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RESEARCH ARTICLE

Multidrug Resistance in Bacterial Strains Isolated from Fish Farming Ponds in Rajshahi, Bangladesh

Sharmin Hafiz[1] and M Habibur Rahman*[2]

[1]Institute of Biological Science, University of Rajshahi, Bangladesh.
[2]Department of Zoology, University of Rajshahi, Bangladesh.

***Correspondence:**
Email: hrzoology@yahoo.com

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Abstract

Understanding the bacteria found in fishponds is essential for fish health management and disease prevention, especially given the increasing public health risks associated with antibiotic resistance in aquatic ecosystems. In this study, the multidrug resistance profile of bacteria in fishpond water was examined, and identification of bacteria was conducted using 16S rRNA sequencing method. The study revealed a worrying trend of antibiotic resistance in bacteria isolated from various fishponds in Rajshahi District, Bangladesh. All the antibiotics tested showed significant resistance, with sulfadiazine-trimethoprim being the highest (86.61%) and ciprofloxacin the lowest (49.187%). Alarmingly, nearly 38.09% of the bacteria were resistant to all the antibiotics tested. The present study identified a diverse bacterial community composed of 37 distinct bacterial strains belonging to 16 different genera. *Enterobacter* emerged as the most abundant genus, followed by *Klebsiella, Bacillus, Escherichia, Acinetobacter, Shigella* and among others. These dominant bacteria genera also demonstrated a high degree of resistance to the antibiotics tested in this study. In addition, the majority of the bacterial isolates identified are known to be pathogenic. The increase in multidrug-resistant bacteria (MRB) in fishponds poses a significant threat to aquatic organisms and human health, as infections can spread through contaminated fish or through direct contact with pond water. This emphasizes the need for judicious use of antibiotics in aquaculture to limit resistance development and its transmission through the food chain.

Keywords: Fish farming, Multidrug Resistant Bacteria, 16S rRNA sequencing, Public Health Risks.

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Introduction

Growing concerns about multidrug resistant bacteria (MRB) and their genes have highlighted the role of the environment in human health issues (Serwecińska 2020). Previously overlooked, the environment is now recognized as a source and pathway for spreading resistance in biota including man and animals. Antibiotics are used medicinally to fight infections and added to livestock feed to promote growth (Ehetasum et al. 2018). These drugs enter the environment through waste products such as urine and feces, contaminating water and wastewater (Lood et al. 2017). The presence of antibiotics in the environment acts as a trigger, encouraging bacteria to develop resistance. Environments teeming with MRB become reservoirs, spreading resistant genes vertically to other bacteria, thus exacerbating the situation. Antibiotics are among the most commonly used chemicals in aquaculture (Lulijwa et al. 2020). These drugs, derived from natural or synthetic sources, can either eliminate harmful pathogens or inhibit their growth. In aquaculture, some antibiotics have a dual purpose: to accelerate fish growth while also being used to treat and prevent diseases. This practice raises concerns, however, due to the potential for misuse and development of antibiotic as well as multidrug resistance (Lulijwa et al. 2020). Several factors contribute to the overuse of antibiotics in aquaculture. These include stress and immune suppression in fishes caused by poor handling practices, overpopulation due to intensification, exploitation of coastal areas, and the lack of appropriate biosecurity measures to prevent disease outbreaks (Naylor and Burke 2005, Lulijwa et al. 2020).

Humans can also be exposed to MRB directly from the environment or indirectly through the food chain (George 2019). The presence of antibiotics in surface water raises concerns about their potential to contribute to

the development of MRB, posing a threat to human and livestock health (Sharma et al. 2016). The presence of antibiotics in ecosystems disrupts the natural balance of microbial communities, potentially harming the overall health of the environment (Berendonk et al. 2015, Sharma et al. 2016). In recent years, the World Health Organization identifies antibiotic resistance as a major public health threat of our time. This concern is amplified by the discovery of MRB in various aquatic environments, where the problem extends beyond just MRB (Leonard et al. 2015). The genes themselves (multidrug resistance genes, MRGs) are becoming environmental pollutants, raising concerns about their potential spread (Hsu et al. 2015). The widespread and often inappropriate use of antibiotics is promoting the rapid development of MRB and MRGs in the environment. This raises serious concerns about the potential spread of this environmental reservoir of resistance to humans (Manaia 2017). Antibiotic use is particularly concentrated in primary animal production, raising public health concerns. For instance, multidrug resistant strains of *Salmonella typhimurium*, a harmful and pathogenic bacterium, can potentially spread from poultry and pork to humans through contaminated food (Verraes et al. 2013). Similar to other food production sectors, aquaculture raises concerns about antimicrobial use and resistance development (Zou et al. 2011, Founou et al. 2016). The widespread practice of using antibiotics to accelerate the growth of food animals and fish contributes significantly to the increase and spread of MRB in the environment (Founou et al. 2016).

The role of the environment in antimicrobial resistance (AMR) is widely recognized, but key questions remain still remained unsolved (Moreno-Switt et al. 2020). While scientists agree the sources and pathways for MRB spread, debate surrounds the biggest contributors and the human health risk that they poses (Bengtsson-Palme et al. 2018, Moreno-Switt et al. 2020). Studies of waterborne multidrug resistance have focused on specific sources such as aquaculture, wastewater treatment, and irrigation. However, our understanding of MRB in both freshwater and marine environments, and the main sources of water contamination, remains limited (Hu et al. 2016, Hoelzer et al. 2017, Hafiz S and Rahman 2024, Haque et al. 2024). The present study aims to address the gap in knowledge regarding the prevalence of MRB in pond water from the Rajshahi District of Bangladesh. Simultaneously, 16S rRNA sequencing was utilized to identify and classify these resistant strains. The findings would contribute valuable scientific data to the current state of the MRB in this specific aquatic environment in the country.

Materials and Methods

Collection of pond water samples and survey

To investigate the abundance of MRB, water samples were collected from 20 fish farming ponds across Rajshahi District mentioned in Table 1, during December 2022 and February 2023. Each sample was carefully transferred to a sterile falcon tube, hermetically sealed, and placed in a cooler for transport. Upon arrival at the Laboratory of Department of Zoology, University of Rajshahi, the samples were promptly stored at -20°C for further analysis.

Table 1: Temperature and pH levels in water samples of twenty ponds of Rajshahi region.

Sl. No.	Sample name	Temperature (°C)	pH
01	Rajshahi University-1 (RU-1)	22.16±0.56	8.13±0.23
02	Rajshahi University-2 (RU-2)	22.66±0.42	8.30±0.20
03	Baghmara (BAP)	24.16±0.76	8.13±0.23
04	Taherpur (TAP)	23.10±0.75	8.50±0.36
05	Rajpara (RAP)	23.23±0.68	8.70±0.26
06	Chapainawabgonj (CHP)	22.66±0.41	8.36±0.40
07	Gomostapur (GOP)	23.60±0.52	8.10±0.36
08	Kashiadanga (KAP)	22.46±0.75	8.60±0.30
09	Tanore (TNP)	23.60±0.52	8.10±0.26
10	Aradighi (ARP)	23.60±0.40	8.10±0.36
11	Durgapur (DUP)	25.13±0.61	8.16±0.30
12	Puthia (PUP)	24.20±0.34	8.26±0.30
13	Nowdapara (NOP)	22.33±0.57	8.13±0.25

Contd. Table 1

14	Kharkhari (KHP)	22.00±0.50	8.03±0.05
15	Shalbagan (SHP)	22.63±0.55	8.33±0.25
16	Helenabad (HEP)	23.40±0.40	8.23±0.25
17	Kadirgonj (KDP)	22.20±0.72	8.03±0.25
18	Padma Abasik (PAP)	22.26±0.46	8.13±0.15
19	Ghoshpara (GPA)	23.50±0.50	8.76±0.21
20	Vatapara (VAP)	24.33±0.42	9.03±0.15

In vitro antimicrobial susceptibility test.

The disc diffusion method was used to assess the effectiveness of antibiotics against the isolated bacterial strains (Bauer et al. 1966). A mixture of bacteria was spread onto a special nutrient plate (Mueller Hinton). Then, small discs containing different antibiotics were placed on the plate, and the whole thing was incubated to see if the bacteria could grow. The transparent circles around the discs, called zones of inhibition, reveal the effectiveness of the antibiotic against the bacteria. A larger zone indicates a more potent antibiotic, as less is needed to stop bacterial growth. To prepare for the test, a sterile cotton swab was dipped into a liquid culture containing the bacteria. Excess liquid was removed by gently pressing or rolling the cotton swab against the inside of the tube. We used the swab to gently and evenly distribute the bacteria on the agar using a back-and-forth motion. This was repeated three times, rotating the plate each time, to ensure an even amount of bacteria in all areas. After letting the plate dry for a few minutes, we carefully placed antibiotic discs on the surface using a special tool. Then each disc was gently pressed onto the agar surface with sterilized tongs to stick it securely. Finally, the plates were placed in an incubator at 37°C for 12 to 18 hours to allow the bacteria to grow. The presence of clear circles around the discs, where bacteria were not able to grow, indicated which antibiotics were effective against the bacteria (Jagessar et al. 2008, Sarker et al. 2014, Islam et al. 2023).

DNA extraction and PCR amplification

The well-established phenol-chloroform technique was used to extract DNA from the bacteria (Wright et al. 2017). Then polymerase chain reaction (PCR) was then used to amplify a specific gene, the 16S rRNA gene, in the bacteria. The PCR reaction was carried out in small tubes containing 50µl of liquid in total. The mixture included 2µl of the extracted DNA, a pre-mixed solution containing all the necessary PCR ingredients (2× PCR Master Mix) at 25µl, and 2µl of each specific primer used in the reaction (each at a concentration of 20 pico moles/µl). Specific primers *viz*., 27F and U1492R to target the 16S rRNA gene, were used which had the following sequences: 27F-(5'-AGAGTTTGATCCTGGCTCAG-3') and U1492R (5'-GGTTACCTTGTTACGACT T-3') (James 2010). To make copies of the 16S rRNA gene, a process called PCR was carried out using a specific heating and cooling program which started with heating the DNA at 94°C for 5 minutes to separate the strands. This process was then repeated 35 times: heating again to 94°C for 1 minute, lowering the temperature to 55°C for 1 minute to allow the primers to bind, and raising the temperature to 72°C for 1 minute to allow the DNA to copy. Finally, the temperature was maintained at 72°C for another 5 minutes to ensure a complete copy.

The results were checked using Gel electrophoresis, which involved placing 5 µl of the PCR product in 1.5% agarose gel and running 90 volts electric current through it for 45 minutes. The DNA fragments were then visualized using UV light, and pictures were taken to record and interpret the results.

Sequencing and phylogenetic analysis

A complete 16S rRNA gene sequencing, covering 1,500 base pairs and including both reverse and forward reactions, was performed using a genetic analyzer at the Central Dogma Lab, Invent Technologies Ltd., Dhaka. The sequencing process involved the use of forward and reverse primers. The sequences were then carefully edited to remove PCR primer binding sites and manually corrected using MEGA software. To confirm the identity of the gene sequences, they were automatically compared to bacterial sequences stored in databases (http://www.ncbi.nlm.nih.gov/) using BLAST analysis. A phylogenetic analysis was then performed using the neighbor-joining algorithm (Hafiz and Rahman 2024).

Results and Discussion

Temperature and pH of the water of sampling pond

The temperature and pH data revealed slight variation across sampling sites, with temperatures ranging from 22.00 ± 0.5°C to 25.13 ± 0.61°C (Table 1). These ranges are within the optimal range for freshwater aquaculture but may influence microbial diversity and antibiotic activity. The pH values, ranging between 8.03 ± 0.05 and 9.03 ± 0.15, indicate alkaline conditions (Table 1). Alkaline water can impact metal solubility, bacterial survival, and the effectiveness of antibiotics, which could be a contributing factor to the persistence of resistant strains in these ecosystems (Mohammad and Haque 2021).

In vitro antimicrobial susceptibility

Eighty-five isolates were tested for resistance to nine antibiotics, including Amoxicillin (AMX 30 µg/disc), Azithromycin (AZM 15 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Erythromycin (ERY 15 µg/disc), Gentamicin (GEN 10 µg/disc), Kanamycin (KAN 30 µg/disc), Oxytetracycline (OTC 15 µg/disc), Sulfadiazine/trimethoprim (SMT 1.25 mg: 23.7 mg/disc) and Tetracycline (TEC 64 mg/ml). Among these, the highest resistance observed was to SMT at 86.61%, while the lowest was to CIP at 49.18% (Fig. 1). The resistance levels for other antibiotics varied: AZM at 84.84%, ERY at 84.76%, AMX at 83.32%, OTC at 78.89%, KAN at 66.14%, and GEN showed at 60.65% resistance rate (Fig. 1). Of the 85 tetracycline (TEC)-resistant bacteria isolates, a common phenomenon was observed; 38.09% of the isolated bacteria showed resistance to all the antibiotics used in this experiment (Table 2). Out of the 37 bacteria examined, a worrying trend emerged: 24 showed resistance to a worrying number of antibiotics (9) used in the study (Table 3, 4 and 5).

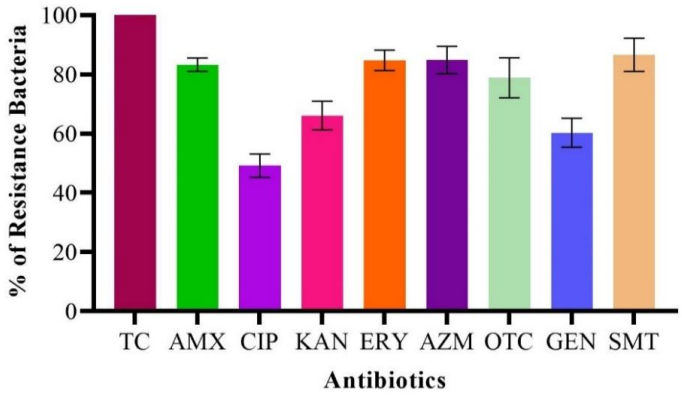

Fig. 1: Pattern of antibiotic resistance in bacteria isolated from fishpond in Rajshahi, Bangladesh.

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

Table 2: The occurrences of MRB in different fish farming ponds in Rajshahi, Bangladesh.

Antibiotics used	% of Resistant bacteria
TEC	100.0
AMX	83.32
AMX -CIP	47.62
AMX-CIP-KAN	42.85
AMX-CIP- ERY-KAN	41.66
AMX-AZM-CIP-ERY-KAN	41.66
AMX-AZM-CIP-ERY-KAN-OTC	40.74
AMX-AZM-CIP-ERY-GEN-KAN-OTC	38.09
AMX-AZM-CIP-ERY-GEN-KAN-OTC-SMT	38.09

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

Table 3: List of pathogenic bacteria that are known to cause disease in humans/animals.

Sample ID.	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR occurrence
S-DUP-5	*Enterobacter asburiae* strain RA11	MT113090.1	97.74%	97%	9(9)
S-RAP-3	*Enterobacter cloacae* strain BY58	MN133905.1	97.65%	95%	7(9)
S-CHP-4	*Enterobacter cloacae* strain ATCC 13047	NR_102794.2	99.78%	98%	9(9)
S-ARP-1	*Enterobacter hormaechei* strain SU103	MT640267.1	96.03%	94%	9(9)
S-PAP-2	*Enterobacter hormaechei* strain 10-17	NR_126208.1	99.71%	98%	9(9)
S-KHP-2	*Enterobacter bugandensis* strain 247BMC	NR_148649.1	97.55%	96%	8(9)
S-TNP-3	*Enterobacter quasihormaechei* strain WCHEs120003	NR_180451.1	99.41%	100%	8(9)
S-ARP-2	*Klebsiella pneumoniae* strain DSM 30104	NR_117683.1	99.06%	97%	9(9)
S-ARP-5	*Klebsiella pneumoniae* strain ATCC 13883	NR_114506.1	99.64%	100%	9(9)
S-TNP-4	*Klebsiella quasipneumoniae* strain 07A044,	NR_134063.1	99.78	100%	8(9)
S-TAP-2	*Acinetobacter baumannii* strain S-X9A	KJ806391.1	98.39%	95%	5 (9)
S-TAP-1	*Acinetobacter gandensis* strain ANC 4864	KM454858.1	98.76%	97%	4(9)
S-CHP-2	*Shigella* sp. strain SCU-C1	MN211528.1	97.16%	97%	9(9)
S-CHP-3	*Shigella flexneri* strain ATCC 29903	NR_026331.1.	95.76%	100%	9(9)
S-GPA-2	*Shigella boydii* strain P288	NR_104901.1	99.41%	99%	8(9)
S-CHP-1	*Escherichia coli* strain ECIUP	MN094133.1	96.43%	95%	9(9)
S-KHP-1	*Escherichia fergusonii* ATCC 35469	NR_074902.1	69.51%	96%	8(9)
S-RAP-2	*Proteus mirabilis* strain ATCC 29906	NR_114419.1	98.19%	97%	8(9)
S-NOP-2	*Proteus mirabilis* strain JCM 1669	NR_113344.1	99.19%	96%	9(9)
S-SHP-1	*Staphylococcus aureus* strain ATCC 12600	NR_118997.2	97.22%	96%	8(9)

*MDR = Multidrug resistance.

These results are consistent with previous research by Lassen et al. (2022), who observed the highest resistance among sulfonamides, reaching 27%. Similarly, Neela et al. (2015) also reported the presence of tetracycline- and ampicillin-resistant bacteria in fish farming environments. A study by Hossain et al. (2018) examined antibiotic resistance in bacteria found in fish from antibiotic-treated ponds. The results showed that these bacteria exhibited high levels of resistance to several classes of antibiotics, including tetracycline, penicillin, cephalosporin, aminoglycosides, and macrolides. They were also highly resistant to sulfonamides and moderately resistant to fluoroquinolones. Interestingly, the bacteria remained fully susceptible to carbapenems, a class of last-resort antibiotics (Hossain et al. 2018, Billah 2024, Haque et al. 2024). These results contribute valuable data to the growing concerns about antibiotic resistance in aquaculture. It highlights the potential public health risks and emphasizes the need for stricter regulations on the use of antibiotics in fish farming ponds in the country.

Molecular identification

A comprehensive study identified 37 isolates from water samples of 20 different fishponds. Agarose gel electrophoresis identified various bacterial strains. After isolating these strains, PCR products, each approximately 1,500 base pairs long, were directly sequenced. The sequencing results demonstrated considerable homology, with similarities ranging from 96.03% to 100%, compared to existing bacterial genera. Presented tables (Table 3, 4 and 5) systematically displayed the sample Id, source, names of the bacterial species with strain Id, accession number, identity percentages and their corresponding homology percentages.

In our research, we conducted a comprehensive study of bacterial diversity and identified a total of 37 distinct bacterial strains spanning over 16 genera. Among these, the genus *Enterobacter* emerged as the most abundant, with a remarkable representation of 8 different strains. These strains included *Enterobacter asburiae* strain RA11, *Enterobacter cloacae* strain BY58, *Enterobacter hormaechei* strain SU103, *Enterobacter cloacae* strain ATCC 13047, *Enterobacter quasihormaechei* strain WCHEs120003, *Enterobacter bugandensis* strain 247BMC, *Enterobacter hormaechei* strain 10-17, and *Enterobacter chuandaensis* strain 090028. Following closely, we observed a significant presence of *Klebsiella* and *Bacillus* strains, identifying four distinct strains of each. The *Klebsiella* strains identified were *Klebsiella quasipneumoniae* strain 07A044, *Klebsiella pneumoniae* strain DSM 30104, *Klebsiella pneumoniae* strain ATCC 13883, and *Klebsiella pasteurii* strain SPARK836C1. The *Bacillus* strains identified were *Bacillus* sp. strain PYCC 8231, *Bacillus cereus* strain HAPH4, *Bacillus altitudinis* strain AS_K1, and *Bacillus wiedmannii* strain FSL W8-0169. Other genera represented in our study included *Escherichia* (3 strains), *Acinetobacter* (3 strains), *Shigella* (3 strains), Proteus (2 strains), *Leclercia* (2 strains), as well as unique strains of *Exiguobacterium*, *Kluyvera*, *Aeromonas*, *Stenotrophomonas*, *Empedobacter*, *Staphylococcus*, *Lysinibacillus*, and *Moraxella*. Most of the identified bacteria were pathogenic and known to cause diseases in human and animals (Table 3). Some opportunistic and non-pathogenic bacteria were also identified in this investigation (Table 4 and 5).

Table 4: List of opportunistic pathogens that infect immunocompromised hosts.

Sample ID.	Identified bacteria	GenBank Accession	Identity	Query coverage	*MDR occurrence
S-BAP-5	*Stenotrophomonas maltophilia* strain ATCC 19861	NR_040804.1	99.63%	100%	8(9)
S-KAP-1	*Aeromonas* sp. strain J16OP4	MN519543.1	97.56%	98%	7(9)
S-RAP-1	*Bacillus cereus* strain HAPH4	MZ443977.1	98.08%	96%	9(9)
S-HEP-1	*Moraxella osloensis* strain A1920	NR_104936.1	97.20%	97%	9(9)
S-TNP-5	*Leclercia adecarboxylata* strain NBRC 102595	NR_114154.1	99.85%	100%	9(9)
S-ARP-4	*Leclercia adecarboxylata* strain CIP 82.92	NR_104933.1	99.85%	100%	9(9)
S-KAP-2	*Kluyvera sichuanensis* strain 090646	NR_181179.1	99.95%	100%	9(9)
S-BAP-2	*Acinetobacter* sp. EU18	JF681283.1	98.46%	96%	7(9)
S-VAP-2	*Enterobacter chuandaensis* strain 090028	NR_180237.1	99.73%	97%	9(9)
S-KAP-5	*Empedobacter falsenii genomovar* 1 strain NF 993.	NR_042430.2	99.95%	99%	9(9)

Table 5: List of non-pathogenic or low pathogenic bacteria that are harmless in humans.

Sample ID.	Identified bacteria	GenBank Accession	Identity	Query Coverage	*MDR occurrence
S-RUP1-3	*Bacillus* sp. strain PYCC 8231	MN510815.1	98.33%	97%	6(9)
S-TNP-1	*Bacillus altitudinis* strain AS_K1	OQ195705.1	97.69%	98%	8(9)
S-NOP-3	*Bacillus wiedmannii* strain FSL W8-0169	NR_152692.1	99.18%	97%	9(9)
S-SHP-2	*Lysinibacillus macroides* strain LMG 18474	NR_114920.1	99.70%	100%	9(9)
S-CHP-6	*Klebsiella pasteurii* strain SPARK836C1	NR_180640.1	99.78%	100%	7(9)
S-RAP-4	*Exiguobacter iumindicum* strain HHS 31	NR_042347.1	99.23%	99%	9(9)

The percentage of identified MRB revealed that *Enterobacter* (21.62%) was the most prevalent genus (Fig. 2). Significant amounts of *Klebsiella* (10.81%), *Bacillus* (10.81%), *Escherichia* (8.11%), *Acinetobacter* (8.11%), and *Shigella* (8.11%) were also present, along with several less common strains. The presence of strains of *Enterobacter, Klebsiella, Escherichia, Acinetobacte*r, and *Shigella* raises serious concerns for the aquatic ecosystem and public health.

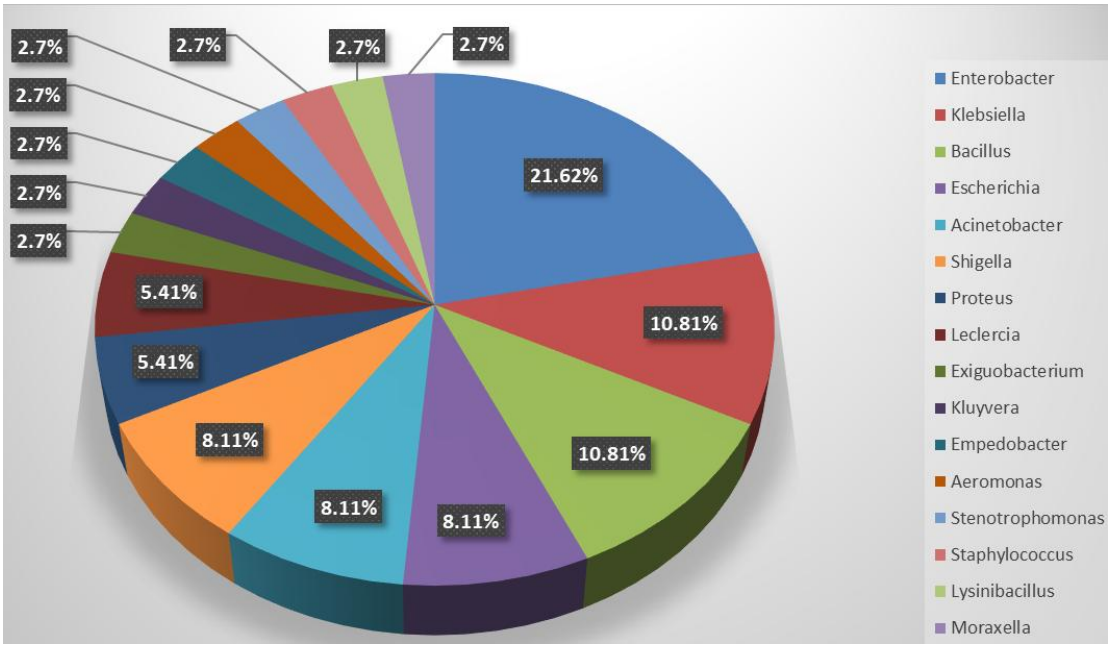

Fig. 2: Prevalence of multidrug resistant bacteria (MRB) in the fish farming ponds in Rajshahi, Bangladesh.

Previous research by Hossain et al. (2018) suggested that the fish farming ponds harbour bacterial species including *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli*, *Vibrio* spp., and *Staphylococcus* spp. were the most abundant genera. These prevalent bacteria exhibited a stronger multidrug resistance profile in fish from antibiotic-treated ponds. A study by Jama et al. (2020) identified various bacterial pathogens in pond water samples. *Escherichia coli* was the most common, accounting for 12.5% of the isolates. Other important pathogens included *Salmonella* spp. (31.25%), *Klebsiella* spp. (6.25%), *Shigella* spp. (18.75%), *Staphylococcus* spp. (18.75%) and *Pseudomonas* spp. (12.5%).

The increasing abundance of multidrug-resistant bacteria in fish farms poses a significant threat to aquatic life and human health (Petersen et al. 2002, Hossain et al. 2017). These bacteria, including *Escherichia*, *Enterobacter*, *Acinetobacter*, and *Vibrio* can cause various diseases in fish, leading to increased mortality and production losses (Kotob et al. 2016). In addition, these bacteria can compromise the immune system of fish, making them more susceptible to other pathogens (Kotob et al. 2016, Jama et al. 2020). The presence of antibiotic-resistant bacteria in fish ponds also poses a potential risk to human health (Klase et al. 2019). Consumption of contaminated fish or exposure to pond water during maintenance activities could introduce these resistant strains into the human body, potentially causing infections that are difficult to treat with conventional antibiotics (Amin et al. 2024). The construction of phylogenetic trees (Fig. 3), facilitated by BLAST results, made it possible to trace the evolutionary lineage of isolates by identifying related sequences between different species or organisms. Around the circle, the tree is divided into different sections, each labeled according to the bacterial species. The length of the branches connecting the species at the center of the tree can indicate their degree of relatedness. Shorter branches can indicate a closer relationship than longer branches.

Fig. 3: Phylogenetic relationship of bacteria isolated from fishponds showing homology with sequences analyzed by the neighbor-joining method sequenced based on 16S rRNA gene sequencing.

The widespread presence of MRB in aquaculture waters raises concerns about the transmission of these resistance genes to fish and, ultimately, to humans via the food chain. This underscores the importance of responsible antibiotic use in aquaculture to minimize the development and spread of antibiotic resistance. However, research on the surveillance of antibiotic resistance in Bangladeshi aquatic environments is limited. To better understand this research, further research is needed to assess the impact of resistant bacteria on fish health and to formulate strategies to reduce the spread of antibiotic resistance in aquaculture environment in the country.

Conclusion

In summary, our study highlights the alarming prevalence of antibiotic resistance in fishponds in Rajshahi, Bangladesh. Widespread resistance to various antibiotics underscores the urgency of action. With a significant proportion of bacteria resistant to all tested antibiotics, the health risks to both fish and humans are significant. These findings underscore the need for stricter regulations on the use of antibiotics in aquaculture. Furthermore, the presence of MRB raises concerns about the transfer of resistance through the food chain, necessitating proactive measures such as judicious use antibiotic use and the exploration of alternative disease control methods. Moving forward, further research is essential to fully understand the extent of antibiotic resistance in fish farming in Bangladeshi. By developing and implementing effective management strategies, we can ensure the sustainability and safety of the aquaculture sector, thereby protecting fish health, production, and public well-being for the future.

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Author's contribution: SH collected samples and data, conducted experiments, writing original draft, formal analysis. HR designed the conceptualization (equal), project administration(equal) supervised the study, writing review and editing the manuscript. All authors have read and approved the final manuscript.

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Data availability: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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