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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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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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.

List of Tables

List of Figures

List of Appendices

Abbreviations

ºC	Degree Celsius
µg	Microgram
µl	Microlitre
µm	Micrometre
AIM	*Aeromonas* isolation medium
BLAST	Basic local alignment search tool
BLASTn	Nucleotide BLAST
BLASTp	Protein BLAST
bp	Base pair
$CaCl_2$	Calcium chloride
CAS	Chromazurol sulfonate
CFU	Colony forming units
cm	Centimetre
CTAB	Cetyl trimethyl ammonium bromide
DF	Detergent-free
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
dNTPs	Deoxyribonucleotide triphosphates
ds	Double-stranded
et al	et alia (and others
g	Gram
GIS	Geographic Information System
h	Hour
HDTMA	Hexadecyltrimethyl-ammoniumbromide
HCl	Hydrochloric acid
kb	Kilobase
KOH	Potassium hydroxide
L	litre
LB	Luria-Bertani
M	Molar
MAR	Multiple antibiotic resistance
mcg	Microgram
MEGA	Molecular evolutionary genetics analysis
mg	Milligram
$MgCl_2$	Magnesium chloride
MHA	Mueller Hinton Agar
mins	Minutes
ml	Millilitre
mM	Millimole
mm	Millimetre
NA	Nutrient agar
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nm	Nanometre
O.D.	Optical density
OD_{260}	Absorbance at 260 nm
OD_{280}	Absorbance at 280 nm
O-F	Oxidative fermentative
PBS	Phosphate buffer saline
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDB	Protein Data Bank
rRNA	Ribosomal RNA
rpm	Rotation per minute
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy
TAE	Tris acetate EDTA
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose
TE	Tris EDTA
TSA	Tryptone soy agar
UV	Ultraviolet
V	Volt
v/v	Volume by volume
w/v	Weight by volume
XLD	Xylose-Lysine Deoxycholate Agar