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RESEARCH ARTICLE

Detection of Multidrug-Resistant *Enterobacter* and *Klebsiella* Bacteria in Dry Fish Collected from Chalan Beel Region, Bangladesh

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Abstract

Dried fish is valued worldwide for its portability and long shelf life, making it an essential source of protein. In this study, dried fish samples from the Chalan Beel region of Bangladesh, were analyzed to investigate the presence of bacteria and their resistance to multiple antibiotics. Additionally, 16S rRNA sequencing identified specific bacterial species. This study revealed a worrying trend of antibiotic resistance among bacteria isolated from dried fish samples. All tested antibiotics showed significant resistance, with sulfadiazine-trimethoprim showing the highest resistance rate (85.29%) and ciprofloxacin the lowest (45.58%). Among other antibiotics, gentamicin (82.35%), oxytetracycline (80.88%), azithromycin (73.52%), erythromycin (72.05%), kanamycin (72.05%) and amoxicillin (69.11%) showed significant resistance. Alarmingly, nearly 19.11% of bacterial isolates were resistant to all tested antibiotics. Molecular identification revealed a high diversity of bacterial communities in the dried fish samples. The study identified 30 isolates belonging to 10 genera, with Enterobacter (11 species) and Klebsiella (8 species) being the most prevalent, followed by Bacillus (3 species), Proteus (2 species), and other genera (6 species). This study thus identified concerning antibiotic resistance among various bacteria present in dried fish from Bangladesh, highlighting the potential risks to public health.

Keywords: Antibiotic Resistance, Dried Fish, Multidrug-Resistant Bacteria, 16S rRNA Sequencing.

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Introduction

Antimicrobial resistance (AMR) represents a major global health threat, causing considerable morbidity and mortality worldwide (Darby et al. 2023). Over the past decades, antibiotics have played a crucial role in saving lives, improving human health, and boosting livestock production, thus contributing significantly to socioeconomic progress (Ding et al. 2023). However, their misuse has exacerbated bacterial resistance, leading to treatment failures, higher morbidity and mortality rates, and increased healthcare costs (Zhou et al. 2022). It is widely recognized that minimal antibiotic concentrations can pose a significant threat to human well-being and the environment (Rodriguez-Mozaz et al. 2015). In 2018, approximately 76,704 tons of veterinary antibiotics were used worldwide (Gehring et al. 2023), while clinical consumption was reported at 14.3 defined daily doses (DDD) per 1000 inhabitants per day (Browne et al. 2021). More than a quarter of healthcare-associated infections are caused by antibiotic-resistant bacteria, which could lead to 10 million deaths by 2050 (O'Neill 2014). The intensive use of antibiotics and their residues can promote antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG), contributing to the acceleration of the development of antibiotic resistance (Ding et al. 2023). The nutrient-rich and temperature-adapted environment of the human gut, as well as its bacterial complexity and diversity, favor the transmission of ARGs and ARGs (Ding et al. 2023). These antibiotic-resistant bacteria and genes consistently pose a significant threat to human health (Zhang et al. 2022).

Recently, convenience food products have become affordable and convenient, gaining popularity. However, consumers remain particularly concerned about animal products, which may harbor antibiotic-resistant bacteria (Abalkhail 2023). Foodborne bacteria play a crucial role in the spread of antibiotic resistance, posing a significant food safety hazard (Helmy et al. 2023). These bacteria can carry antibiotic resistance genes, which can be transmitted throughout the food chain, from production to consumption (Sagar et al. 2023). Most foodborne pathogens are bacteria, responsible for various diseases in humans and animals (Elbehiry et al. 2019). Common bacteria often detected in food include *Salmonella*, various *Shigella* species, *Listeria monocytogenes*, several

Bacillus sp., *Yersinia* sp., *Clostridium botulinum, Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, various *Campylobacter* species, and *Vibrio cholera* (Elbehiry et al. 2023). This underscores the importance of strict food safety measures to curb the spread of antibiotic resistance by foodborne bacteria. Asia is home to 56% of the world's pig population and 54% of the world's chicken population. Unsurprisingly, Asia leads the way in antimicrobial resistance in animals (Sagar et al. 2023). The World Health Organization (WHO) reports that more than half of foodborne illnesses cause diarrhea, resulting in approximately 2 million deaths each year (Qi et al. 2022). "Shutki," the local name for "dried fish," is preserved using a conventional method that involves sun and air drying. Dried fish is a key source of protein, essential fatty acids, vitamins, and minerals in Bangladesh, especially in regions where alternative protein sources are limited or expensive (Majumdar et al. 2023). Dried fish products may pose health risks due to potential contamination during processing and storage (Majumdar et al. 2023). In addition, insect infestations, microbial contamination, pesticide residues, and lipid oxidation can have a significant negative impact on food safety (Hasan et al. 2016). In Bangladesh, producers often use traditional drying methods to increase fish weight and thus increase profits. Furthermore, during storage, they may use harmful insecticides such as DDT and Nogos to prevent insect infestations in dried fish, which can compromise its nutritional quality and pose public health risks (Majumdar et al. 2023). Therefore, regular scientific evaluations are essential to assess the quality and safety of sun-dried fish and mitigate potential health risks.

Every year, a considerable amount of fish is produced in Chalan Beel (Samad et al. 2009). The Chalan Beel region is full of small beels and canals. It turns into a huge water body covering more than 375 km^2 during the rainy season and then from July to November, but shrinks to only 85 km^2 the rest of the year (Nahiduzzaman et al. 2020). During the rainy season, Chalan Beel serves as a breeding ground for native fish species. Local residents, including commercial fishermen and the general public, catch large quantities of fish (Nahiduzzaman et al. 2020). Most fish are dried using traditional sun-drying methods, which are generally rudimentary and often lack good hygiene practices. During the rainy season, high humidity can prevent complete drying, resulting in moisture reabsorption by the stored fish and making it vulnerable to bacteria, fungi, and insect infestations (Samad et al. 2009). Although dried fish is of cultural and economic importance in the region, there are notable gaps in scientific knowledge regarding its microbial safety, particularly in terms of antibiotic resistance. Fish drying has become a small-scale activity of considerable economic importance to the local people. However, most of them lack knowledge about the microbial safety of dried fish, particularly with regard to antibiotic resistance. This research aims to comprehensively study the presence, diversity, and profiles of antibiotic-resistant bacteria in different types of dried fish from the Chalan Beel region, using 16S rRNA sequencing.

Materials and Methods

Sample collection and preliminary surveys

Dried fish samples were collected from various producers in the Chalan Beel region of Bangladesh, between January and February. The samples were carefully sealed in sterile zippered bags and stored in a cooler during transport to the laboratory of the Department of Zoology, University of Rajshahi. Upon arrival, the samples were stored at -20°C for further experiments. Ten grams of dried fish were taken from each sample, to which 90 ml of distilled water was added using a vortex mixer. The mixture was homogenized for 1 to 2 minutes before being placed in sterile containers and stored at -20°C until use. Table 1 shows the dried fish species identified in Chalan Beel, of which twelve species were selected for this study.

Table 1: Samples of dried fish collected from Chalan Beel region with local, English and scientific names.

Sl. No.	Local name	English name	Scientific name
1	Punti	Barb	*Puntius* sp.
2	Raikhor	Reba carp	*Cirrhinus reba*
3	Phul chela	Minnow	*Salmostoma phulo*
4	Silver carp	Exotic carp	*Hypophthalmicthys molitrix*
5	Darkina	Flying barb	*Esomus danricus*
6	Mola	Mola carplet	*Amblypharyngodon mola*
7	Taki	Spotted snakehead	*Channa punctatus*
8	Shoal	Snakehead	*Channa striatus*
9	Guchi	Striped spiny eel	*Mastacembelus pancalus*
10	Tara baim	Lesser spiny eel	*Mastacembelus aculeatus*

Contd. Table 1

11	Gutumpuiya	Guntea loach	*Lepidocephalus guntea*
12	Bou mach	Bangla loach	*Botia dario*
13	Icha chingri	Freshwater prawn	*Macrobrachium* sp.
14	Chanda	Elongate glassy perchlet	*Chanda nama*
15	Kholisha	Goramy	*Colish faciatus*
16	Tengra	River catfish	*Mystus* sp.
17	Kakila	Needlefish	*Xenentodon cancila*
18	Chapila	Shad	*Gudusia chapra*
19	Bele	Tank goby	*Glossogobius giuris*
20	Baspata	Gangetic ailia	*Ailia coila*
21	Boal	Helicopter catfish	*Wallago attu*

Antimicrobial susceptibility testing

The disk diffusion method was used to assess the effectiveness of various antibiotics against a bacterial strain (Bauer et al. 1966). Bacteria were spread on a Mueller-Hinton agar plate, and disks containing antibiotics were placed on the surface. The plate was incubated to observe bacterial growth. Clear areas surrounding the disks, called zones of inhibition, indicated antibiotic activity, with larger areas indicating stronger antibacterial activity. To prepare the test, we dipped a sterile cotton swab into a liquid bacterial culture and removed the excess liquid by gently pressing the swab against the inside wall of the tube. The bacteria were then spread evenly on the agar by a back-and-forth motion repeated three times while rotating the plate to ensure even coverage. The plate was dried for a few minutes, then antibiotic discs were carefully placed on the surface using a special tool and gently pressed with sterilized forceps to ensure adhesion. To promote bacterial growth, the plates were incubated at 37°C for 12 to 18 hours. The appearance of clear areas around the discs indicated the effectiveness of the antibiotics against the bacteria (Jagessar et al. 2008).

DNA extraction and PCR amplification and gel electrophoresis

The phenol-chloroform method was used to extract DNA from bacterial strains (Wright et al. 2017). The 16S rRNA gene was then amplified by polymerase chain reaction (PCR). Thermal cyclers were used for amplification. PCR was performed in a 50 µL reaction mixture containing 2 µL of each primer (20 pmol/µL), 2 µL of DNA template, and 25 µL of 2x PCR reaction mixture. Primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and U1492R (5'-GGTTACCTTGTTACGACTT-3') were used (James 2010). The PCR protocol included an initial denaturation at 94°C for 5 minutes, then 35 cycles of 1-minute denaturation at 94°C, followed by 1-minute annealing at 55°C and 1-minute elongation at 72°C, ending with a final elongation at 72°C for 5 minutes. 5 µL of the PCR product was then subjected to 1.5% agarose gel electrophoresis at 90 volts for 45 minutes. The gel was visualized under UV transillumination. The results were carefully recorded during the process.

Sequence analysis and phylogenetic relationship construction

Complete 1,500 base pair 16S rRNA gene sequencing was performed using a genetic analyzer at the Central Dogma Lab, Invent Technologies Ltd., Dhaka. Forward and reverse primers were used. The sequences were carefully edited to remove PCR primer binding sites. At the same time, Snap Gene Viewer software was used to manually correct the sequences. To identify genetic sequences, BLAST analysis was performed to automatically compare them to bacterial sequences. MEGA software was then used to develop a phylogenetic tree using the neighbor-joining method.

Results and Discussion

Antibiotic susceptibility assessment

In this study, sixty-eight samples were assessed for resistance to nine antibiotics, including tetracycline (64 mg/ml), erythromycin (15 µg/disc), amoxicillin (30 µg/disc), ciprofloxacin (5 µg/disc), azithromycin (15 µg/disc), oxytetracycline (15 µg/disc), gentamicin (10 µg/disc), kanamycin (30 µg/disc), and sulfadiazine/trimethoprim (1.25 mg: 23.7 mg/disc). The highest resistance rate was observed for sulfadiazine-trimethoprim (85.29%), while ciprofloxacin showed the lowest resistance (45.58%) (Fig. 1). Resistance rates to other antibiotics were: gentamicin 82.35%, oxytetracycline 80.88%, azithromycin 73.52%, erythromycin 72.05%, kanamycin 72.05%, and amoxicillin 69.11% (Fig. 1). Among the 68 tetracycline-resistant bacterial isolates, 19.11% showed resistance to all antibiotics tested (Table 2). Furthermore, of the 30 bacteria analyzed, 13 showed resistance to eight of the antibiotics used in this study (Table 3), indicating significant concern. Multidrug-

resistant bacteria were identified in all samples using antibacterial susceptibility testing. Previous studies have shown that the majority of bacterial species isolated from dried fish samples showed resistance to a range of antibiotics including amoxicillin, nalidixic acid, penicillin-G, cefuroxime sodium, cephalexin, kanamycin, cephradine, azithromycin, and erythromycin (Begum et al. 2021). These antibiotic-resistant strains of bacteria pose potential risks to public health (Begum et al. 2021).

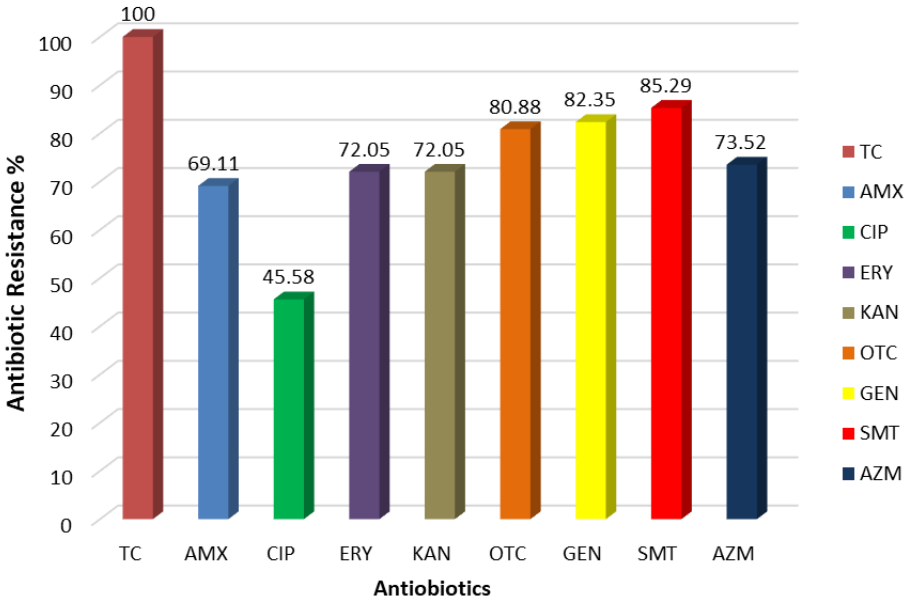

Fig. 1: Antibiotic resistance profile of bacteria isolated from dried fish samples.

Table 2: Presence of drug-resistant bacteria in different dried fish samples in the Chalan Beel region.

Resistance to	% of Resistant bacteria
TC	100.0
TC-AMX	69.11
TC-AMX -CIP	30.88
TC-AMX-CIP-ERY	20.58
TC-AMX-CIP-ERY-KAN	20.58
TC-AMX-CIP-ERY-KAN-OTC	19.11
TC-AMX-CIP-ERY-KAN-OTC-GEN	19.11
TC-AMX-CIP-ERY-KAN-OTC-GEN-SMT	19.11
TC-AMX-CIP-ERY-KAN-OTC-GEN-SMT-AZM	19.11

TC = Tetracycline, AMX = Amoxicillin, CIP = Ciprofloxacin, ERY = Erythromycin, KAN = Kanamycin, OTC = Oxytetracycline, GEN = Gentamycin, SMT = Sulfadiazine-trimethoprim and AZM = Azithromycin.

Molecular identification

A comprehensive study resulted in the collection of 38 bacterial isolates from dried fish samples from the Chalan Beel region. After isolation, PCR products of approximately 1,500 base pairs were directly sequenced. The sequencing data revealed high similarity, ranging from 96.78% to 100%, with known bacterial genera. Tables (Table 3-11) present bacterial species names with strain IDs, accession numbers, identity percentages, and corresponding homology percentages, categorized according to the dried fish names. This study examined dried fish samples from the Chalan Beel region of Bangladesh, revealing a diverse bacterial community using molecular identification techniques. A total of 30 bacteria spanning 10 different genera were identified. Among these, *Enterobacter* was the most prevalent genus, with eleven species detected, including *Enterobacter quasihormaechei* strain WCHEs120003, *Enterobacter bugandensis* strain 247BMC, *Enterobacter hormaechei* strain AUH-ENM30, *Enterobacter chuandaensis* strain 090028, *Enterobacter* sp. M3, *Enterobacter hormaechei* subsp. strain *xiangfangensis* 10-17, strain *Enterobacter hormaechei* RB13, strain *Enterobacter ludwigii* JCR-38, strain *Enterobacter hormaechei* UCHE20, strain *Enterobacter hormaechei* 20710 and strain *Enterobacter*

sichuanensis WCHECL1597. *Klebsiella* was the second most common genus, comprising eight bacteria: *Klebsiella pneumoniae* strain DSM 30104, *Klebsiella pneumoniae* strain NBRC 14940, *Klebsiella pneumoniae* strain JCM 1662, Klebsiella pneumoniae strain ATCC 13883, *Klebsiella variicola*, *Klebsiella pneumoniae* strain KPN6328, *Klebsiella quasipneumoniae* subsp. *Similipneumoniae* strain 07A044, and *Klebsiella pasteurii* strain SPARK836C1.

The study also identified three *Bacillus* strains: *Bacillus* sp. strain 201709CJKOP-109, *Bacillus subtilis* strain BCRC 10255, and *Bacillus pacificus* strain MCCC 1A06182. Two strains of *Proteus* were found: *Proteus mirabilis* strain HH140 and *Proteus mirabilis* strain ATCC 29906. In addition, a single strain of *Macrococcus caseolyticus* strain GCF1S3, *Pantoea dispersa* strain LMG 2603, *Pluralibacter gergoviae* ATCC 33028 = NBRC 105706, *Lysinibacillus capsici* strain PB300, *Pseudomonas fulva* strain NBRC 16637 and *Acinetobacter johnsonii* strain I22 were identified. This study detects a significant presence of *Enterobacter* and *Klebsiella* in dried fish samples. Both *Enterobacter* and *Klebsiella* are known to cause various infections in humans, including urinary tract infections, bloodstream infections, and respiratory infections (Khairy et al. 2020). For example, *Klebsiella pneumoniae* is a notorious pathogen responsible for serious hospital-acquired infections (Sekyere et al. 2024). Many strains of these bacteria are resistant to multiple antibiotics, complicating treatment and increasing the risk of complications, especially in individuals who are immunocompromised or have underlying health conditions (Khairy et al. 2020). These bacteria can spread to humans through direct contact with contaminated water or fish, consumption of contaminated products, and improper handling during food preparation. Results of this study identified four types of bacteria in both raw and dried fish samples, which is consistent with the study conducted by Nur et al. (2020). *E. coli*, *Staphylococcus* spp., *Pseudomonas* spp., and *Salmonella* spp. are the four types of bacteria identified. However, another study identified seven genera of bacteria in dried fish and two species of bacteria in cooked fish samples (Begum et al. 2021).

Table 3: Bacteria identified from dried fish (Boal) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Boal (*Wallago attu*)	*Proteus mirabilis* strain HH140	HQ-407310.1	98.75%	97%	5(8)
	E. quasihormaechei strain WCHEs120003	NR-180451.1	99.75%	99%	7(8)
	K. pneumoniae strain DSM 30104	NR-117686.1	97.17%	97%	8(8)
	E. quasihormaechei strain WCHEs120003	NR-180451.1	100%	99%	8(8)
	Proteus mirabilis strain ATCC 29906	NR-114419.1	99.78%	99%	7(8)

*MDR: multi-drug resistance.

Table 4: Bacteria identified from dried fish (Kholisha) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Kholisha (*Colish faciatus*)	*M. caseolyticus* strain GCF1S3	MG-719986.1	98.39%	98%	7(8)
	K. pneumoniae strain NBRC 14940	NR-113702.1	99.42%	100%	8(8)
	K. pneumoniae strain JCM 1662	NR-113240.1	99.36%	100%	8(8)
	K. pneumoniae strain DSM 30104	NR-117686.1	99.49%	99%	8(8)
	K. pneumoniae strain ATCC 13883	NR-114506.1	99.89%	100%	7(8)

*MDR: Multi-drug Resistance.

Table 5: Bacteria identified from dried fish (Darkina) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Darkina (*Esomus danricus*)	*Klebsiella variicola*	MT-256078.1	98.37%	97%	7(8)
	Klebsiella pneumoniae strain KPN6328	CP-131955.1	98.49%	98%	7(8)
	Klebsiella quasipneumoniae strain 07A044	NR-134063.1	99.17%	99%	7(8)
	Enterobacter bugandensis strain 247BMC	NR-148649.1	99.27%	99%	(8)

*MDR: Multi-drug resistance.

Table 6: Bacteria identified from dried fish (Taki) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank accession	Identity	Query coverage	*MDR
Taki (*Channa punctatus*)	*E. hormaechei* strain AUH-ENM30	CP-045611.1	98.25%	97%	8(8)
	E. chuandaensis strain 090028	NR-180237.1	97.96%	97%	5(8)
	E. chuandaensis strain 090028	NR-180237.1	98.57%	97%	5(8)
	E. chuandaensis strain 090028	NR-180237.1	99.29%	97%	6(8)

*MDR: Multi-drug Resistance.

Table 7: Bacteria identified from dried fish (Mola) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Mola (*Amlypharyngodon mola*)	*E.* sp. M3	JX-081544.1	97.90%	97%	6(8)
	Bacillus sp. strain 201709CJKOP-109	MG-867559.1	96.78%	97%	7(8)
	E. hormaechei strain 10-17	NR-126208.1	98.86%	100%	7(8)
	E. quasihormaechei strain WCHEs120003	NR-180451.1	99.78%	100%	8(8)

*MDR: Multi-drug resistance.

Table 8: Bacteria identified from dried fish (Khoyra) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Khoyra (*Sardinella fimbriata*)	*E. hormaechei* strain UCHE20	MW-548183.1	99.15%	97%	8(8)
	E. quasihormaechei strain WCHEs120003	MW-548183.1	99.15%	97%	8(8)
	E. hormaechei strain 20710	NR-180451.1	99.42%	99%	6(8)
	E. quasihormaechei strain WCHEs120003	NR-180451.1	99.85%	100%	8(8)

*MDR: Multi-drug Resistance.

Table 9: Bacteria identified from dried fish (Chela) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Chela (*Salmostoma phulo*)	*E. hormaechei* strain 10-17	NR-126208.1	99.93%	99%	7(8)
	E. hormaechei strain RB13	KC-431791.1	97.04%	97%	7(8)
	E. ludwigii strain JCR-38	KU-714599.1	97.13%	98%	6(8)

*MDR: Multi-drug Resistance.

Table 10: Bacteria identified from dried fish (Bele, Guchi and Pabda) using 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Bele (*Glossogobius giuris*)	*P. dispersa* strain LMG 2603	NR-116755.1	98.95	97%	7(8)
	B. subtilis strain BCRC 10255	NR-116017.1	98.06	97	7(8)
	Pluralibacter gergoviae ATCC 33028 = NBRC 105706	NR-024641.1	98.95	98	8(8)
Guchi (*Mastacembelus pancalus*)	*L. capsici* strain PB300	NR-181788.1	98.67	99	8(8)
	Bacillus pacificus strain MCCC 1A06182	NR-157733.1	99.50%	98%	4(8)
Pabda (*Ompok bimaculatus*)	*Klebsiella pasteurii* strain SPARK836C1	NR-180640.1	98.64%	97%	6(8)

*MDR: Multi-drug Resistance.

Table 11: Bacteria identified from dried fish (Chingri, Kakila and Tengra) using the 16s rRNA gene sequencing methods.

Fish sample	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR
Chingri (*Fenneropenaeus indicus*)	*Pseudomonas fulva* strain NBRC 16637	NR-113857.1	100%	97%	6(8)
Kakila (*Xenetodon cancila*)	*E. sichuanensis* strain WCHECL1597	NR-179946.1	98.37%	97%	6(8)
Tangra (*Mystus vittus*)	*A. johnsonii* strain I22	KT-767799.1	98.47%	99%	6(8)

*MDR: Multi-drug resistance.

Table 3-11 describes specific types of bacteria (including strains) identified from thirteen types of dried fish samples. The corresponding accession numbers show the closest matches between 16S rRNA gene sequences retrieved from GenBank database and obtained sequence data from the isolated bacterial strains. High values of identity (97.04% to 100%) and query coverage (97% to 100%) confirm the accuracy and reliability of bacterial identification using 16S rRNA gene sequencing. Tables also show that all identified strains are resistant to at least 4 antibiotics out of 8 antibiotics, which represents an important finding.

Phylogenetic tree was constructed by using BLAST results tracing evolutionary lineage of the isolates across various species. The phylogenetic tree clearly shows that organisms largely cluster by their genus. For instance, *Klebsiella* species form a strong group. Many of the branching points have high bootstrap values (e.g., 89%, 99%), particularly for the *Klebsiella* clade and the *Macrococcus/Bacillus* clade meaning these relationships are statistically well-supported and robust. Two *Enterobacter hormaechei* entries (MW548183.1) show a branch length of 0.0000, indicating they are genetically identical in the sequenced region. One strain, CP045611.1 *Enterobacter hormaechei* AUH-ENM30, branches off very early with a long branch length (0.8204) and is highly divergent from the other *Enterobacter hormaechei* strains in the upper cluster. This suggests considerable genetic diversity within the species *Enterobacter hormaechei*. The differing branch lengths indicate varying degrees of evolutionary change. Short branches mean high similarity (e.g., within the *Enterobacter hormaechei* cluster), while long branches signify greater divergence (e.g., for the AUH-ENM30 strain).

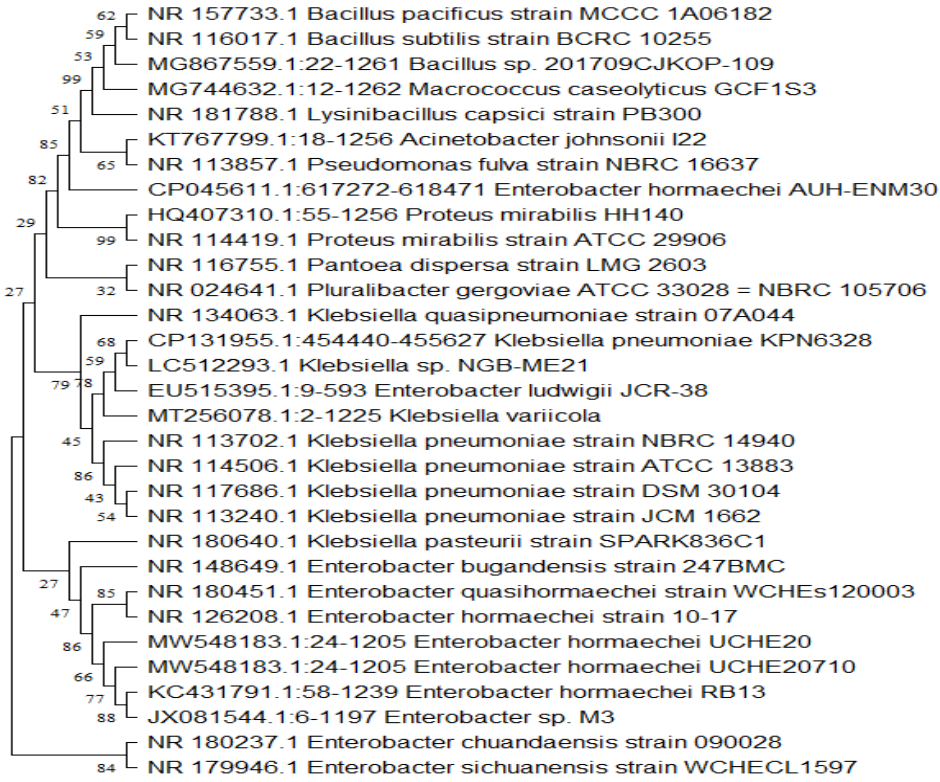

Fig. 3: Phylogenetic tree of bacteria identified from dried fish samples using 16s rRNA sequencing.

Conclusion

In conclusion, this research revealed a concerning pattern of antibiotic resistance among various bacterial populations observed in dried fish samples collected from the Chalan Beel region, Bangladesh. All antibiotics tested exhibited notable levels of resistance, with a significant presence of multidrug-resistant bacteria, representing nearly 19% of isolates. Enterobacter and Klebsiella, both recognized human pathogens, were the main genera observed. These findings highlight the potential public health risk associated with dried fish consumption and underscore the need to strengthen hygiene measures throughout the production chain to safeguard consumer well-being. Further research is needed to analyze the human circulation pathway of these pathogenic bacteria and their consequences for human health.

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Author's contribution: MHR designed the experiment, supervised the study, and finally corrected the article. FM conducted experiments, collected data statistical analysis procedure and completed the draft of the article.

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References

Abalkhail A (2023). Frequency and antimicrobial resistance patterns of foodborne pathogens in ready-to-eat foods: An evolving public health challenge. Applied Sciences 13(23): 12846.

Bauer AW, Kirby WM, Sherris JC and Turck M (1996). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45(4): 493-496.

Begum MD, Muniruzzaman M, Salauddin M and Rahman MM (2021). Detection and antibiogram study of bacteria isolated from dried and cooked fish. Veterinary Sciences: Research and Reviews 7(2): 134-142.

Browne AJ, Chipeta MG, Haines-Woodhouse G, Kumaran EP, Hamadani BH, Zaraa S, Henry NJ, Deshpande A, Reiner RC, Day NP and Lopez AD (2021). Global antibiotic consumption and usage in humans, 2000-18: a spatial modelling study. The Lancet Planetary Health 5(12): e893-904.

Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA and Blair JM (2023). Molecular mechanisms of antibiotic resistance revisited. Nature Reviews Microbiology 21 (5): 280-295.

Ding D, Wang B, Zhang X, Zhang J, Zhang H, Liu X, Gao Z and Yu Z (2023). The spread of antibiotic resistance to humans and potential protection strategies. Ecotoxicology and Environmental Safety 254: 114734.

Elbehiry A, Abalkhail A, Marzouk E, Elmanssury AE, Almuzaini AM, Alfheeaid H, Alshahrani MT, Huraysh N, Ibrahem M, Alzaben F and Alanazi F (2023). An overview of the public health challenges in diagnosing and controlling human food borne pathogens. Vaccines (4): 725.

Elbehiry A, Marzouk E, Abdeen E, Al-Dubaib M, Alsayeqh A, Ibrahem M, Hamada M, Alenzi A, Moussa I and Hemeg HA (2019). Proteomic characterization and discrimination of Aeromonas species recovered from meat and water samples with a spotlight on the antimicrobial resistance of Aeromonas hydrophila. Microbiologyopen 8(11): e782.

Gehring R, Mochel JP and Schmerold I (2023). Understanding the background and clinical significance of the WHO, WOAH, and EMA classifications of antimicrobials to mitigate antimicrobial resistance. Frontiers in Veterinary Science 10: 1153048.

Hasan MM, Rasul MG, Ferdausi HJ, Hossain MM, Shah AK and Bapary MA (2016). Present status of dried fish markets in Sylhet of Bangladesh. Progressive Agriculture 27(2): 235-241.

Helmy YA, Taha-Abdelaziz K, Hawwas HA, Ghosh S, AlKafaas SS, Moawad MM, Saied EM, Kassem II and Mawad AM (2023). Antimicrobial resistance and recent alternatives to antibiotics for the control of bacterial pathogens with an emphasis on foodborne pathogens. Antibiotics 12(2): 274.

Jagessar RC, Mars A and Gomes G (2008). Selective antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans, Escherichia coli* and *Staphylococcus aureus* using stokes disc diffusion, well diffusion, Streak plate and a dilution method. Nature and Science 6(2): 24-38.

James G (2010) Universal bacterial identification by PCR and DNA sequencing of 16S rRNA Gene. *In*: Schuller M, Sloots PT, James SG, Halliday LC and Carter WJI, Eds., PCR for Clinical Microbiology, Springer, Dordrecht, 209-214. https://doi.org/10.1007/978-90-481-9039-3_28.

Khairy RM, Mahmoud MS, Shady RR and Esmail MA (2020). Multidrug-resistant Klebsiella pneumoniae in hospital-acquired infections: concomitant analysis of antimicrobial resistant strains. International Journal of Clinical Practice 74(4): e13463.

Majumdar BC, Ahammad B, Kabir IE, Mollik JR, Baidya A, Hossain MF, Asadujjaman M, Roy TK and Paul SI (2023). Sensorial, physicochemical and microbial quality evaluations of sun-dried marine fishes available in the Bay of Bengal of Bangladesh. Applied Food Research 3(2): 100369.

Nahiduzzaman M, Flowra FA, Hossen MN, Awal MR, Rahman MA and Akter S (2020). Traditional fish drying method practiced by the farmers of Chalan beel (Singra Upazila) and their socio-economic status. International Journal of Fisheries and Aquatic Studies 8: 184-190.

Nur IT, Ghosh BK and Acharjee M (2020). Comparative microbiological analysis of raw fishes and sun-dried fishes collected from the Kawran bazaar in Dhaka city, Bangladesh. Food Research 4(3): 846-851.

O'neill JI (2014). Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance: 1-16.

Qi X, Alifu X, Chen J, Luo W, Wang J, Yu Y and Zhang R (2022). Descriptive study of foodborne disease using disease monitoring data in Zhejiang Province, China, 2016-2020. BMC Public Health 22(1): 1831.

Rodriguez-Mozaz S, Chamorro S, Marti E, Huerta B, Gros M, Sànchez-Melsió A, Borrego CM, Barceló D and Balcázar JL (2015). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Research 69: 234-242.

Sagar P, Aseem A, Banjara SK and Veleri S (2023). The role of food chain in antimicrobial resistance spread and One Health approach to reduce risks. International Journal of Food Microbiology 391: 110148.

Samad MA, Galib SM and Flowra FA (2009). Fish drying in Chalan Beel areas. Bangladesh Journal of Scientific and Industrial Research 44(4):461-466.

Sekyere OJ, Mmatli M, Bosch A, Ntsoane RV, Naidoo H, Doyisa S, Maningi NE, Mbelle NM and Said M (2024). Molecular epidemiology of multidrug-resistant *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli* outbreak among neonates in Tembisa hospital, South Africa. Frontiers in Cellular and Infection Microbiology14: 1328123.

Zhang Y, Xu S, Yang Y, Chou SH and He J (2022). A 'time bomb in the human intestine—the multiple emergence and spread of antibiotic resistant bacteria. Environmental Microbiology 24(3): 1231-1246.

Zhou J, Wang W, Zhang Q, Zhang Z, Guo J and Yan F (2022). Oxygen-supplied mesoporous carbon nanoparticles for enhanced photothermal/photodynamic synergetic therapy against antibiotic-resistant bacterial infections. Chemical Science 13(23): 6967-6981.