for isolation, identification and antibiogram study of the isolated bacteria. For total aerobic viable microbial load detection Total Viable Plate Count was performed (TVC) in Plate Count Agar (PCA) media and counted by digital colony counter (Start Doc-It Colony Counter). The TVC result vary from 1.28×10^{7}CFU/g to 3.74×10^{9}CFU/g. Highest microbial load found in Lottya and lowest TVC count in Ruhi dried fish sample. This study indicated that the different dried fish samples showed wide range of TVC which exceed accept level. Hazard Analysis and Critical Control Point-Total Quality Management (HACCP-TQM) technical guidelines rates microbial quality for raw foods containing aerobic plate count of $<10^{4}$ cfu/g as "Good", 10^{4}-5×10^{6} cfu/g as "Average", 5×10^{6}-5×10^{7} cfu/g as "Poor" and $>5\times10^{7}$cfu/g as "Spoilt" Which can be said to be extremely hazardous for public health.

Table 2: *TVC result of cooked fish sample.*

Sample No	Cooked fish local name	Dried fish name (Scientific Name)	Dilution	Number of colonies	Total viable count cfu/gm
S_1R_1	Mirigel/Mirka	Mirgal Carp (*Cirrhinus cirrhinus*)	10^{-2}	89	1.78×10^5
S_2R_2	Chingri+Ptato Fry	Shrimp/ Prawn (*Penaneus monodon*)	10^{-3}	71	1.42×10^6
S_3R_3	Ruhi	Rohu Carp/ Rohu (*Labeo rohita*)	10^{-2}	63	1.26×10^5
S_4R_4	Puti	Punti (*Puntius sphore*)	10^{-3}	171	3.42×10^5
S_5R_5	Ruhi	Rohu Carp/ Rohu (*Labeo rohita*)	10^{-2}	98	1.96×10^5
S_6R_6	Bata+ Potato	Labeo bata	10^{-2}	123	2.48×10^5

Table 3: *Summary of isolated bacteria from different dried fish samples.*

Fish sample	Isolated presumptive organisms	Fish sample	Isolated presumptive organisms	Fish sample	Isolated presumptive organisms
Boal	*Escherichia coli* *Salmonella* spp. *Pseudomonas* spp. *Vibrio* spp.	Rup Chads	*Escherichia coli* *Staphylococcus* spp. *Salmonella* spp. *Pseudomonas* spp. *Vibrio* spp.	Cheli	*Escherichia coli* *Staphylococcus* spp. *Pseudomonas* spp.
Shol/ Shoal	*Escherichia coli* *Staphylococcus* spp. *Shigella* spp. *Pseudomonas* spp. *Vibrio* spp.	Bhetki	*Escherichia coli* *Staphylococcus* spp. *Salmonella* spp.	Baim	*Escherichia coli* *Salmonella* spp.
Baspata	*Escherichia coli*	Mola/ Ulfa	*Escherichia coli* *Salmonella* spp.	Lakhoa/ lokkha	*Escherichia coli* *Staphylococcus* spp. *Salmonella* spp. *Vibrio* spp.
Darikana/ Dariki	*Staphylococcus* spp. *Shigella* spp. *Pseudomonas* spp.	Ruhi	*Escherichia coli* *Staphylococcus* spp. *Pseudomonas* spp. *Vibrio* spp.	Loitta/ Lotey	*Klebsiella* spp. *Vibrio* spp. *Shigella* spp.
Ilish	*Escherichia coli* *Staphylococcus* spp. *Salmonella* spp. *Pseudomonas* spp. *Vibrio* spp.	Churi	*Escherichia coli* *Staphylococcus* spp. *Pseudomonas* spp. *Vibrio* spp.	Kechhki	*Staphylococcus* spp. *Klebsiella* spp. *Shigella* spp. *Pseudomonas* spp.
Taki	*Vibrio* spp.	Chingri	N/B	Chapila	*Shigella* spp. *Vibrio* spp. *Pseudomonas* spp.

Table 4: *Summary of isolated bacteria from different cooked fish samples.*

Cooked fish sample	Isolated presumptive organisms
Mirigel/Mirka	*Escherichia coli*
Chingri+Ptato Fry	*Escherichia coli, Shigella* spp.
Ruhi	*Escherichia coli*
Puti+Potato	*Escherichia coli, Shigella* spp.
Silver Carp	*Escherichia coli*
Bata+ Potato	*Escherichia coli, Shigella* spp.

All samples were inoculated into various selective media such as Eosin Methylene Blue (EMB) agar, MacConkey agar, Salmonella Shigella (SS) agar, and Mannitol Salt (MS) agar, Cetrimide agar and Thiosulfate Citrate Bile Salts Sucrose (TCBA). Among 54 dried samples all (100%) and 20% of 25 cooked fish sample had found contaminated. A total of 168 bacterial isolates belong to seven genera (*Staphylococcus* spp., *Vibrio* spp., *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp. and *Shigella* spp.) were identified from dried fish. The prevalence was 21.43% *Escherichia coli*,18.45% *Vibrio* spp., 17.86% *Staphylococcus* spp., 17.86% *Pseudomonas* spp., 12.5% *Salmonella* spp., 8.93% *Shigella* spp. and 2.9% *Klebsiella* spp. In case of cooked fish samples 9 bacterial isolates of two species (*E. coli* and *Shigella* spp.)

Table 5: *Results of antibiotic sensitivity test for gram negative organisms.*

Bacterial Species	Antibacterial agents and diameter of zone of inhibition (mm) with Interpretation								
	GEN	S	CH	E	CFM	CN	K	NA	CL
E. coli	45(S)	37(S)	R	R	R	R	R	R	22(S)
Shigella spp.	18(S)	16(S)	R	R	14(I)	R	22(S)	17(I)	19(S)
Klebsiella spp.	35(S)	16(S)	R	R	R	R	R	R	27(S)
Salmonella spp.	28(S)	9(R)	R	16(I)	12(R)	R	R	R	26(S)
Pseudomonas spp.	19(S)	R	8(R)	39(S)	R	R	20(S)	R	R
Vibrio spp.	24(S)	20(s)	R	17(I)	R	R	20(S)	15(I)	R

GEN: Gentamicin; S: Streptomycin; CH: Cephradine; E: Erythromycin; CFM: Cefixime; K: Kanamycin; NA: Nalidixic Acid; Cl: Colistin; I: Intermediate; S: Susceptible; R: Resistance.

Table 6: *Results of antibiotic sensitivity test for gram positive organism.*

Bacterial species	Antibacterial agents diameter of zone of inhibition (mm) interpretation								
	GEN	S	E	AMX	CL	AZM	P	CXM	CN
Staphylococcus spp.	23(S)	18(S)	18(I)	R	14(S)	22(S)	R	R	R

G: Gentamycin; S: Streptomycin; E: Erythromycin; AMX: Amoxicillin; CL: Clindamycin; AZM: Azithromycin; P: Penicillin; G CXM: Cefuroxime Sodium; CN: Cephalexin; S: Sensitive; R: Resistant; –: No zone of inhibition.

Table 7: *Result of antibiotic sensitivity test for gram negative organism isolated from cooked fish.*

Bacterial species	Antibacterial agents and diameter of zone of inhibition (mm) with interpretation								
	GEN	S	CH	E	CFM	CN	K	NA	CL
E. coli	41(S)	11(R)	R	R	R	R	R	22(S)	22(S)
Shigella spp.	18(S)	16(S)	R	R	14(I)	R	22(S)	17(I)	19(S)

GEN: Gentamicin; S: Streptomycin; CH: Cephradine; E: Erythromycin; CFM: Cefixime; K: Kanamycin; NA: Nalidixic Acid; Cl: Colistin; I: Intermediate; S: Susceptible; R: Resistance.

were found with a prevalence 66.66% *Escherichia coli* and 33.34% *Shigella* spp. According to Hassan *et al.* (2021) highest occurrence was *Staphylococcus* spp., *Bacillus* spp., *Salmonella* spp. and *E. coli*. In the current work *Staphylococcus* sp., *E. coli* and *Salmonella* sp. notoriously appeared. Our findings are in agreement with the findings of Sulieman *et al.* (2014) in which they isolated *Escherichia coli*, *Staphylococcus* spp., *Salmonella* spp., *Enterobacter cloacae*, *Klebsiella* spp., *Proteus* spp., *Bacillus cereus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Pseudomonas* spp. from dried fish. Logesh *et al.* (2012) also studied the microbiological quality of salted dried Marin fish according to their study they isolated *Escherichia coli*, *Staphylococcus* spp., *Salmonella* spp. and *Vibrio cholerae* which are known as human pathogenic organisms.

Above all reports are more or less similar to this experiment. Result of the present study indicates that all of the seven different types of bacteria were commonly present in dried cooked fish sample. The *in vitro* antibiotic sensitivity test of 7 different types of bacterial isolates to 9 different antibiotics for gram negative organisms such as gentamicin, streptomycin, cephalexin, erythromycin, cefixime, kanamycin, Cephradine, nalidixic acid, Colistin and 9 for gram positive organism such as gentamycin, Streptomycin, Erythromycin, Amoxicillin, Colistin, Azithromycin, Penicillin G, Cefuroxime Sodium and Cephalexin. The antibacterial sensitivity of isolates in this study to antibiotics was variable. Antimicrobial sensitivity test result showed that all of the sample harbor multidrug resistant food borne bacteria which might cause public health hazards if these antibiotic resistant transfer to human. Hence, it is recommended that a closer supervision of such food type should be carried out by relevant authorities to avoid any future pathogen outbreaks.

Conclusions and Recommendations

The present study was conducted for detection and antibiogram study of the bacteria isolated from different dried fishes and cooked fish samples.

Presence of coliforms in the sample might be due to poor quality of water, unhygienic processing, handling, packaging transport, surrounding places and poor personal hygiene of personals involve in fish and fish product processing unit. Most dried fish producer and restaurant cook are illiterate and they did not have a clear hygienic knowledge about the preparation, storage and serving of the food. The results of this study suggested that a good source of animal protein may not be healthy due to lack of hygienic measures, dirty utensils, and cook 's hygiene. These factors contributing many species of bacteria but major pathogens are *E. coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Vibrio* spp. Basic and main source of bacterial infection is poor hygienic measures and this problem may be solved by improving supervision in food handling procedure, extended consumer education on transmission of enteric food borne diseases and food safety risks. So that fish drying and cooking should be manufactured under Good Hygienic Practices and conservation practices should be developed in order to minimize the microbial contamination of food.

Acknowledgements

Authors are really thankful to the Department of Microbiology, Faculty of Veterinary and Animal Science. HSTU, Bangladesh, for the laboratory support to this work.

Novelty Statement

Current research work helps to estimate the pathogenic bacteria in dried and cooked fish at Dinajpur, Bangladesh. This article independently focuses on the microbial load, quality of dried and cooked fish as well as creating public awareness against antimicrobial resistance (AMR). Lastly this work helps to aware about human health concern against fish born multi drug resistance bacteria.

Author's Contribution

All authors contributed to write and read the manuscript and agree to be responsible for any aspect of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

Ahmad, R., 2017. Fish revolution, Bangladesh sees 25-fold growth in commercial fish farming in 3 decades. Daily Star., 9: 1-2. Available from: https://www.thedailystar.net/frontpage/fish-revolution-1445770

Austin, B., 2006. The bacterial microflora of fish, revised. Sci. World J., vol. 6. https://doi.org/10.1100/tsw.2006.181

Boylan, S., 2011. Zoonoses associated with fish. The veterinary clinics of North America. Exot. Anim. Pract., 14: 427-438. https://doi.org/10.1016/j.cvex.2011.05.003

Buxton, A., and Fraser, G., 1977. Animal microbiology. Vol. 1. Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne. pp. 400-480.

Cappuccino, J.G., and Sherman, N., 1996. Microbiology a laboratory manual. The Benjamin Cummings Publishing Co. Inc., San Francisco.

DoF, 2016. National fish week, compendium (In Bengali). Dhaka: Department of Fisheries, Ministry of Fisheries and Livestock, Government of Bangladesh.

DoF, 2017. Annual report 2017 Dhaka. Department of Fisheries, Ministry of Fisheries and Livestock, Government of Bangladesh.

EUCAST, 2015. Breakpoint tables for interpretation of MICs and zone diameters, version 5.0.

FAO, 2016. Food and Agriculture Organization of United Nation. The State of World Fisheries and Aquaculture 2016 (SOFIA). 176-182. Available here: http://www.fao.org/3/a-i5555e.pdf

Hassan, M.K., Poppy, M., Ahamed, T., Morsheda, F., Yadav, R., Hasan, A., and Siddique, M.P., 2021. Bacterial flora isolated from dried fishes sold at retail markets within Dhaka city corporation of Bangladesh. Res. Agric. Livest. Fish., 8(1): 109-115. https://doi.org/10.3329/ralf.v8i1.53272

Hudzicki, J., 2009. Kirby-Bauer disk diffusion susceptibility test protocol. Am. Soc. Microbiol.,

ISO, 1995. Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6. The Netherlands. pp. 10-18.

Logesh, A.R., Pravinkumar, M., Raffi, S.M., and Kalaiselvam, M., 2012. An investigation on

microbial screening on salt dried marine fishes. J. Food Resour. Sci., 1(1): 15-21. https://doi.org/10.3923/jfrs.2012.15.21

Merchant, I.A., and Packer, R.A., 1967. Veterinary bacteriology and virology. 7th edition, The Iowa University Press, Ames, IA, pp. 286–306.

Mozumder, M.M.H., Uddin, M.M., Schneider, P., Islam, M.M., Shamsuzzaman, M., 2018. Fisheries-based ecotourism in Bangladesh. Potentials and challenges. Resources, 7(4): 61. https://doi.org/10.3390/resources7040061

Novotny, L., Dvorska, L., Lorencova, A., Beran, V., and Pavlik, I., 2017. Fish: a potential source of bacterial pathogens for human beings. A review. Veterinarni Medicina-UZPI (Czech Republic). 2004. Present status and future direction. Aquac. Fish., 2017 May 23.

Pal, M., Ketema, A., Anberber, M., Mulu, S., and Dutta, Y., 2016. Microbial quality of fish and fish products. Beverage Food World, 43(2): 46-49.

Shamsuzzaman, M.M., Islam, M.M., Tania, N.J., Al-Mamun, M.A., Barman, PP., and Xu, X., 2017. Fisheries resources of Bangladesh: Present status and future direction. Aquac. Fish., 2(4): 145-156. https://doi.org/10.1016/j.aaf.2017.03.006

Sohana, S.A., and Karim, M.E., 2016. Microbiological quality assessment of frozen fish and fish processing materials from Bangladesh international scholarly research; Available from: http://www.hindawi.com/jpurnals/ijfs/2016/8605689/cta https://doi.org/10.1155/2016/8605689

Sulieman, A.M., Hassan, Z.M., and Elkhalifa, E.A., 2014. Microbial safety of dried fish meat (Kejeik) produced in Sudan. Food Nutr. Sci., 5(7): 606. https://doi.org/10.4236/fns.2014.57071