

## DECLARATION

I, Preeti Mangar, hereby declare that the work embodied in my thesis entitled **“Studies on the occurrence of antibiotic resistance and virulence in motile *Aeromonas* species from fish farming environments in sub-Himalayan West Bengal”** has been carried out by me under the supervision of Dr. Aniruddha Saha, Professor, Department of Botany, University of North Bengal for the award of the Degree of Doctor of Philosophy in Botany. I also declare that, this thesis or any part thereof has not been submitted for any other degree/diploma either to this or any other university.

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### TO WHOM IT MAY CONCERN

This is to certify that Mrs. Preeti Mangar, M.Sc. has worked under my supervision at the Department of Botany, University of North Bengal for Ph.D. thesis entitled "**Studies on the occurrence of antibiotic resistance and virulence in motile *Aeromonas* species from fish farming environments in sub-Himalayan West Bengal**". I am forwarding her thesis for the Ph. D. Degree (Science) in Botany of the University of North Bengal. I recommend that she has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in her thesis.

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# Acknowledgement

*First and foremost, I would like to express my sincere gratitude to my supervisor Dr. Aniruddha Saha, Professor, Department of Botany, University of North Bengal for his invaluable advice, continuous support, motivation, and immense knowledge and patience during my PhD study. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study.*

*In addition, I would like to mention Dr. Dipanwita Saha, Professor, Department of Biotechnology, University of North Bengal to guide me well throughout the research work from title's selection to interpreting the results. Her vast knowledge, unwavering support and remarkable patience has given me more power and spirit to excel in the research writing. Conducting the academic study regarding such a difficult topic couldn't be as simple as she made this for me. She has given me much food for thought, which helped in making improvements to the final version of this thesis. She is my mentor and a better advisor for my doctorate study beyond the imagination.*

*It has been an amazing experience working between two research groups across different departments. I would like to express my heartiest gratitude to Dr. M. Chowdhury, Head, Department of Botany, University of North Bengal, Prof. A. Sen, Prof. S.C. Roy, Late Dr. P. Mandal, Dr. J. B. Bhandari, Dr. S. Roy and Dr. P. Mathur, the faculties of our department for their valuable advice and encouragement throughout the course of this work. My thanks also go for all the non-teaching staff of the Department of Botany for their various types of assistance.*

*I want to give my deepest appreciation to Dr. Anoop Kumar, Head, Department of Biotechnology, University of North Bengal, Prof. Ranadhir Chakraborty, Prof. Shilpi Ghosh and Dr. Manab Deb Adhikary, the faculties of Department of Biotechnology for their valuable advice and encouragement throughout the course of this work. My thanks also go for all the non-teaching staff of the Department of Biotechnology for their various kinds of assistance.*

*I would always remember my fellow lab mates from Department of Biotechnology, Ms Smriti Pradhan, Ms Sushmita Das, Ms Enakshi Sadhu, Mr Abhinandan Chowdhury, Ms Dipanwita Ghosh and Mrs Khushboo Lepcha, Mrs Vijeta Rai for the fun-time we spent together, tiresome days that gave us the courage to complete tasks before deadlines and for stimulating the discussions and for being a great bunch of people in and out of the lab. I would also like to thank my Seniors Dr. Gargee Dhar Purkayastha, Dr. Ramashish Kumar, Dr. Sima Mondal, Ms Anindita Chakraborty, Dr. Bhusan Gurung for their practical guidance and futuristic knowledge during initial days of my research work. I would also*

like to thank dissertation students Partha Barman, Cessna Bhattacharya, Aindrila Mazumdar and Monisha Dey for their contributions.

I would also like to extend my thanks to the Research scholars, Department of Botany, University of North Bengal Dr. Shibu Das, Dr. Arnab Saha, Dr. Prosenjit Chakraborty, Mr. Arup Karmakar, Ms. Tanushree Sarkar, Dr. Hrisikesh Mandal, Dr. Bikram Saha, Mrs. Piyali Sarkar, Mr. Asit Ray, Ms. Suyojna Tamang, Mr. Praveen Mandal, Mr. Biswajit Paul, Ms. Ankita Roy, Mrs. Ritabrita Saha, Mr. Kalyan Roy and Mr. Subhrajyoti Saha for their continuous support in various aspect of my research work.

I am very thankful to Ms Aditi Rai Research Scholar taking care of me and offering me invaluable advice that will benefit me throughout my life. I would also like to extend my heartfelt gratitude to Dr. Vivek Kumar Ranjan for helping me understand the practical aspects of certain critical issues in my research.

I am grateful to my parents Mr Bel Kumar Mangar and Mrs Kumari Mangar, my siblings Sandhya Mangar and Minu Mangar, I consider myself nothing without them. They gave me enough moral support, encouragement and motivation to accomplish the personal goals. My two lifelines (parents) have always supported me so that I could completely focus on my studies and achieve my objectives without any obstacles on the way. I would also like to thank my extended family members, my brother in laws, Mr. Ranjit Chettri and Mr. Arbind Allay for giving me positive advice that helped me in my voyage. I would also like thank youngest member Oshin Allay for her innocence acts which always energized me with positive feelings.

I would also like to mention my husband Hansen Agnelo Vaz for his contribution throughout this journey and always being there by my side and also not forgetting my mother-in-law Mrs. Lynette Vaz for her constant encouragement and the support. I would also like to mention Mr Jane Alam Khan for his constant medical advice and support whenever required.

My friends, Mrs. Amina Sultana, Mr. Abdul Hamid, Mrs. Sandhani Saikia and Mrs. Chandni Sharma who were family like friends and acquaintances who remembered me in their prayers for the ultimate success.

Last, I must thank University Grants Commission (UGC) for providing financial support in the form of a research fellowship (UGC-BSR) during the tenure of my work.

*Preeti Mangar*

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Dated: 06.12.2022

### Document Information

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Analyzed document	Preeti Mangar_Botany.pdf (D150515242)
Submitted	2022-11-22 07:23:00
Submitted by	University of North Bengal
Submitter email	nbuplg@nbu.ac.in
Similarity	0%
Analysis address	nbuplg.nbu@analysis.arkund.com

### Sources included in the report

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<b>W</b>	URL: <a href="https://www.researchgate.net/publication/11464938_Aeromonas_Species_in_Foods">https://www.researchgate.net/publication/11464938_Aeromonas_Species_in_Foods</a> Fetched: 2021-04-06 07:40:44	 5

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# *Preface*

With the ever-increasing world population and increasing food requirements, fisheries and aquaculture serve to fulfil the nutritional demand of the world. Apart from this, aquaculture provides alternative avenues for employment and livelihood for a considerable proportion of the world population. The world has witnessed a boost in the aquaculture produce by a surprising scale up production by 527% between 1990-2018. Indeed, countries have formulated policies and strategies for growth and management of fisheries to increase their output. Aquaculture rather than captivity of wild fish contributes significantly to the overall output. India is among the leading producer of fish and occupies the second position after China in world fish production. However, fish diseases with numerous causative agents around the world affects both quality and yield of fish produce.

In India, as in many other nations around the world, disease is a major impediment to aquaculture and a limiting factor for economic as well as socio-economic growth. Not only the livelihood of the fish farmers is impacted, but fish disease also stunts the potential growth of this sector and affects the consumers. The present-day diseases are mainly due to intensification of culture without proper knowledge on the equilibrium of host, pathogen and its environment. In India, a large proportion of the rural population is dependent on traditional and indigenous farming methods. The size of fish farms, fish seed source, stocking density, type of fish species, nursery management, pond preparation, frequency of harvesting, awareness of chosen methods and fish diseases, management, etc. all have significant roles in fish production. With very less availability of resources and minimal understanding on fish health management and controlling disease outbreaks, rural farmers incur heavy economic losses due to yield drop.

Among the fish diseases, bacterial diseases are quite common and difficult to control. Generally, the bacteria are saprophytic and may become pathogenic under physiological imbalance, nutritional deficiency and other stressful

conditions in the host. Often fish eggs, fry and fingerlings develop bacterial infections and cause mortality. The prevalence of epizootic ulcerative syndrome and motile aeromonad septicemia in a number of Southeast Asian nations as well as in India has drawn a great deal of attention to the threat that disease epidemics pose to farmers. In this context, the relentless use of antibiotics to control the diseases without proper knowledge on the hazards of excess antibiotic usage has come to forefront. Widespread use antibiotics leads to development of antibiotic resistance in pathogens, raises residual content, and unnecessarily exposes non-target organisms to high antibiotic doses which may pose health risks for aquatic life. The disease-causing bacteria employ a subset of factors responsible for virulence and host establishment. Therefore, understanding the molecular makeup of bacterial pathogens may provide a proper insight and help in development of appropriate public health programmes.

In this light, the present study focuses on the isolation of a pathogenic bacterium *Aeromonas* sp. from fish farming environments of sub-Himalayan West Bengal. The history of fish disease outbreaks in this region have been commonly associated with this pathogen. The virulence markers and its phenotypic expression was studied along with the antibiotic resistance that exists among the aeromonads. A detailed review and the methods followed and the supporting results have been documented in this study. However, the status of aeromonads infecting fishes and also the antibiotic resistance among pathogens in aquatic reservoirs need constant surveillance from time to time.

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# Abbreviations

<b>°C</b>	Degree Celsius	<b>MgCl<sub>2</sub></b>	Magnesium chloride
<b>µg</b>	Microgram	<b>MHA</b>	Mueller Hinton Agar
<b>µl</b>	Microlitre	<b>mins</b>	Minutes
<b>µm</b>	Micrometre	<b>ml</b>	Millilitre
<b>AIM</b>	<i>Aeromonas</i> isolation medium	<b>mM</b>	Millimole
<b>BLAST</b>	Basic local alignment search tool	<b>mm</b>	Millimetre
<b>BLAST<sub>n</sub></b>	Nucleotide BLAST	<b>NA</b>	Nutrient agar
<b>BLAST<sub>p</sub></b>	Protein BLAST	<b>NaCl</b>	Sodium chloride
<b>bp</b>	Base pair	<b>NCBI</b>	National Centre for Biotechnology Information
<b>CaCl<sub>2</sub></b>	Calcium chloride	<b>ng</b>	Nanogram
<b>CAS</b>	Chromazurol sulfonate	<b>nm</b>	Nanometre
<b>CFU</b>	Colony forming units	<b>O.D.</b>	Optical density
<b>cm</b>	Centimetre	<b>OD<sub>260</sub></b>	Absorbance at 260 nm
<b>CTAB</b>	Cetyl trimethyl ammonium bromide	<b>OD<sub>280</sub></b>	Absorbance at 280 nm
<b>DF</b>	Detergent-free	<b>O-F</b>	Oxidative fermentative
<b>DNA</b>	Deoxyribonucleic acid	<b>PBS</b>	Phosphate buffer saline
<b>DNase</b>	Deoxyribonuclease	<b>PCA</b>	Principal component analysis
<b>dNTPs</b>	Deoxyribonucleotide triphosphates	<b>PCR</b>	Polymerase chain reaction
<b>ds</b>	Double-stranded	<b>PDB</b>	Protein Data Bank
<b>et al</b>	et alia (and others)	<b>rRNA</b>	Ribosomal RNA
<b>g</b>	Gram	<b>rpm</b>	Rotation per minute
<b>GIS</b>	Geographic Information System	<b>SD</b>	Standard deviation
<b>h</b>	Hour	<b>SDS</b>	Sodium dodecyl sulphate
<b>HDTMA</b>	Hexadecyltrimethyl-ammoniumbromide	<b>SEM</b>	Scanning electron microscopy
<b>HCl</b>	Hydrochloric acid	<b>TAE</b>	Tris acetate EDTA
<b>kb</b>	Kilobase	<b>TCBS</b>	Thiosulfate-Citrate-Bile Salts-Sucrose
<b>KOH</b>	Potassium hydroxide	<b>TE</b>	Tris EDTA
<b>L</b>	litre	<b>TSA</b>	Tryptone soy agar
<b>LB</b>	Luria-Bertani	<b>UV</b>	Ultraviolet
<b>M</b>	Molar	<b>V</b>	Volt
<b>MAR</b>	Multiple antibiotic resistance	<b>v/v</b>	Volume by volume
<b>mcg</b>	Microgram	<b>w/v</b>	Weight by volume
<b>MEGA</b>	Molecular evolutionary genetics analysis	<b>XLD</b>	Xylose-Lysine Deoxycholate Agar
<b>mg</b>	Milligram		