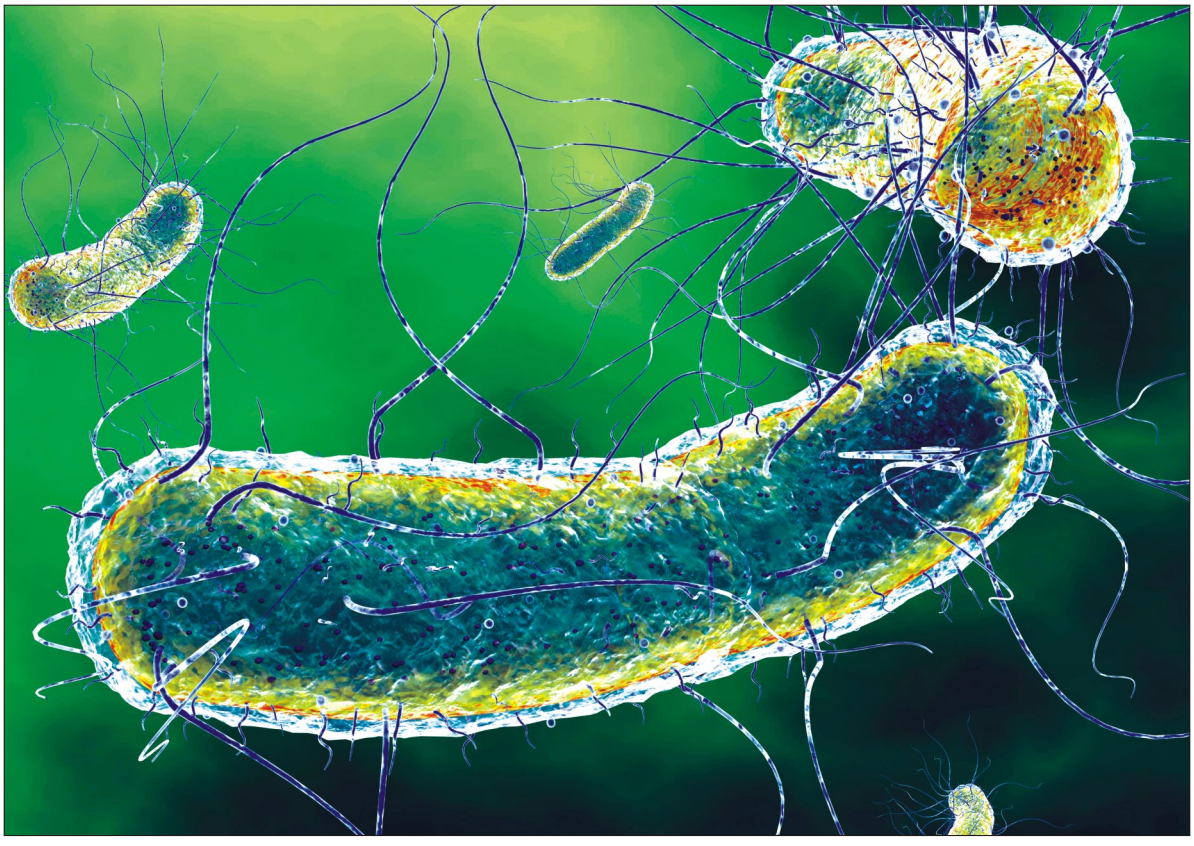

Current Research in Bacteriology

ISSN 1994-5426

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Current Research in Bacteriology

ISSN 1994-5426
DOI: 10.3923/crb.2023.27.36

Research Article
Characterization and Molecular Identification of Bacterial Isolates from Tail and Fin Rot Infected Silver Carp

[1,2]Dilruba Afrin, [3]Md. Ashikul Islam, [1,2]Md. Nazmul Islam Bappy, [4]Md. Shahadat Hossain, [1,2]Kazi Md. Ali Zinnah, [3]Biswanath Sikdar, [3]Md. Asadul Islam, [3]Md. Khalekuzzaman and [1,5]Shamsunnahar Mukta

[1]Faculty of Biotechnology and Genetic Engineering, Sylhet Agricultural University, Sylhet 3100, Bangladesh
[2]Department of Animal and Fish Biotechnology, Sylhet Agricultural University, Sylhet 3100, Bangladesh
[3]Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205, Bangladesh
[4]Department of Biotechnology and Genetic Engineering, Noakhali Science and Technology University, Noakhali 3814, Bangladesh
[5]Department of Plant and Environmental Biotechnology, Sylhet Agricultural University, Sylhet 3100, Bangladesh

Abstract

Background and Objective: Silver carp is considered one of the most cultivated farm fish of Bangladesh but bacterial diseases like fin and tail rot disease make it difficult for the farmers to maintain the silver carp properly. Therefore, this study tried to isolate and detect bacteria from the tail and fin rot disease-infected silver carp collected from the Sylhet Region of Bangladesh. **Materials and Methods:** Isolated bacteria were subjected to different morphological and biochemical tests for their preliminary identification. The recovered isolates were then sequenced for the 16S rRNA to confirm identification and to understand subsequent evolution using phylogenetic relationships. In addition, antibiotic sensitivity of the isolated strains was carried out to screen potential antibiotics against those foes. **Results:** The biochemical test and the molecular identification confirmed the presence of *Kurthia gibsonii* (Accession Number: MN658386), *Klebsiella pneumoniae* (Accession Number: MN658387) and *Bacillus subtilis* (Accession Number: MN658386) in the diseased fish. The antibiogram test revealed that all three isolates were susceptible to cefotaxime and kanamycin. However, the isolated strains also exhibited resistance to particular antibiotics at certain degrees which indicated the probable failure of those antibiotics to combat those pathogens. **Conclusion:** The detection and antibiogram profiling of isolated bacteria from tail and fin rot diseased silver carp will help to identify proper treatment options against this disease.

Key words: Silver carp, tail and fin rot disease, biochemical characterization, molecular identification, phylogenetic analysis, antibiotic sensitivity

Citation: Afrin, D., M.A. Islam, M.N.I. Bappy, M.S. Hossain and K.M.A. Zinnah *et al.*, 2023. Characterization and molecular identification of bacterial isolates from tail and fin rot infected silver carp. Curr. Res. Bacteriol., 16: 27-36.

Corresponding Author: Dilruba Afrin, Department of Animal and Fish Biotechnology, Sylhet Agricultural University, Sylhet 3100, Bangladesh

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fishes are one of the most common vertebrates that are consumed worldwide as an important nutrient-rich food. Aquaculture is growing rapidly in many developing nations, concerning both quantity and variety of species. However, natural reserves in many developing countries are decreasing at an alarming rate due to increased human consumption, lack of cost-effective wastewater treatments and urbanization[1]. Along with these factors, fish susceptibility to pathogens contributed to a greater portion of losses from aquaculture. Bacterial infections are devastating to both marine and freshwater cultured fish and their annual economic impact on aquaculture production is estimated to be in the hundreds of millions to billions of dollars range[2]. Fish farms frequently experience fish infections such as cottonmouth, gill infection, itch, swelling, tail and fin rot, fungal disease, pop and cloudy eye, swim bladder disease, lice and nematode worm infestations, water quality-induced disease, alimentary stoppages, anorexia, chilodonella, ergasilus, TB, glugea, henneguya, Hexamita, injuries, leeches in aquariums. The increase in disease occurrences that threaten global aquaculture productivity, profitability and sustainability coincides with recent advancements in aquaculture production. Diseases caused by bacteria can kill a lot of fish, both wild and farmed. Infections and illnesses caused by bacteria are quite common and are very widespread[3].

Among the diverse fish species cultured worldwide, cyprinids are considered the largest group of species cultivated. Silver carp, a planktivorous freshwater cyprinid fish species, plays an economically vital role in the aquaculture sector because of a shorter rearing period and lower of cost feed consumption. Importation was typically done for aquaculture purposes, although occasionally it was also done to promote wild fish populations and regulate water supply. Their capacity to directly consume large amounts of phytoplankton and change the water environment makes them valuable as manipulative filter feeders to manage blast algal blooms in addition to providing economic advantages[4].

However, the intensification and spread of silver carp aquaculture, which has a higher susceptibility to numerous bacterial diseases, is seriously endangering this sector in Bangladesh and India[5]. The production rate of silver carp has been reduced due to various disease-causing agents. Mass mortalities and poor growth are mostly caused by several bacterial illnesses in combination with environmental conditions, which have an impact on this fish's production and marketability[6]. The common pathogens of silver carp include bacteria, viruses, fungi and protozoans. The diseases caused by bacteria in silver carp are commonly tail and fin rot, septicaemia, columnaris and gills disease. Several bacterial species: *Plesiomonas shigelloides*, *Aeromonas* spp., *Vibrio* spp., *Pseudomonas* spp., *Kurthia gibsonii* and *Edwardsiella tarda* have been found to be involved in diseases of silver carp.

Freshwater fish are susceptible to bacterial infection known as tail and fin rot disease. It exists in both tropical and arctic climates. Skin erosion, grayish-white lesions on the body and rotting are signs of infection. The majority of fish species are susceptible to this potentially lethal sickness[7]. Although, no systematic investigations have been carried out to determine the precise pathogen causing the disease. It is believed that the infestation of a disease similar to tail and fin rot has grown in many fish species in Bangladesh in recent decades.

Diseases are a crucial factor thwarting the development and sustainability of aquaculture. Diverse fish diseases that pose serious dangers to the aquaculture industry have impeded the economic and social growth of aquaculture markets[6]. Identification of the pathogens behind those diseases is highly requisite to take preventive measures and screen the therapeutics against those pathogens. Since silver carps are important to Bangladesh's aquaculture production, the goal of this research is to isolate and identify the pathogenic bacteria that cause silver carp tail and fin disease in Bangladesh as well as to evaluate these bacteria's sensitivity to antibiotics. Furthermore, another primary issue in aquaculture is the emergence of Antimicrobial Resistance (AMR) in cultivated fishes. Because of the high prevalence of bacterial infections in fish, antibiotics are frequently used and thus persist in aquatic habitats, causing the spread of microorganism's resistant to antibiotics. Therefore, diseased fish samples were collected and then isolated bacteria were subjected to different biochemical tests for their identification and finally confirmed by DNA sequencing. Then the tests for antibiotic sensitivity have also been carried out to create a profile of those organisms' antibiotic resistance and to identify the best antibiotics to combat it.

MATERIALS AND METHODS

Diseased silver carp were collected to examine the bacterial pathogen responsible for the disease. The study was conducted from July, 2017 to June, 2018. The steps to identify the pathogen were as follows:

Ethics statement: The Cruelty to Animals Act of 1920, Act No. I of 1920 of the Government of the People's Republic of Bangladesh, was followed when conducting this study in terms of animal handling. The Sylhet Agricultural University Ethics Committee in Bangladesh approved this work.

Fig. 1: Diseased silver carp (*Hypophthalmichthys molitrix*)

Collection of fish samples: This study focused on silver carp (*Hypophthalmichthys molitrix*) with tail and fin rot infection. Twenty five infected fish (Fig. 1) were collected from several cultivated ponds in Sylhet, Bangladesh. The samples were then transported to the Laboratory of Animal and Fish Biotechnology, Sylhet Agricultural University, Bangladesh, in a sterile plastic container with a maintained temperature for bacteriological analysis. Measures were also taken to avoid touch during the collection process.

Sample preparation and isolation of bacteria: The fish were given a 50 1/L dosage of clove oil to make them unconscious. Before being dissected, the fish were washed with alcohol. The diseased parts of the collected samples were isolated with a sterilized scalpel and minced appropriately and grinded well. A small portion (approximately 10 gm) of them was taken in an LB (Luria-Bertani) liquid medium and mixed bacterial cultures were grown. Then, cultivated samples were serially diluted up to 10^{-4} before being poured into sterile petri-plates with nutritional agar using one mL of the diluted bacterial cell suspension. For 48 hrs, the inoculation plates were left incubated at 37°C. Pure cultures were then cultivated on nutrient agar (NA) plates supplemented with peptone 1 gm/100 mL, yeast extract 0.5 gm/100 mL and agar 2 gm/100 mL. The plates were kept for incubation at 37°C for 24 hrs before single colonies with varying morphological characteristics were picked for further investigation.

Morphology and biochemical characterization: Biochemical tests in microbiology are a set of chemical tests used to identify microorganisms in a sample, these microorganisms are typically bacteria. The selection of these tests, however, is based on preliminary findings, such as the Gram staining pattern and growth traits, which allow the bacteria to be classified. Both solid and liquid LB media were used to inoculate the isolates. After 24 hrs of incubation at 28°C, the isolates' colony morphologies on the LB medium were examined and the patterns of growth in both kinds of media were observed as shown in Table 1. Gram staining and several biochemical tests such as MacConkey agar, oxidase, catalase, KOH, sulfide indole motility (SIM), triple sugar iron (TSI), Simmons citrate, Kligler Iron agar, Urea Hydrolysis and Methyl Red were performed to primarily identify the isolates that were enlisted in Table 2[8]. The results were then compared with those of biochemical assays given in Bergey's protocol[9].

Molecular identification:

- **DNA extraction:** Using the easy pure bacterial genomic DNA kit (TransGen, China), bacterial genomic DNA was isolated from isolates by the manufacturer's instructions.
- **DNA amplification:** To identify microorganisms at the genus, family and species categories, 16S rDNA has reportedly been frequently utilized. Therefore, universal primers, primer 27 F (Forward primer: 5'-AGAGTTTGATCMTGGCTCAG-3) and primer 1492 R (Reverse primer: 3'-GGTTACCTTGTTACGACTT-5') were used to amplify the 16S rDNA gene of the isolates[10]. The PCRs were conducted with a 20 µL reaction volume with the use of a thermal cycler. Promega Taq Polymerase was used to amplify the DNA that can amplify 1000 bases per min and our targeted PCR product size was around 1465 bases. Therefore, a minimum extension time of 90 sec was required as the extension time is directly proportional to product size. The PCR procedure involved a 3 min preheating period at 95°C, 35 cycles of 95°C for 30 sec, 48°C for 30 sec and 72°C for 1 min and a final extension period of 10 min at 72°C.
- **Quantification and purification:** Concentration and the quality of the PCR products were then analyzed with the aid of gel electrophoresis and visualized under a UV trans-illuminator (Analytik Jena, Germany)[11]. Another step involved using a PCR purification kit to clean up the PCR products.
- **Sequencing of 16S rRNA:** The 16S rRNA sequences were analyzed using the single capillary ABI 3100 DNA analyzer after the reaction mixture had been purified. The 10 µL of supernatant were extracted, placed on a transparent plate, coated with a septa mat and then put in the sequencing device. The Chromas Software Version 2.5.1 was used to read the chromatogram of the raw sequence files.

Antibiotic profiling: Each of the identified bacteria was tested for antibiotic sensitivity using the agar well diffusion method. The resistance profile of the isolated bacteria was checked against 15 commonly used antibiotics. After the overnight-grown bacteria were adjusted to 0.5 McFarland turbidity standards, 100 µL of the broth cultures were equally dispersed on Mueller-Hinton agar plates. Agar plates with inoculations were then provided with antibiotic discs (HiMedia, India), which were then incubated at 37°C for 24 hrs after being air-dried for 10 min. After incubation, clear zones indicated that the bacteria's development had been stopped. Depending on the diameter of the zone of inhibition, antibiotic sensitivity against each bacterium was evaluated as resistant (R), sensitive (S) or intermediate (I). An isolate was considered an MDR when it went resistant against at least three groups of antibiotics according to the guideline of the Clinical and Laboratory Standards Institute, CLSI (2021) and European Committee on Antimicrobial Susceptibility Testing, EUCAST (2021)[12].

Phylogenetic analysis: The pairwise sequence alignment tool, Basic Local Alignment Search Software (BLAST) was used to retrieve closely related sequences from the NCBI database. The MEGA-11 software was then utilized to perform multiple sequence alignment using CLUSTAL-O[13] and MUSCLE[14] and a FASTA file was created for further investigation.

The phylogenetic tree was constructed using the MEGA-11 software's maximum likelihood (ML) algorithm employing aligned sequences[15]. Moreover, the analysis of 1000 bootstrapped input datasets was used to verify the phylogenetic trees. Finally, the built tree was visualized and edited in the iTOL (The Interactive Tree of Life) server[16].

RESULTS

Isolation of the bacteria: Several different bacterial colonies were observed on LB agar media after incubation of pure culture at 37°C for 48 hrs. Depending on the features of colony morphologies, three different colonies were selected for further analysis and they were then labeled as S1, S2 and S3. The results showed that the colonies morphology of these isolated strains (S 1-3) were >2>1 and >1 mm in sizes, entire, entire, erratic in margins, white, yellow and white in color, smooth, smooth and rough in surface, sticky, non-sticky and non-sticky in consistency, respectively (Table 1 and Fig. 2(a-c), respectively).

Table 1: Morphological features of the isolated colonies

Characteristics	S1	S2	S3
Size	>2 mm	>1 mm	>2 mm
Colony shape	Round	Round	Erratic
Color	White	Yellow	White
Margin	Entire	Entire	Erratic
Surface	Smooth	Smooth	Rough
Opacity	Opaque	Opaque	Opaque
Consistency	Sticky	Non-sticky	Non-sticky

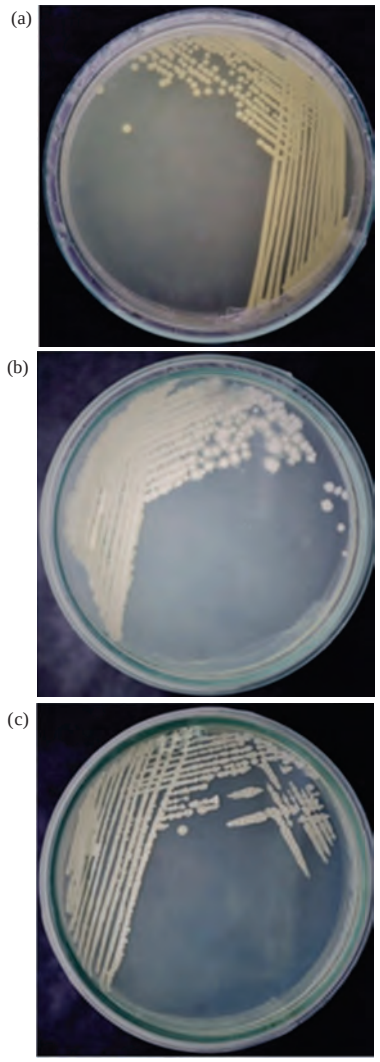

Fig. 2(a-c): Isolated bacteria on nutrient agar media, (a) S1, (b) S2 and (c) S3

Cellular morphology: The microscopic observation of isolated bacterial pathogens revealed that out of the three bacterial isolates, bacterium S1 (Fig. 3a) and S3 (Fig. 3c) were Gram-positive (+ve) coccoids while bacterium S2 was Gram-negative (-ve) rod-shaped (Fig. 3b). However, all the bacterial strains were found to be non-motile in nature (Fig. 3).

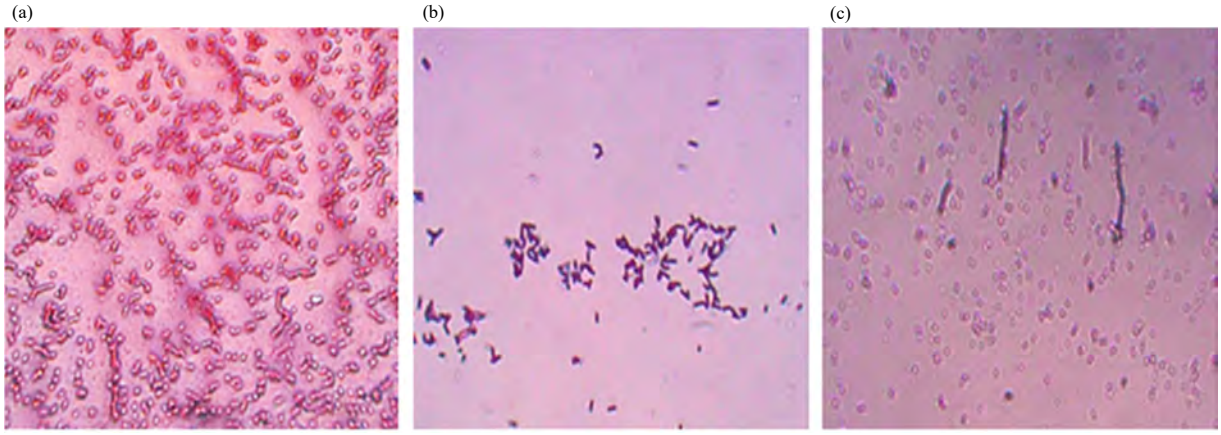

Fig. 3(a-c): Microscopic view of, (a) S1, (b) S2 and (c) S3

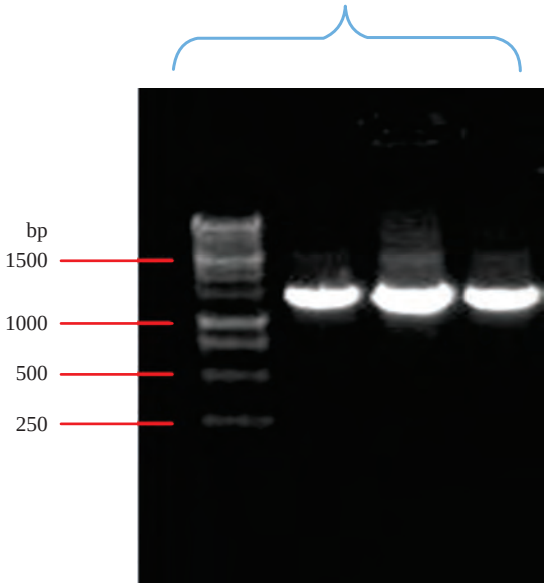

Fig. 4: Results of the isolates S1, S2 and S3's 16s rRNA on agarose gel electrophoresis

Table 2: Biochemical tests for the isolated strains

Name of test		S1	S2	S3
Oxidase test		-T	-T	-
Catalase test		+T	+T	+
KOH test		+	-	-
Triple sugar iron (TSI) agar test	Gas production	-	-X	-
	H_2S production	-	-T	-
	Sugar fermentation	+	-	-
Sulfide indole motility (SIM) test	Sulfide	-	-	-
	Indole	-	-T	-
	Motility	+	-T	-
Simmon citrate utilization test		+	-X	-
MacConkey test		+	+	-
Kligler Iron test		+	-	-
Methyl red test		+	-T	-
Urease test		+	-X	-
Interpretations		*Kurthia* spp.	*Klebsiella* spp.	*Bacillus* spp.

A positive reaction is denoted by (+) sign, while negative reaction is denoted by (-) sign

Table 3: Summary of antibiotic sensitivity test

Antibiotic discs	Disc concentration	Zone of inhibition (mm) (sensitivity)		
		Kurthia gibsonii	*Klebsiella pneumoniae*	*Bacillus subtilis*
Tetracycline	30 µg	12 (R)	16 (S)	13 (R)
Oxytetracycline	30 µg	14 (R)	16 (S)	8.5 (R)
Cefixime	5 µg	14 (R)	26 (S)	34 (S)
Cefotaxime	30 µg	18 (S)	28 (S)	40 (S)
Nalidixic acid	30 µg	12 (R)	14 (R)	16 (S)
Kanamycin	30 µg	16 (S)	18 (S)	26 (S)
Vancomycin	30 µg	-	12.5 (R)	18 (S)
Doxycycline	30 µg	12 (R)	15 (I)	18 (S)
Neomycin	30 µg	11 (R)	14 (R)	22 (S)
Gentamycin	10 µg	12 (R)	15 (I)	25 (S)
Erythromycin	15 µg	5 (R)	20 (S)	31 (S)
Sulfonamide	300 IU	6 (R)	22 (S)	18 (S)
Amoxicillin	30 µg	-	18 (S)	15 (I)
Penicillin-G	10 µg	-	27 (S)	17 (S)
Rifampicin	5 µg	-	20 (S)	18 (S)

R: Resistant, S: Sensitive and I: Intermediate

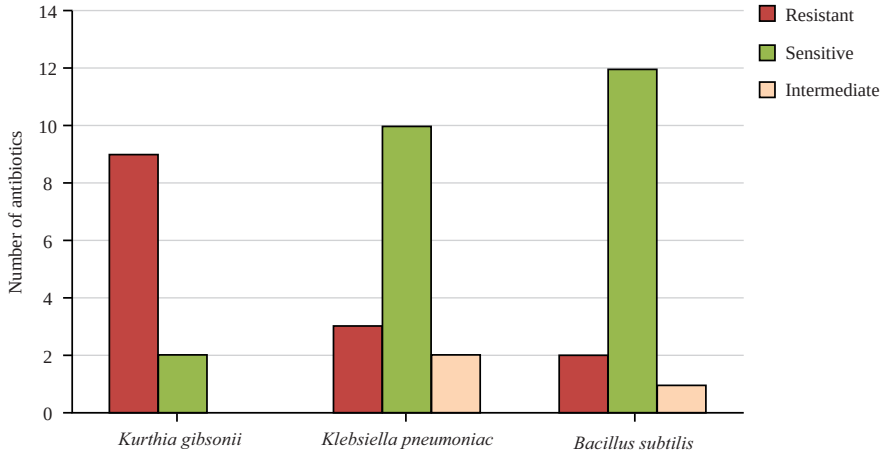

Fig. 5: Antibiogram profile of isolated strains

Biochemical identification of the isolates: Different biochemical tests were carried out on the isolates and the results were compared with those of biochemical assays given in Bergey's protocol. The majority of the isolate's traits were discovered to be similar to those of the species described in Bergey's guide. Results of biochemical tests for the isolated bacterial strains were enlisted in Table 2.

Molecular confirmation of the isolated strains: Following the extraction of the genomic DNA from all 3 isolated bacteria, the 16S rRNA gene was amplified, yielding approximately 1450 bp of DNA for each isolate using forward and reverse primers (Fig. 4). These amplified DNA segments were then subjected to DNA sequencing and then performed BLAST in NCBI to detect the bacterial isolates. The results revealed that the isolated DNA fragment from S1 was 1423 bp long (GenBank Accession Number: MN658386), S2 DNA contains 1407 bp (MN658387) and S3 fragment has 1411 bp (MN658388) (Fig. 4). Finally, the results from BLAST analysis confirmed the isolated strains belong to the species *Kurthia gibsonii*, *Klebsiella pneumoniae* and *Bacillus subtilis*, respectively.

Antibiotic susceptibility of isolated bacterial strains: In this study, the patterns of susceptibility of the three isolated bacteria to 15 different antibiotics were observed by following the disk diffusion method. The area of inhibition was evaluated by comparing it to CLSI (Clinical and Laboratory Standards Institute) standard. The result showed that all three isolates were sensitive to Cefotaxime and kanamycin three isolates have the highest sensitivity to cefotaxime (Table 3 and Fig. 5).

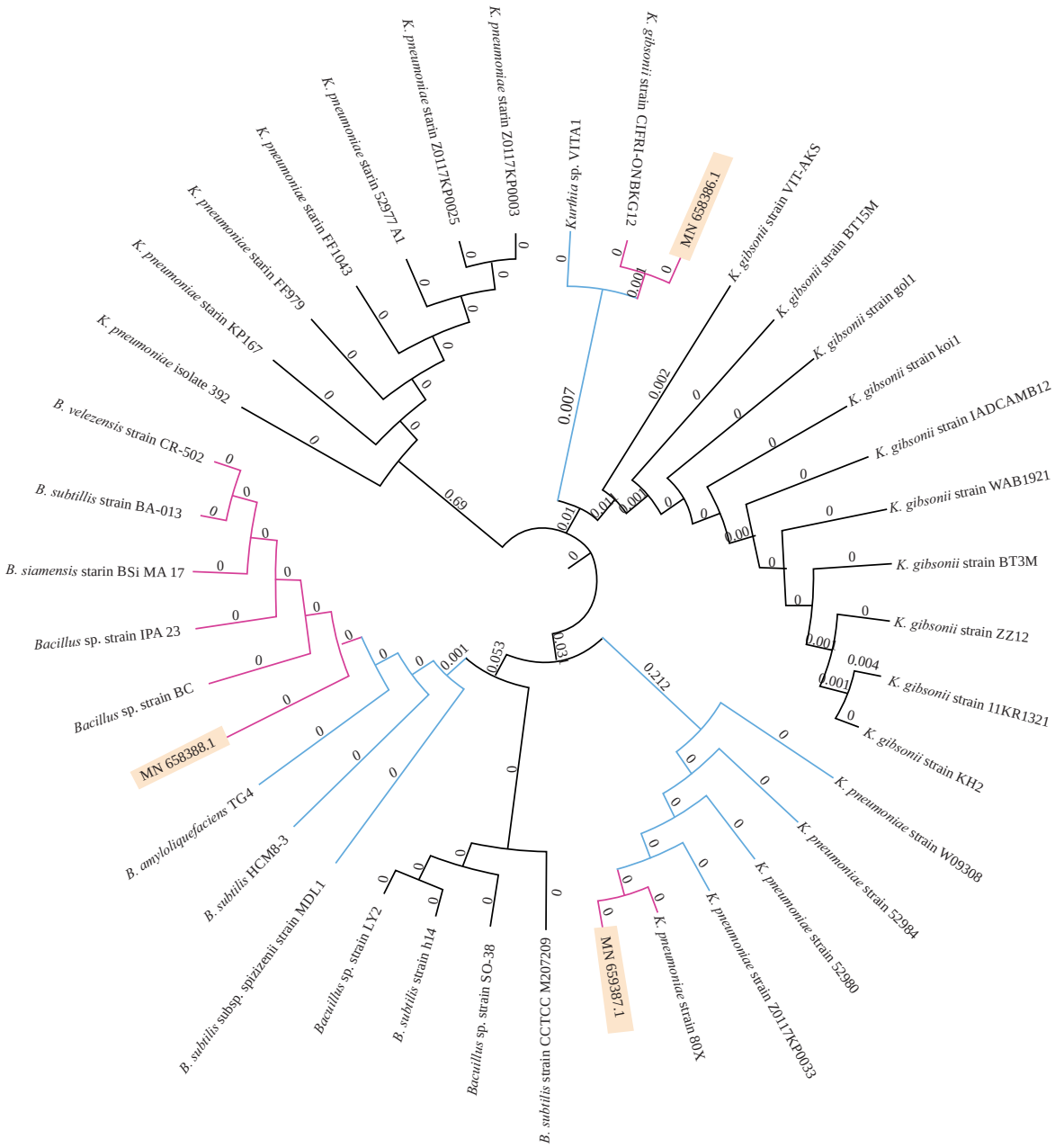

Fig. 6: Phylogenetic study of bacterial isolates, position of the isolated strains within the tree is highlighted by a red mark

Phylogenetic analysis: Homologous sequences of *Kurthia gibsonii* (supplementary file 1), *Klebsiella pneumoniae* (supplementary file 2) and *Bacillus subtilis* (supplementary file 3) were collected after the BLAST was done that were then aligned in the MEGA-11 software and used for building the tree.

The phylogenetic tree distance coefficient ranged from 0.0 to 0.69 as a whole where the efficiency for *Kurthia* was between 0 and 0.1 and 0.0 to 0.69 for *Klebsiella* and 0.0 to 0.53 for *Bacillus subtilis*. The whole tree contained 3 major clades and interestingly some of the *Klebsiella pneumoniae* strains including our isolated one showed more similarity with *Bacillus* species and lay closely with *Bacillus* species in the same clades (Fig. 6).

DISCUSSION

The biochemical test of this study provided a presumption that the isolate strains belong to the genus *Kurthia*, *Klebsiella* and *Bacillus*. The amplification and

sequence analysis of the 16S rRNA gene is a specific and effective method for determining diverse bacterial isolates, providing validation at the species and genus level and thereby identifying unique to biochemical characterization[17]. In general, all strains' 16S rRNA gene sequences have more than 97% (the minimum for genus level similarity) and equivalent to 99% (the minimum for species level similarity) homology with known sequences, which is widely recognized as the optimal threshold for identifying bacteria[18-20]. The isolated strains in this study shared more than 99% homology with previously published 16S rRNA gene sequences of *Kurthia gibsonii*, *Klebsiella pneumoniae* and *Bacillus subtilis*, revealing that the identification is accurate. Thus, the molecular identification confirmed the species level of the identified strains and the isolated strains were *Kurthia gibsonii*, *Klebsiella pneumoniae* and *Bacillus subtilis*, respectively.

Aeromonas and *Pseudomonas* are prevalent bacterial pathogens isolated from diseased fish around the world, including Bangladesh. *Aeromonas hydrophila, Aeromonas salmonicida, Flexibacter columnaris* and *Pseudomonas* spp. were isolated from diseased fish by Nusbaum and Shotts[21]. In a study with *Oreochromis niloticus*, *Aeromonas sobria* was found associated with fin and rot disease[22]. Haldar *et al.*[23] confirmed the presence of *Vibrio harveyi* in tail and fin rot disease in sea bream (*Sparus aurata*) in a hatchery environment in Malta. These results were indicating the presence of several different types of bacterial species as the causative agents depending on locations and fish types.

Earlier, Hemamalini *et al.*[24] revealed the presence of *Kurthia gibsonii* and *Bacillus subtilis* in the infected ornamental fish Guppy (*Poecilia reticulata*) and Molly (*P. sphenops*). The potential involvement of the enteric organism, *K. pneumoniae*, on the other hand, was unique to fish pathology until its presence was confirmed by Daskalov *et al.*[25] when they worked with diseased tails as well as fins of rainbow trout. Certainly, the organism is a common inhabitant of polluted freshwater which could be the infection's reservoir[26]. Nonetheless, a growing number of Enterobacteriaceae members, including *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Hufnia*, *Providencia*, *Salmonella*, *Serratia* and *Yersinia*, have been identified as fish pathogens. As a result, the appearance of *Klebsiella* should not come as a surprise. It is possible that the organisms were previously misidentified, as there has been a tendency to associate the causative agents of fin and/or tail rot with extremely broad taxonomic groups[27].

One of the most serious threats to aquaculture systems is antimicrobial resistance. *Kurthia gibsonii* exhibited the highest percentages of resistance cases at 81% (resistant against 9 out of 11 antibiotics) while the percentages for *Klebsiella pneumoniae* and *Bacillus subtilis* were very low at 20 and 13%, respectively. The latter two strains were sensitive to most of the tested antibiotics i.e. 67% for *K. pneumoniae* and 80% for *B. subtilis* similar to the finding of Kathleen *et al.*[28]. *Klebsiella pneumoniae*, *Bacillus subtilis* exhibited similar results to Kathleen *et al.*[28] against nalidixic acid, kanamycin, doxycycline, gentamycin, erythromycin, penicillin-g and rifampicin. However, the results of *Kurthia gibsonii* are threatening as it showed resistance to 9 antibiotics while sensitivity was found against only cefotaxime and kanamycin and a similar result was found by Preena *et al.*[29]. Antibiotic resistance highlights the importance of suitable surveillance and constant monitoring programs in fish farms, as well as the use of other effective alternatives.

The study lacks confirmation of the association of the identified bacteria. Therefore, future studies should focus on inoculating the isolated bacteria on healthy fish and confirming their pathogenicity. However, the findings from the study will help to manage the cultural conditions and to select the proper treatment options. Before choosing an antibiotic for treatment, considerable thought should be given to preventing the development of antibiotic resistance. Additionally, to ensure the maximum yields and the best quality of the products, illness prevention of various indigenous fishes should be carried out by using superior cultural methods and health management.

CONCLUSION

The study has revealed that *Kurthia gibsonii, Klebsiella pneumoniae* and *Bacillus subtilis* are associated with the tail and fin rot disease of silver carp of Bangladesh. However, the concerning matter is that the associated bacteria have developed resistance against some commonly used antibiotics and also developed resistance against other antibiotics. Investigations of these pathogens in inland open waters like wetlands and lakes will facilitate pathogenesis and pathology management of the disease outbreak in aquatic habitats. Additionally, the antibiotics susceptibility test and phylogenetic studies may aid in the creation of therapeutics for such pathogens.

SIGNIFICANCE STATEMENT

This study helped to identify the pathogens responsible for tail and fin rot diseased silver carp in the local area. This information provides a baseline for future assessment and is crucial for the management of fish disease in the nation. Furthermore, the detection and antibiogram profiling of isolated bacteria from the diseased fish will help to identify proper treatment options for this disease.

REFERENCES

1. Carr, R.M., U.J. Blumenthal and D.D. Mara, 2004. Guidelines for the safe use of wastewater in agriculture: Revisiting WHO guidelines. Water Sci. Technol., 50: 31-38.
2. Pridgeon, J.W. and P.H. Klesius, 2012. Major bacterial diseases in aquaculture and their vaccine development. CABI Rev., Vol. 2012. 10.1079/PAVSNNR20127048.
3. Tanjung, C.F., 2023. Bacterial fish diseases: Environmental and economic constraints. 2022. Edited by Gowhar Hamid Dar, Rouf Ahmad Bhat, Humaira Qadri, Khalid M. Al-Ghamdi, Khalid Rehman Hakeem. Academic Press, Elsevier Inc. 460 pages. ISBN: 978-0-323-85624-9 (Paperback). J. Fish Dis., 46: 707-708.
4. Guo, L., Q. Wang, P. Xie, M. Tao, J. Zhang, Y. Niu and Z. Ma, 2015. A non-classical biomanipulation experiment in Gonghu Bay of Lake Taihu: Control of blooms using silver and bighead carp. Aquacult. Res., 46: 2211-2224.
5. Mishra, S.S., R. Das, P. Choudhary, J. Debbarma and S.N. Sahoo *et al*., 2018. Present status of fisheries and impact of emerging diseases of fish and shellfish in indian aquaculture. J. Aquat. Res. Mar. Sci., 1: 5-26.
6. Bagum, N., M.S. Monir and M.H. Khan, 2013. Present status of fish diseases and economic losses due to incidence of disease in rural freshwater aquaculture of Bangladesh. J. Innovation Dev. Strategy, 7: 48-53.
7. Frerichs, G.N. and R.J. Roberts, 1989. The Bacteriology of Teleosts. In: Fish Pathology, Roberts, R.J. (Ed.). Bailliere Tindall, London, pp: 289-291.
8. Krovacek, K., L.M. Eriksson, C. Gonzalez-Rey, J. Rosinsky and I. Ciznar, 2000. Isolation, biochemical and serological characterisation of *Plesiomonas shigelloides* from freshwater in Northern Europe. Comp. Immununol. Microbiol. Infect. Dis., 23: 45-51.
9. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Willams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams & Wilkins, Philadelphia, ISBN-10: 9780683006032, Pages: 787.
10. Behera, B.K., P. Paria, A. Das, S. Bhowmick, A.K. Sahoo and B.K. Das, 2017. Molecular characterization and pathogenicity of a virulent *Acinetobacter baumannii* associated with mortality of farmed Indian major carp *Labeo rohita* (Hamilton 1822). Aquaculture, 471: 157-162.
11. Clsi, 2021. M100: Performance Standards for Antimicrobial Susceptability Testing. Clinical & Laboratory Standards Institute, Wayne, ISBN: 9781684401048, Pages: 352.
12. Das, A., S. Acharya, B.K. Behera, P. Paria, S. Bhowmick, P.K. Parida and B.K. Das, 2018. Isolation, identification and characterization of *Klebsiella pneumoniae* from infected farmed Indian major carp *Labeo rohita* (Hamilton 1822) in West Bengal, India. Aquaculture, 482: 111-116.
13. Sievers, F., A. Wilm, D. Dineen, T.J. Gibson and K. Karplus *et al*., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol., 7: 539-544.
14. Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res., 32: 1792-1797.
15. Tamura, K., 2000. On the estimation of the rate of nucleotide substitution for the control region of human mitochondrial DNA. Gene, 259: 189-197.
16. Letunic, I. and P. Bork, 2019. Interactive Tree Of Life (iTOL) v4: Recent updates and new developments. Nucleic Acids Res., 47: W256-W259.
17. Nisha, R.G., V. Rajathi, R. Manikandan and N.M. Prabhu, 2014. Isolation of *Plesiomonas shigelloides* from infected cichlid fishes using 16s rRNA characterization and its control with probiotic sp. Acta Sci. Vet., Vol. 42.
18. Stackebrandt, E., W. Frederiksen, G.M. Garrity, P.A.D. Grimont and P. Kampfer *et al*., 2002. Report of the *ad hoc* committee for the re-evaluation of the species definition in bacteriology. Int. J. Syst. Evol. Microbiol., 52: 1043-1047.
19. Harris, K.A. and J.C. Hartley, 2003. Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. J. Med. Microbiol., 52: 685-691.
20. Clarridge, J.E., 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin. Microbiol. Rev., 17: 840-862.
21. Nusbaum, K.E. and E.B. Shotts, 1981. Action of selected antibiotics on four common bacteria associated with diseases of fish. J. Fish Dis., 4: 397-404.
22. Li, Y. and S.H. Cai, 2011. Identification and pathogenicity of *Aeromonas sobria* on tail-rot disease in Juvenile Tilapia *Oreochromis niloticus*. Curr. Microbiol., 62: 623-627.

23. Haldar, S., A. Maharajan, S. Chatterjee, S.A. Hunter and N. Chowdhury *et al*., 2010. Identification of *Vibrio harveyi* as a causative bacterium for a tail rot disease of sea bream *Sparus aurata* from research hatchery in Malta. Microbiol. Res., 165: 639-648.
24. Hemamalini, N., S.A. Shanmugam, A. Kathirvelpandian, A. Deepak, V. Kaliyamurthi, E. Suresh and S. Ezhilmathi, 2022. Prevalence, antimicrobial resistance and resistance gene cassettes detection in bacterial pathogens isolated from freshwater ornamental fishes. Indian J. Anim. Res., 10.18805/IJAR.B-4903.
25. Daskalov, H., M. Stobie and B. Austin, 1998. *Klebsiella pneumoniae*: A pathogen of rainbow trout (*Oncorhynchus mykiss*, Walbaum)? Bull. Eur. Assoc. Fish Pathol., Vol. 18.
26. Allen, D.A, B. Austin and R.R. Colwell, 1983. Numerical taxonomy of bacterial isolates associated with a freshwater fishery. J. Gen. Microbiol., 129: 2043-2062.
27. Austin, B. and D.A. Austin, 1993. Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. Taylor and Francis, United Kingdom, ISBN-13: 9780130594945, Pages: 356.
28. Kathleen, M.M., L. Samuel, C. Felecia, E.L. Reagan, A. Kasing, M. Lesley and S.C. Toh, 2016. Antibiotic resistance of diverse bacteria from aquaculture in Borneo. Int. J. Microbiol., Vol. 2016. 10.1155/2016/2164761.
29. Preena, P.G., A. Dharmaratnam, N.S. Raj, T.V.A. Kumar, S.A. Raja and T.R. Swaminathan, 2019. Antibiotic susceptibility pattern of bacteria isolated from freshwater ornamental fish, guppy showing bacterial disease. Biologia, 74: 1055-1062.