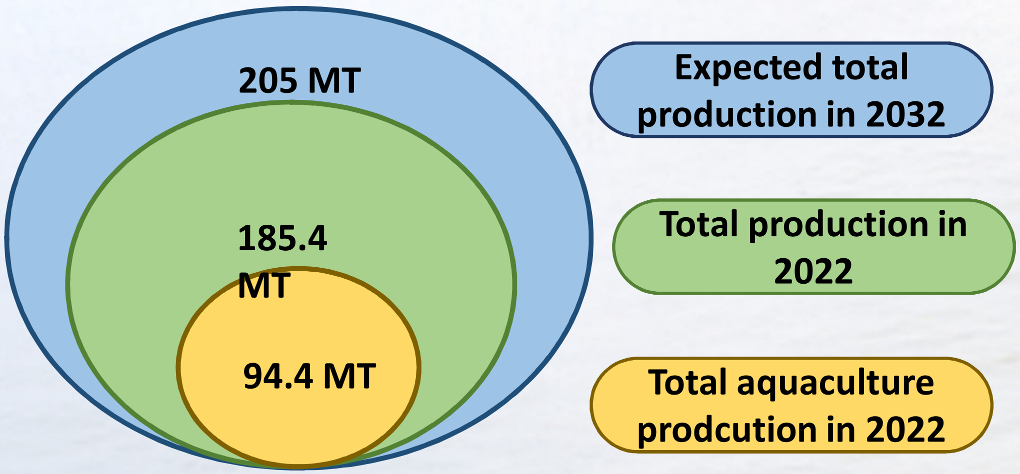
WORKSHOP & TRAINING (RESIDENTIAL)

THE INDIAN FRESHWATER FISHERIES SECTOR

How to scale up preventive approaches to minimise antibiotic use

Management Practices in Freshwater Fish Farms and Hatcheries

Current trend and projection of World aquaculture



The key drivers:

- Rising Global Population & economic growth
- Changing Dietary Habits
- Food Security
- Sustainability Concerns

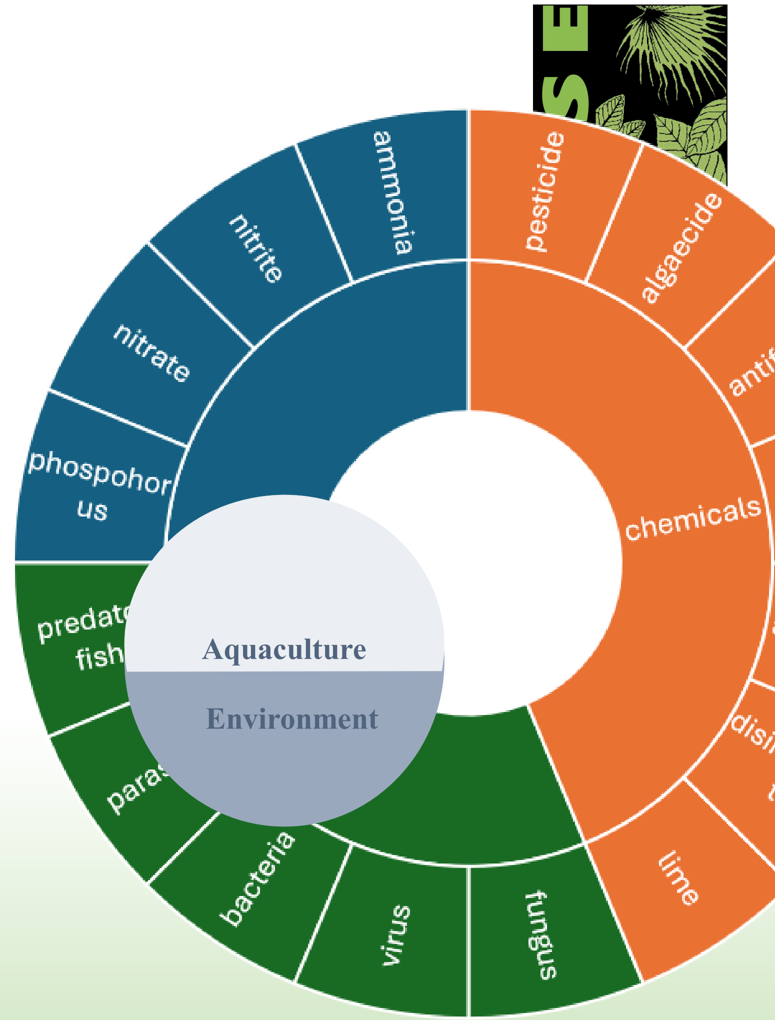
FAO advocates Blue Transformation, to meet the overall requirements of better production, better nutrition, a better environment and a better life, leaving no one behind.

Challenges

- **Aquaculture contributes more than half of the world's fish production, and India is the second-largest producer, contributing to this blue food basket.**
- **Due to the incidence of bacterial diseases, the potential contribution of the Indian aquaculture is often limited.**
- **The economic loss due to diseases in Indian major carp farms is US$996.01 M, from which the bacterial disease alone is US$351.43 M, 35.28% followed by parasitic (Argulosis alone caused a loss of US$ 615 ha^{-1} to Indian carp farms (Sahoo et al., 2013, Patil *et al*, 2025).**
- **Among the farming systems, bacterial infections were dominant in pangasius (cage) (38.46%), followed by marine fishes in cages (28.92%), IMC (25.84%), IMC+ (10.43%) and tilapia (7.14%) farms.**
- **The economic loss was dominated by diseases of multiple etiology (US$ 468.27 M), bacterial hemorrhagic septicemia (US$ 326.47 M), and epizootic ulcerative syndrome (US$ 88.12 M) in finfish (Patil *et al*, 2025).**
- **The major contributors to the disease burden included production loss (23.90%), expenses on prophylactics (50.31%) and therapeutics (17.26%). (Patil *et al*, 2025).**

- **Disease prophylaxis in Indian aquaculture mainly comprises good management practices.**
- **Antimicrobial agents of natural and chemical origin are used in the therapeutic management of bacterial diseases in Indian carp culture operations.**
- **Prophylactic application of environmental modifiers amounting to US$ 13.23 t^{-1} in IMC and immunostimulants amounting to US$ 26.63 t^{-1} in IMC+ were effective in reducing the incidence of diseases.**
- **In pangasius (pond), environmental modifiers worth US$ 5.41 t^{-1}, antiparasitic drug worth US$ 5.41 t^{-1}, and nutritional supplements worth US$ 7.63 t^{-1}) was helpful in managing disease occurrence.**
- **Environmental modifiers worth US$ 17.93 t^{-1}, probiotics worth US$ 7.33 t^{-1}, and nutritional supplements worth US$ 9.05 t^{-1} is spent to control disease outbreak in tilapia farms.**
- **Notably, in shrimp farms, higher expenditure on environmental modifiers (US$ 129.79 t^{-1})**

- **Aquaculture can have environmental impacts, such as pollution from waste, the potential for habitat destruction and Climate change**
- **The overuse and misuse of antibiotics is believed to be the main reason to induce a high level of antimicrobial resistance (AMR) in aquaculture.**
- **Some studies in SEA countries reported the antibiotic susceptibility and the multiple antibiotic resistance (MAR) index, ranged between 0.13 and 0.88 with 74.7 % of the isolates having MAR values higher than 0.2 (Nhinh et al., 2021)**
- **The industry needs to adopt sustainable practices to minimize environmental impacts and ensure long-term viability.**



Disease issues

- A multitude of factors have contributed to the fish health problems currently faced in aquaculture.
- The key to successful aquaculture is maintaining of healthy stock in a healthy environment.
- A wide range of pathogens (viruses, bacteria, parasites etc.), environmental factors (water quality, soil quality, organic pollution etc.) and even husbandry practices been responsible for outbreak of diseases.
- Risk of emergence of exotic and transboundary pathogens

Common clinical signs & symptoms and possible causes

Common clinical signs & symptoms	Possible causes
Sudden death of fish without much preceding symptoms	Low oxygen level (DO) Exogenous toxins Poisoning Per-acute bacterial and viral disease
Gasping, coming to surface, crowding at inlets or corner of the pond	Low DO Gill parasites/Gill disease Bacterial gill disease
Jumping, flashing, rubbing	Ectoparasites, toxins, irritants in water
Fin rot	Bacterial disease, Cytophaga, Saprolegnia infection, Physical damage, Cannibalism
Changes in head and body ratio, loosening of scales	Malnutrition/nutritional imbalances
Dropsy	Bacterial Infections

Common clinical signs & symptoms	Possible causes
Scoliosis, lordosis	Vitamin deficiency
Necrotic lesion on operculum	Myxobacterial infection, VDN
Haemorrhages on anus/vent	Septicaemic bacterial infection
Opaque eye/white cover on eye	Streptococcal infection
Excess production of mucus from skin/ gills	Ectoparasites, toxins, irritants in water, myxobacterial infections
White cotton-wool growth on skin	Fungal infection (Saprolegenia), Cytophaga infection Columnaris disease
White spots on skin	Parasitic disease (Icthhyopthirius) , Myxobolus infection
Swelling on skin	Parasitic cysts, tumours
Haemorrhages on scales, fins	Bacterial infections, Ectoparasites with secondary bacterial infections
Skin lesions/ ulcers	EUS, Ectoparasites with secondary bacterial infections,

Bacterial Diseases

Aeromoniasis (BHS/Infectious dropsy/Red sore/Ulcer diseases)

This is probably the most common bacterial disease of freshwater fish and is often confused with red disease.

The disease affects fish of all ages and is favoured by environmental stress factors, *viz*., poor water quality, low D.O, Low pH, Low alkalinity (less than 70 ppm), high organic load, high H_2S level, and fall in water temperature.

Several members of the genus *Aeromonas,* including *A. hydrophila, A. sobria, A. caviae, A. schuberti,* and *A. veronii* are associated with the disease

Tail rot/Fin rot/ulcer

The disease have typical symptoms like erosion of tail part or loss of fins.

The disease is due to group of pathogenic bacterial strains like *Pseudomonas* sp. Sometimes total loss of fins is also recorded.

Columnaris disease/Saddle back disease

Columnaris, is a common bacterial disease that affects the skin or gills of freshwater fish and is caused by *Flavobacterium columnare*.

Bacterial Gill disease

Flavobacterium branchiophilum **is the aetiological agent**

The disease is closely linked to environmental conditions and other stress factors like high organic load and overcrowding etc. The disease is characterized by attachment and proliferation of the filamentous bacteria on the gill mucoid surface.

Edwardsiellosis

Septicaemic condition caused due to *Edwardsiella tarda/ Edwardsiella piscicida*, generally termed as "emphysematous putrefactive disease (EPD)".

It affects all cultured freshwater and brackish water fishes of all stages where mortality goes up to 100% in fry/fingerlings and juveniles.

The infection leads to emaciation, anaemia, loss of skin, peeling off and dropping of skin, gas-filled, foul-smelling abscesses.

Parasitic Diseases

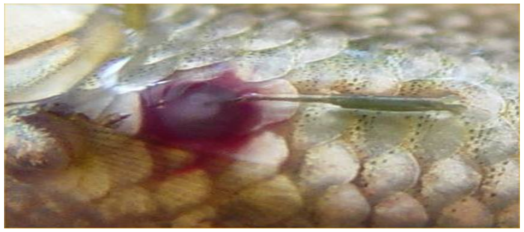
Argulosis:

Causative agent: It is caused by species of the genus *Argulus*, also commonly known as fish louse.
Symptoms include small red patches on skin, gills and fins.

Argulus parasite has hook like limbs and a sucking feeding apparatus and can cause open wounds that can lead to secondary infection.

Feeding sites are marked by haemorrhagic spots, hyperplasia of epidermis at the margins of wound.

Lernaeasis:



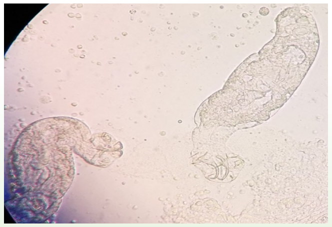
Lernaeasis is caused by species of the genus *Lernaea sp.*

This parasite is commonly known as "anchor worm".

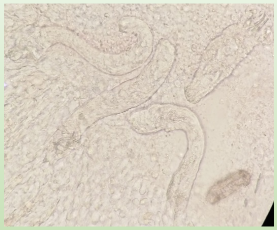
The mature female parasite (5-15 mm long) penetrates the skin of fish and remains attached to the host with its head buried in the muscles and the elongated body protrudes outside with a pair of egg sacs attached to the host.

Gyrodactylosis and Dactylogyrosis

The disease is caused by monogenetic trematode, mainly *Dactylogyrus* sp. (infest the gills) and some extent *Gyrodactylus* sp. (infest both skin and gills).

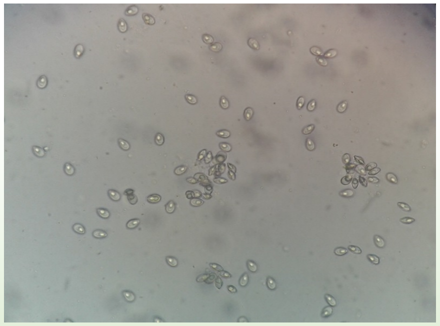


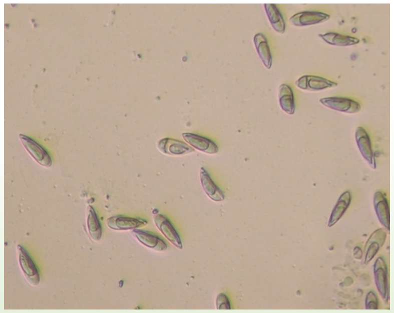
The parasite is about 1 mm long and dorsoventrally flattened. *Gyrodactylus* is viviparous and no eye spots whereas *Dactylogyrus* is oviparous and with four eyespots. The parasites cause haemorrhage, necrosis and damage to the gill, skin and loss of health.

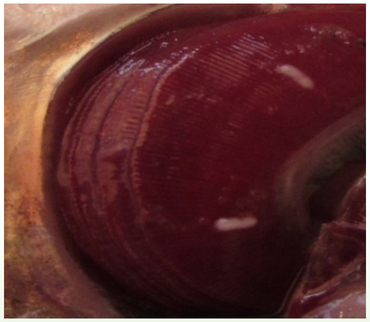


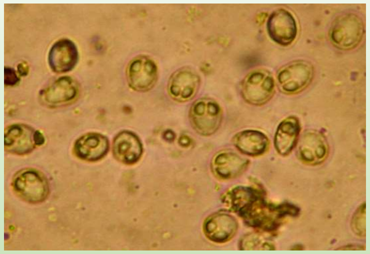
Myxozoan infection:

There are group of parasites viz., *Myxobolus, Henneguya, Sphaerospora, Thelohanellus* sp. Many species of this group infest mostly, gills, skin, fins and the kidney, liver of fish.



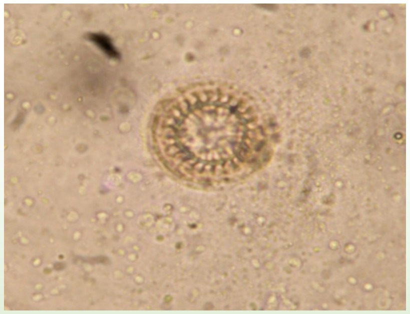






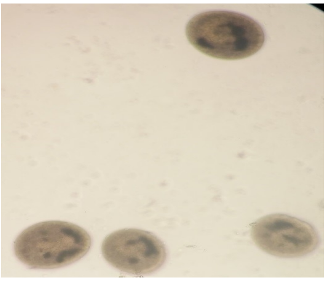
Trichodinosis

The disease is caused by the spherical ciliate protozoa of the species *Trichodina, Trichodinella, Tripartiaella and Glossatella* which infest mostly skin, fins and gills of fish.



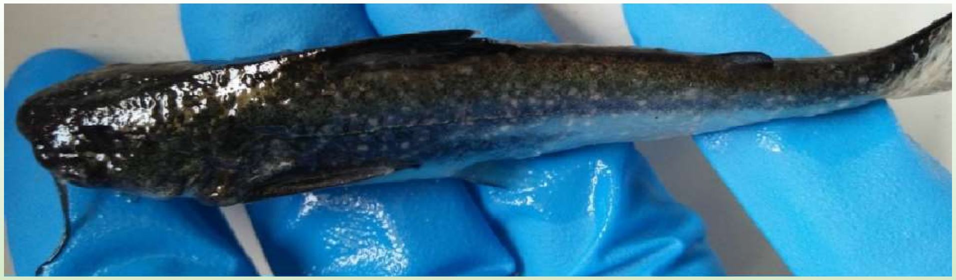
Due to sucking action of these parasites the skin of the host is irritated with heavy mucus secretion and ultimately leads to death of the fish.

Ichthyophthiriosis (Ich or Freshwater white spot disease)



Infectious disease, caused by *Ichthyophthirius multifilis.*

It is a round to oval-shaped holotrichous ciliate protozoan, 0.5-1.5 mm wide, characterised by its horseshoe-shaped macronucleus.



Fungal Diseases

Branchiomycosis (Gill rot disease)

***Branchiomyces* sp.**

Appearance of white necrotic tips of primary gill lamellae, fusion of gill lamellae, necrosis of gill filaments, gills turns yellowish brown.

Saprolegniasis/Cotton wool disease:

Saprolegnia parasitica

Epizootic Ulcerative syndrome

Aphanomyces invadans, a fungus as the necessary causative factor.

It affects both wild and cultured fish. Affected fish show abnormal swimming behaviour with head projected out of water.

Localized red spots appear on the body that spread to become ulcers and ultimately turn out to deep sores.

Viral Diseases

- **Tilapia Lake Virus** – Skin lesions, ulcers, haemorrhages
- **Tilapia Parvo Virus** - Scale loss, redness on the body with haemorrhages on the operculum, base of fins and ventral part, opaqueness of the eyes
- **Carp Edema Virus/Koi sleepy disease (KSD)** which is characterized by congestion of gills, skin alterations at the fins base or around the mouth, and lethargic behavior of fish.
- **CyHV2/Goldfish haematopoietic necrosis herpes virus**

- **No treatment only management.**

- *Macrobrachium rosenbergii* nodavirus/ White muscle disease

Macrobrachium rosenbergii Golda virus

Disease Management

All India Network Project on Fish Health: Recommendation

Antimicrobial agents for controlling bacterial infections in fish

Sl. No	Chemical substance	Mode of application	Dosage	Withdrawal period
1.	Oxytetracycline Dihydrate	Oral through feed	80-100 mg/kg fish biomass/day for 10 d	Warm water fish - 7 days Coldwater fish 21 days
	Oxytetracycline HCl	Immersion	20-50 ppm for 1 hour static bath for 4 consecutive days	Not applicable
2.	Florfenicol	Oral through feed	10-15 mg/kg fish biomass per day for 10 d	7 days
3.	Sulfadimethoxine+ Trimethoprim	Oral through feed	50 mg/kg fish biomass per day for 5-7 days	Warm water fish - 7 days Coldwater fish 21 days
4.	Sulfadimethoxine + Ormetoprim	Oral through feed	50 mg/kg fish biomass per day for 5 d	Warm water fish - 7 days Coldwater fish 21 days
5.	Oxolinic acid	Oral through feed	12 mg/kg fish biomass per day for 7 d	Warm water fish - 7 days Coldwater fish 21 days

All India Network Project on Fish Health: Recommendation

Potential antiparasitic drugs for management of parasitic diseases

Sl.	Chemical	Mode of application	Dosage	Withdrawal period
1.	Formalin	Immersion	100-200 ppm 1 h, once every 3 d	Not required
		Immersion	100 ppm 30 min for 5 days	Not required
		Immersion	Fertilized eggs: 100 ppm for 1 min Nauplii:400ppm for 30-60 sec	Not required
2.	Hydrogen peroxide	Immersion (egg wash)	500-1000 ppm 15 min	Not required
		Immersion	50-100 ppm for 60 min	Not required
3.	Emamectin benzoate	Oral through feed	50 µg/kg biomass/day for 7-10 d	Not required
4.	Lufenuron	Oral through feed	5 mg/kg biomass/day for 7 days	14 days

All India Network Project on Fish Health: Recommendation

Potential antiparasitic drugs for management of parasitic diseases

5.	Albendazole	Oral through feed	10 mg/kg biomass repeat after 2 w	Warm water fish - 7 days Coldwater fish 21 days
6.	Fenbendazole	Oral through feed	40 mg/kg biomass (twice at interval of 4 days)	Warm water fish 7 d Coldwater fish 21 daya
7.	Ivermectin	Oral through feed	0.5-0.7 mg/kg biomass once weekly for 3 weeks	Not required
8.	Praziquantel	Immersion	40 ppm for 1 h	Not required
		Oral through feed	50 mg/kg fish biomass/ day for 5 days	Warm water fish - 7 days Coldwater fish 21 days

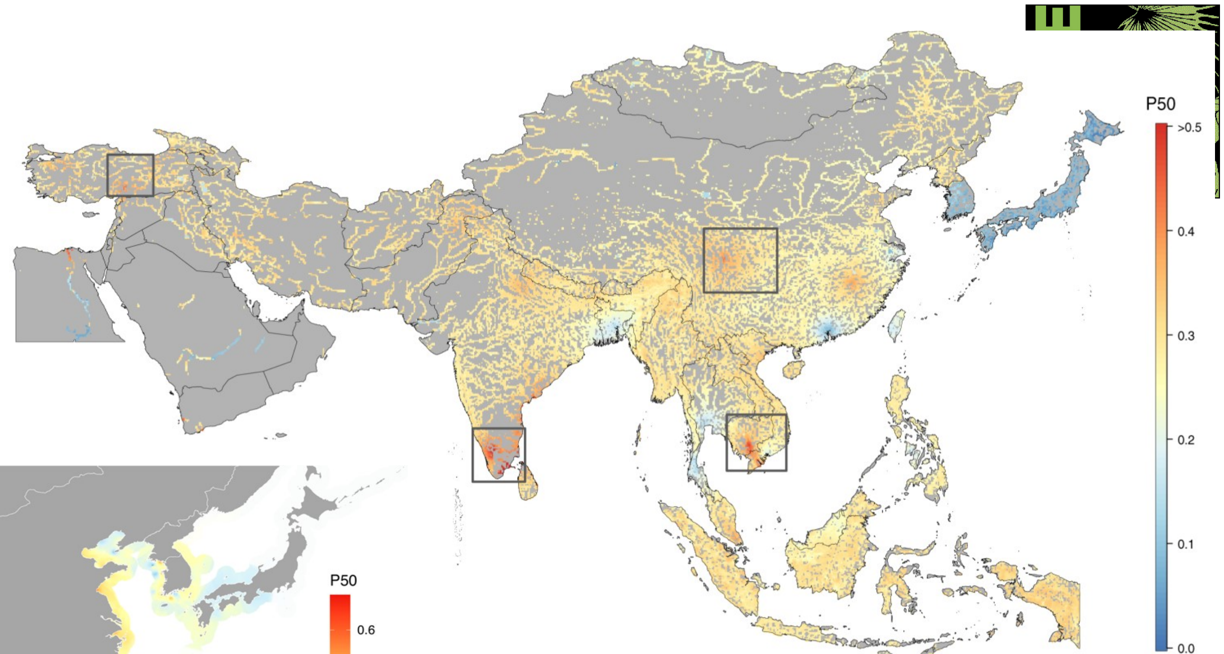
AMR - A global concern

- Antimicrobial medicines are the cornerstone of modern medicine. The emergence and spread of drug-resistant pathogens threatens our ability to treat common infections and to perform life-saving procedures including cancer chemotherapy and caesarean section, hip replacements, organ transplantation and other surgeries
- Drug-resistant infections impact the health of animals and plants, reduce productivity in farms, and threaten food security.
- AMR has significant costs for both health systems and national economies overall
- AMR is a problem for all countries at all income levels. Its spread does not recognize country borders.
- Contributing factors include lack of access to clean water, sanitation and hygiene (WASH) for both humans and animals; poor infection and disease prevention and control in homes, healthcare facilities and farms; poor access to quality and affordable vaccines, diagnostics and medicines; lack of awareness and knowledge; and lack of enforcement of relevant legislation., improper prescription, Dumping in animal health care products and farm activities and awareness

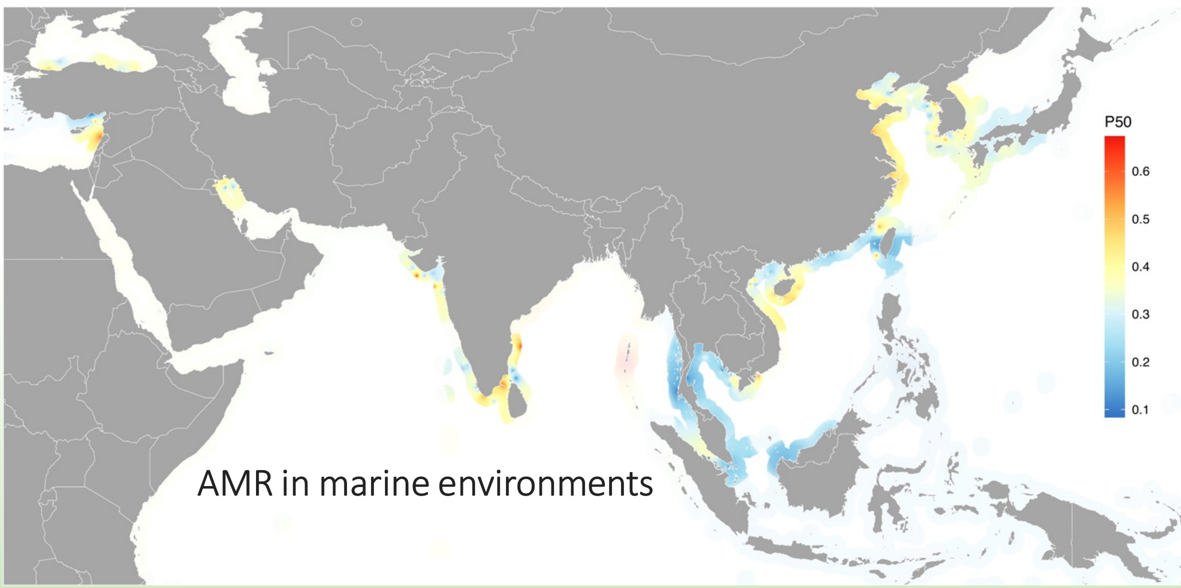
Present situation?

- 76 countries of 42% for third-generation cephalosporin-resistant *E. coli* and 35% for methicillin-resistant *Staphylococcus aureus* are a major concern
- *Klebsiella pneumoniae*, a common intestinal bacterium, also showed elevated resistance levels against critical antibiotics
- The emergence and spread of multi-drug resistant *Candida auris*, an invasive fungal infection
- Drug resistance in HIV, tuberculosis (MDR-TB) and malaria (*Plasmodium falciparum)*
- Drug resistance in neglected tropical diseases (NTDs)

AMR in aquatic environment and species



AMR in freshwater environments



AMR in marine environments

Nat Commun 2021; 12: 5384

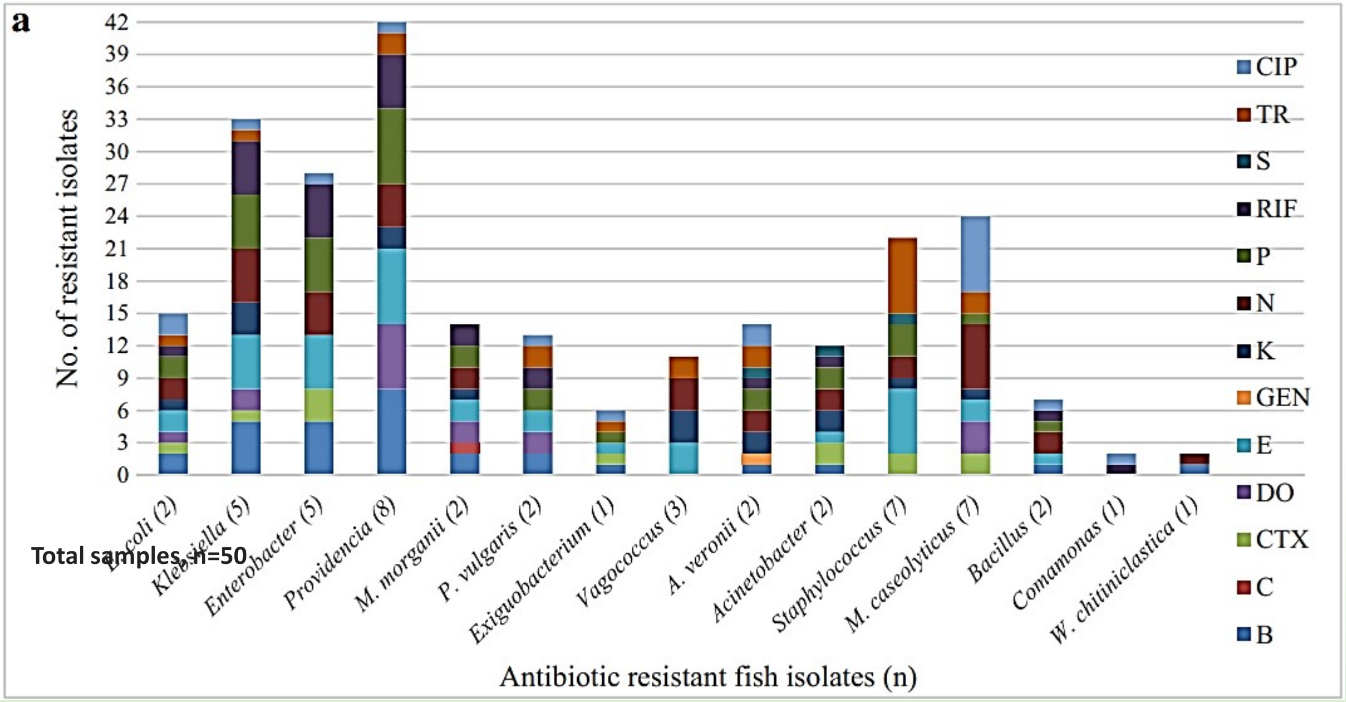
Resistant bacteria isolates from fishes in retail market

ABR in freshwater fishes

Name and dose of antibiotics	Percentage of resistant
Augmentin (AMC) 30 µg	73.68
Colistin (CL) 10 µg	26.32
Cefpodoxime (CPD) 10 µg	22.81
Nitrofurantoin (NIT) 300 µg	10.53
Co-Trimoxazole (COT) 25 µg	7.02
Nalidixic acid (NA) 30 µg	5.26
Imipenem (IPM) 10 µg	3.51
Levofloxacin (LE) 5 µg	1.75
Cefoxitin (CX) 30 µg	1.75
Gentamicin (GEN) 10 µg	1.75
Ciprofloxacin (CIP) 5 µg	0.00
Tobramycin (TOB) 10 µg	0.00
Moxifloxacin (MO) 5 µg	0.00
Ofloxacin (OF) 5 µg	0.00
Ceftazidime (CAZ) 30 µg	0.00
Norfloxacin (NX) 10 µg	0.00
Gatifloxacin (GAT) 5 µg	0.00
Amikacin (AK) 30 µg	0.00
Aztreonam (AT) 30 µg	0.00
Ceftriaxone (CTR) 30 µg	0.00

Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. 2019; 89:559–564

ABR in marine water fishes

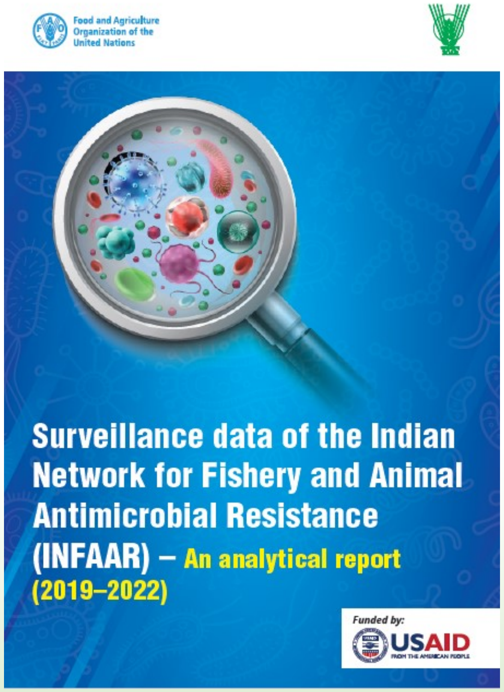


Environ Sci Pollut Res Int. 2018; 25(7):6228-6239

ALL INDIA NETWORK PROJECT on AMR in FISHERIES and LIVESTOCK/INFAAR

Objectives:

- To undertake **surveillance** of antimicrobial resistance (AMR) in target microorganisms isolated from healthy farmed animals and fish/shellfish with an aim to quantify its burden and monitor the spatial and temporal trends of AMR in India.
- To strengthen the knowledge on **determinants of AMR** in target microorganisms isolated from healthy farmed animals and fish/shellfish with an aim to understand the spread of AMR and its risk factors in context of its **One health** implications in India.
- To improve **awareness** and understanding of AMR among the farming community, veterinary and fish health professionals and policymakers, through effective communication, education and training so as to promote the judicious use of antimicrobials in farmed food animals and fish.

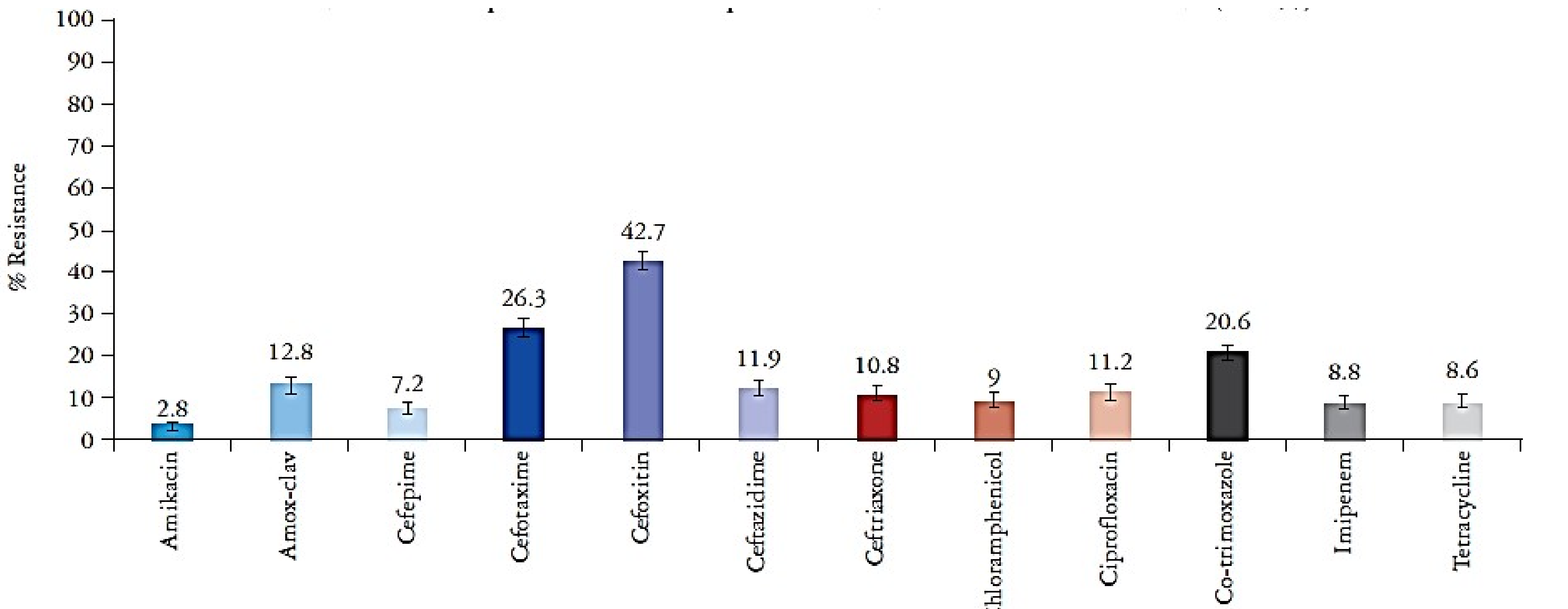


Members of INFAAR (2019–2022)

ICAR-CIFA, Bhubaneshwar, Orissa	Dr S.S. Mishra	Mr Satyanarayan Sahoo Dr P. Swain

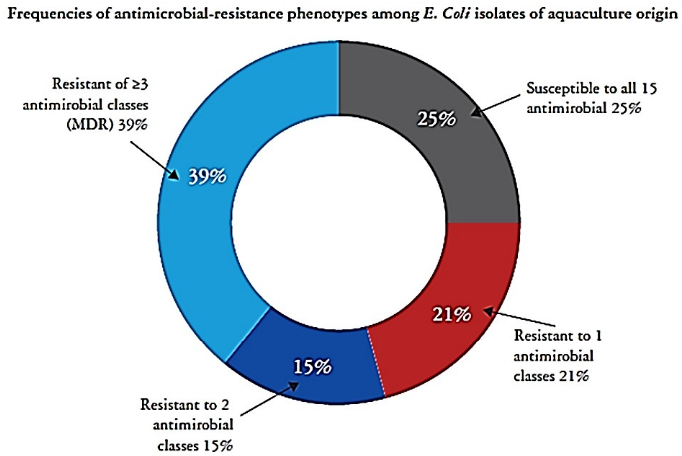
FAO, 2024. *Surveillance data of the Indian Network for Fishery and Animal Antimicrobial Resistance (INFAAR) – An analytical report 2019–2022*. New Delhi.

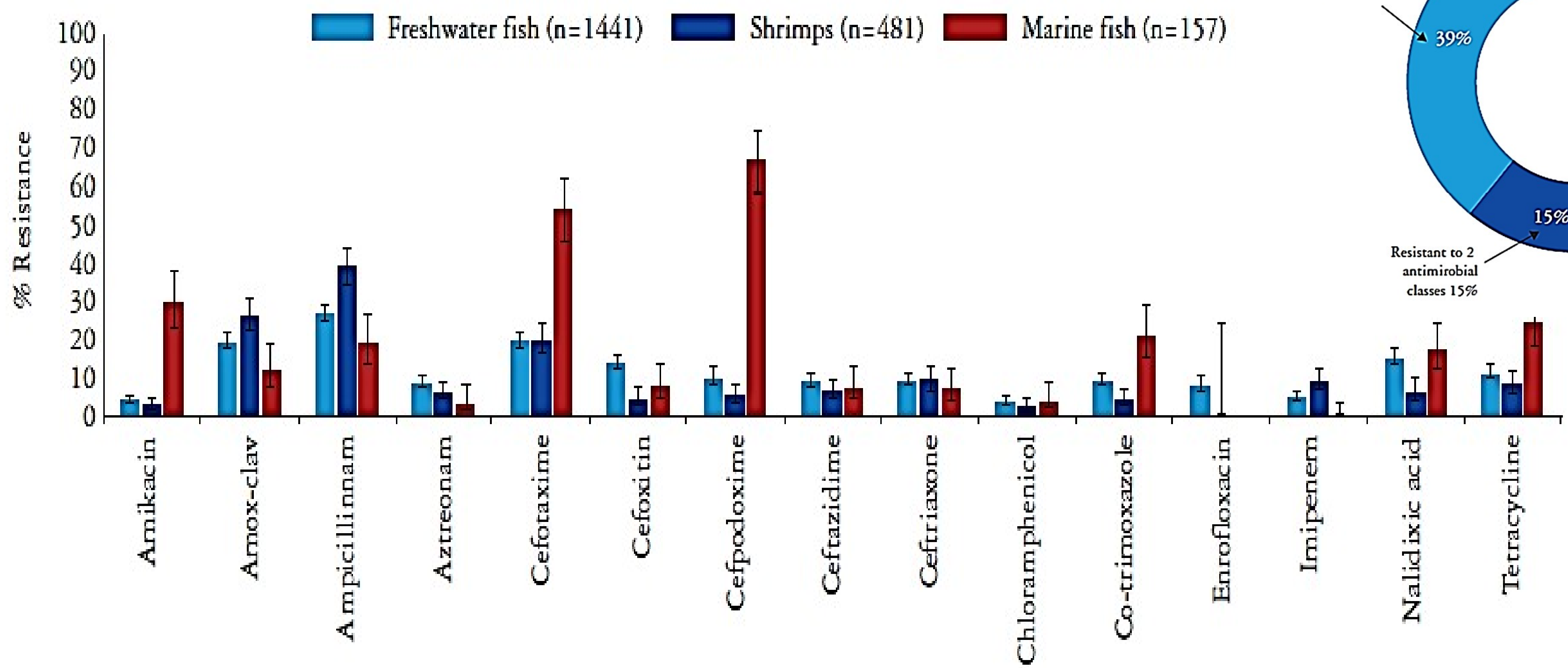
AMR profile of *Aeromonas* species isolated from freshwater fish India (n=1657)



FAO, 2024. *Surveillance data of the Indian Network for Fishery and Animal Antimicrobial Resistance (INFAAR) – An analytical report 2019–2022*. New Delhi.

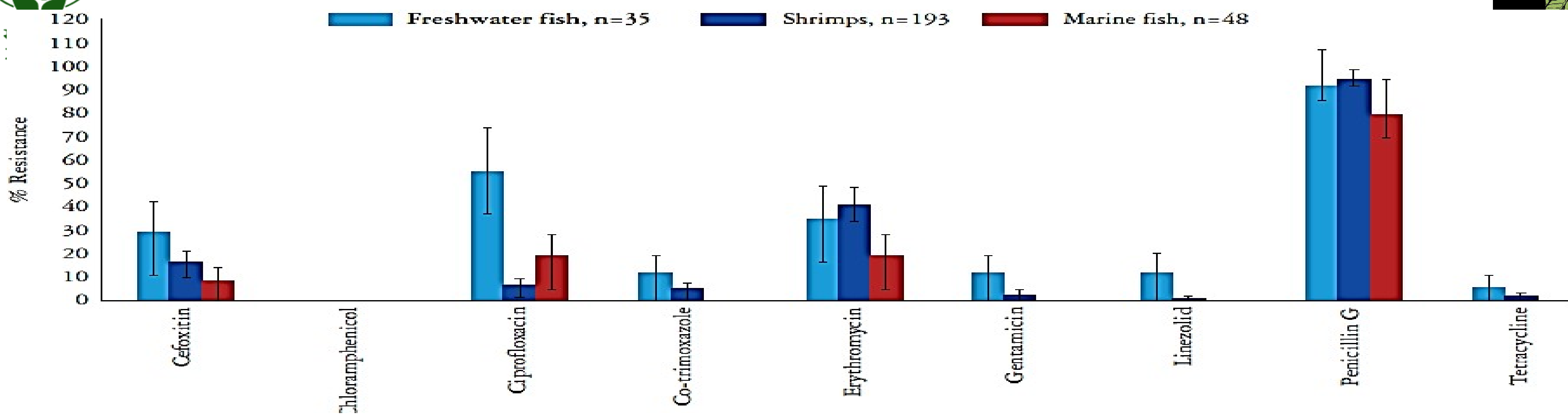
Comparative AMR profile of *E. coli* isolated from cultured freshwater fish, shrimp and marine fish of India (n=2079)



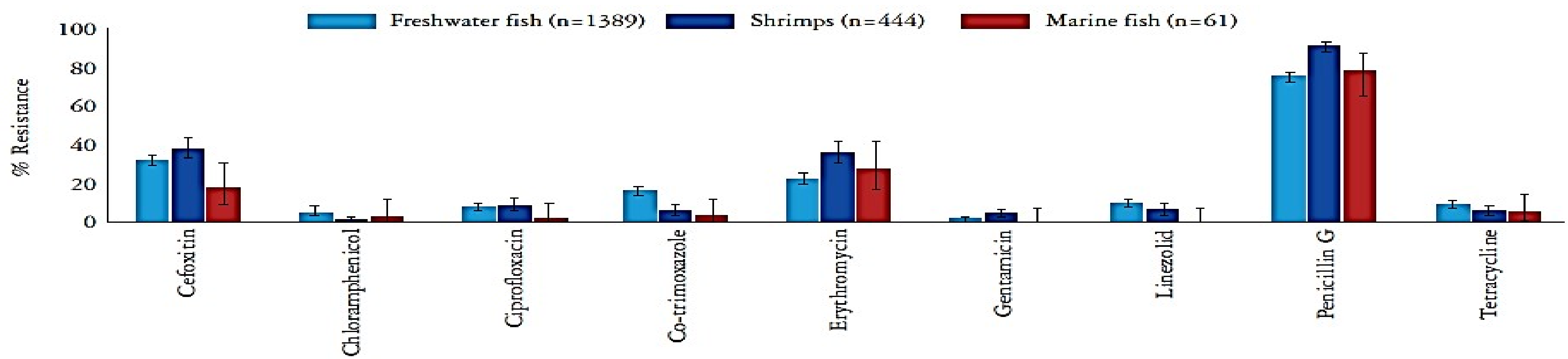


FAO, 2024. *Surveillance data of the Indian Network for Fishery and Animal Antimicrobial Resistance (INFAAR) – An analytical report 2019–2022*. New Delhi.

Comparative AMR profile of *S. aureus* isolated from cultured freshwater fish, shrimp and marine fish of India (n=276)



Comparative AMR profile of CONS isolated from cultured freshwater fish, shrimp and marine fish of India (n=1894)



Harmonized One health Trans-species and Community Surveillance for Tackling Antibacterial Resistance in India (HOTSTAR-India) (2019-21)

Collaborative Effort by

- **All India Institute of Medical Sciences-Bhubaneswar,**
- **Kalinga Institute of Medical Sciences,**
- **Institute of Medical Sciences & SUM Hospital**
- **Hi-Tech Medical College**
- **College of Veterinary Science & Animal Husbandry, OUAT**
- **Central Institute of Freshwater Aquaculture-ICAR**
- **The INCLEN Trust International**
- **Supported by BIRAC-DBT (Grand Challenges India on AMR)**

- **Objectives**

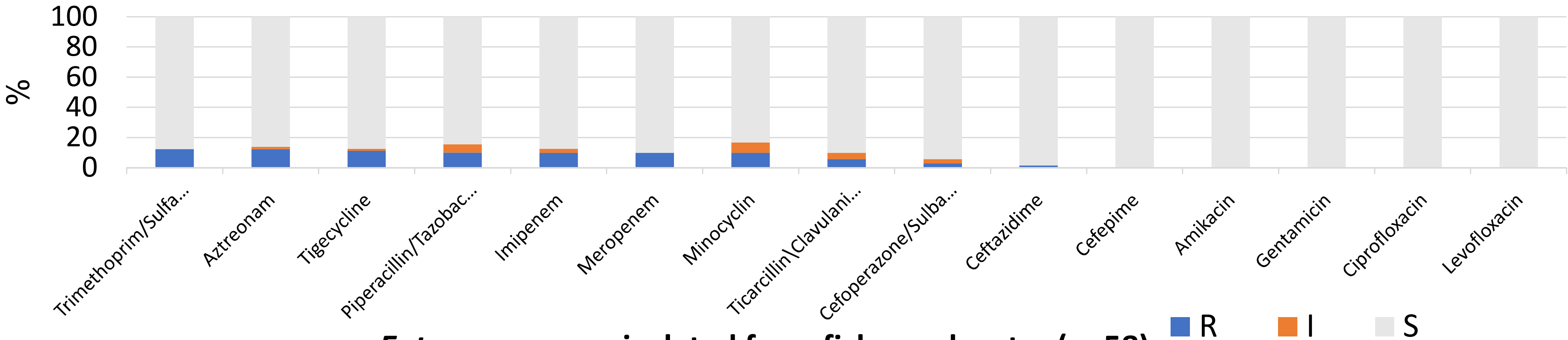
(1) To document the pattern of infections because of the 7 index bacteria in humans, animals (including livestock, food chain, and pets), birds (food chain birds such aschicken), and freshwater fishes sharing the same environment and their resistance patterns.

(2) To document the risk factors for ABR at the individual and community level related to health, antibiotic consumption, and antibiotic usage in food animal breeding and agriculture.

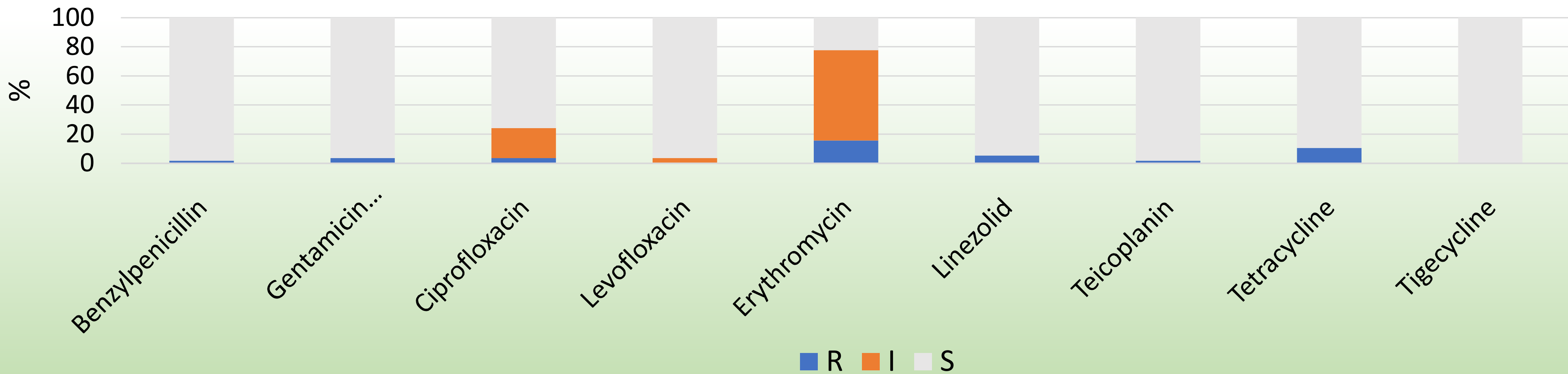
(3) Apply geospatial epidemiology analytical methodology to improve the understanding of bacterial infections and ABR.

- During 2019-21, A total 72 farms (Khordha 27 and Puri 45) from Odisha were surveyed, fish and water samples were collected for isolation of the index bacteria and AST was analyzed.

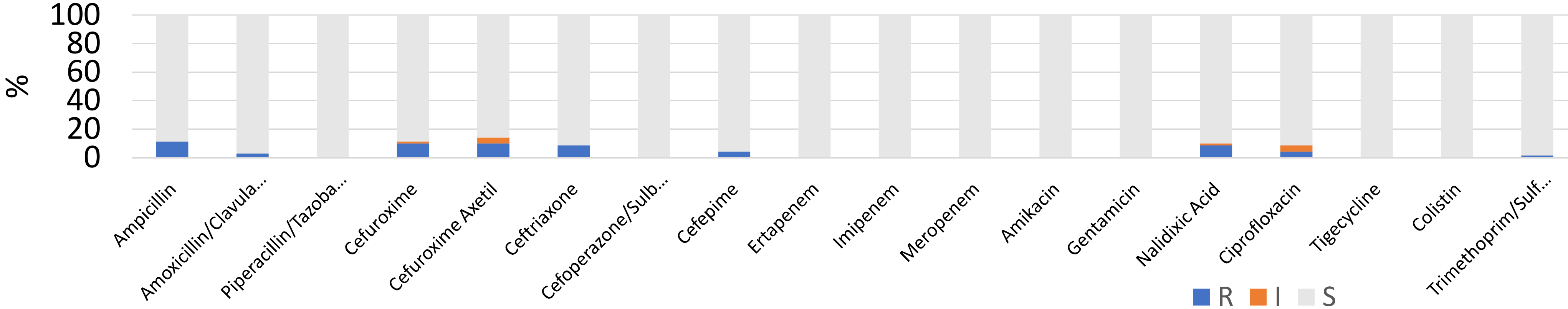
Pseudomonas sp. isolated from fish pond water (n=73)



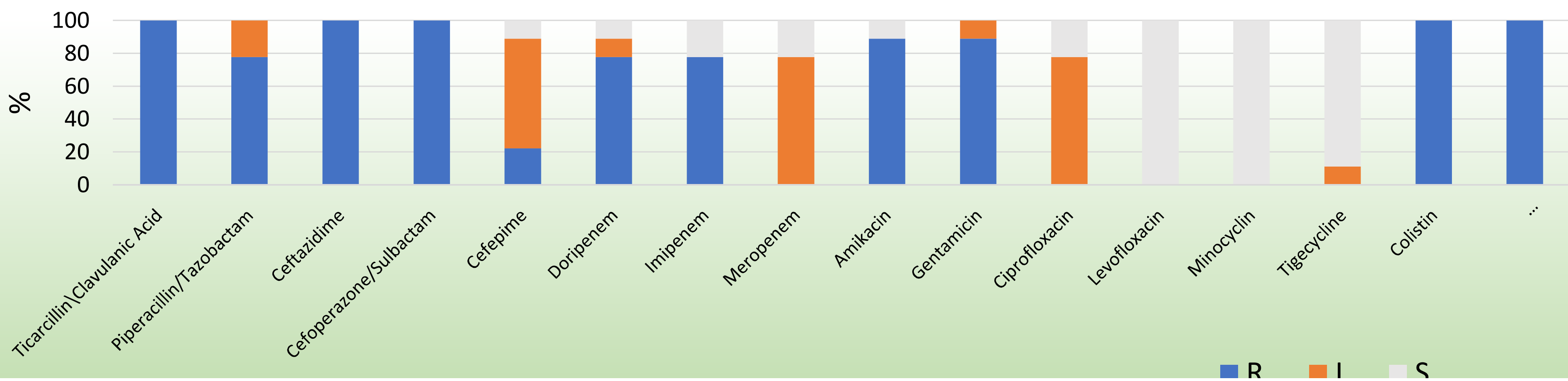
Enterococcus sp. isolated from fish pond water (n=58)



E. coli isolated from fish pond water (n=73)



Acinetobacter sp. isolated from fish pond water (n=9)



Preventive methods to be adopted to prevent occurrence of disease:

- **Regular monitoring of water quality parameters is utmost important to control disease outbreak. The pH should be within 7.5-8.5 and Total alkalinity should always be > 100 ppm in culture ponds.**
- **Proper liming and periodical fertilization with Cow dung, DAP-Urea should be done to help plankton growth**
- **Proper stocking density should be maintained (4000 Nos. fingerlings in one Acre pond water spread area)**
- **Stock only certified seeds/ seeds from disease free hatchery stock.**
- **Proper pond preparation should be followed after each culture cycle: removal of unwanted material such as weeds, humus, silt, predatory fish, insects, mollusks and if possible keep pond bottom for sun drying minimum 7-10 days after one cycle of fish culture.**
- **Application of bleaching powder / calcium oxide for pond disinfection is a good practice.**

- Ponds with history of repeated occurrence of obligate parasites should be kept without fish for at least 7-10 days before stocking. This practice helps to eliminate the obligate parasites.
- Quarantine checks and prophylactic measures like bath treatment with antiseptic solutions should be done before releasing fish into the culture pond.
- Liming, fertilizer application etc. should be done depending on the water quality parameters and as per the actual requirement of the particular water area.
- Frequent observation of the fish as far as possible through periodic netting is a good practice for taking timely corrective measures. Fish pathologist/ Expert advice should be immediately sought in case of any abnormality is noticed either in pond environment or with the stocked fish.
- Dead or moribund fish should be immediately removed and suitably disposed-off or buried away from culture site, to prevent spread of the disease.

Optimum Water Quality Parameters for Fresh Water Aquaculture

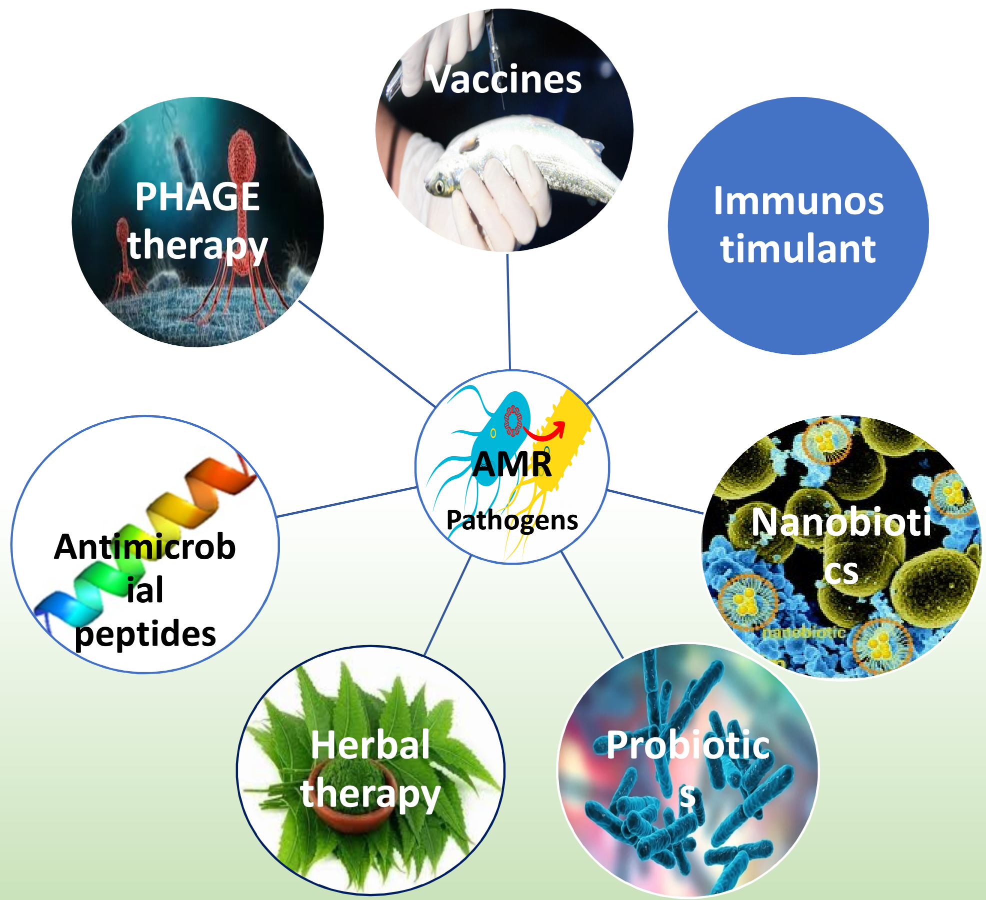
Sl. No.	Parameter	Optimum Range
1	pH	7.5-8.5
2	Total alkalinity	80-200 ppm
3	Hardness	100-180 ppm
4	Dissolved oxygen	>5 ppm
5	Unionized NH_3	0-0.1 ppm
6	Ionized NH_3	0-1 ppm
7	NO_2-N	0-0.5 ppm
8	NO_3-N	0.1-3 ppm
9	Hydrogen sulphide	<0.002 ppm

Prevention strategies – Better management

- Hatchery accreditation for better seed certification-SPF/SPR
- Seed disinfection-$KMnO_4$ dip, Salt dip, Quarantine
- Pond sterilization (Pre and post-stocking)-CaO, Bleaching powder
- Diligent Surveillance-Routine Health checkup and Prompt diagnosis
- Pond water corrections-Optimum water quality
- Appropriate Stocking and feeding-10000 fingerlings/ha for carps
- Training of farmers and mid workers

Alternative approaches

- Given the complexity of the emergence and transmission of antibiotic resistance among aquatic bacteria, no single solution can be identified.
- Since both disease and environment concerns are significant, it is suggested that finding innovative solutions is crucial to tackle these problems effectively

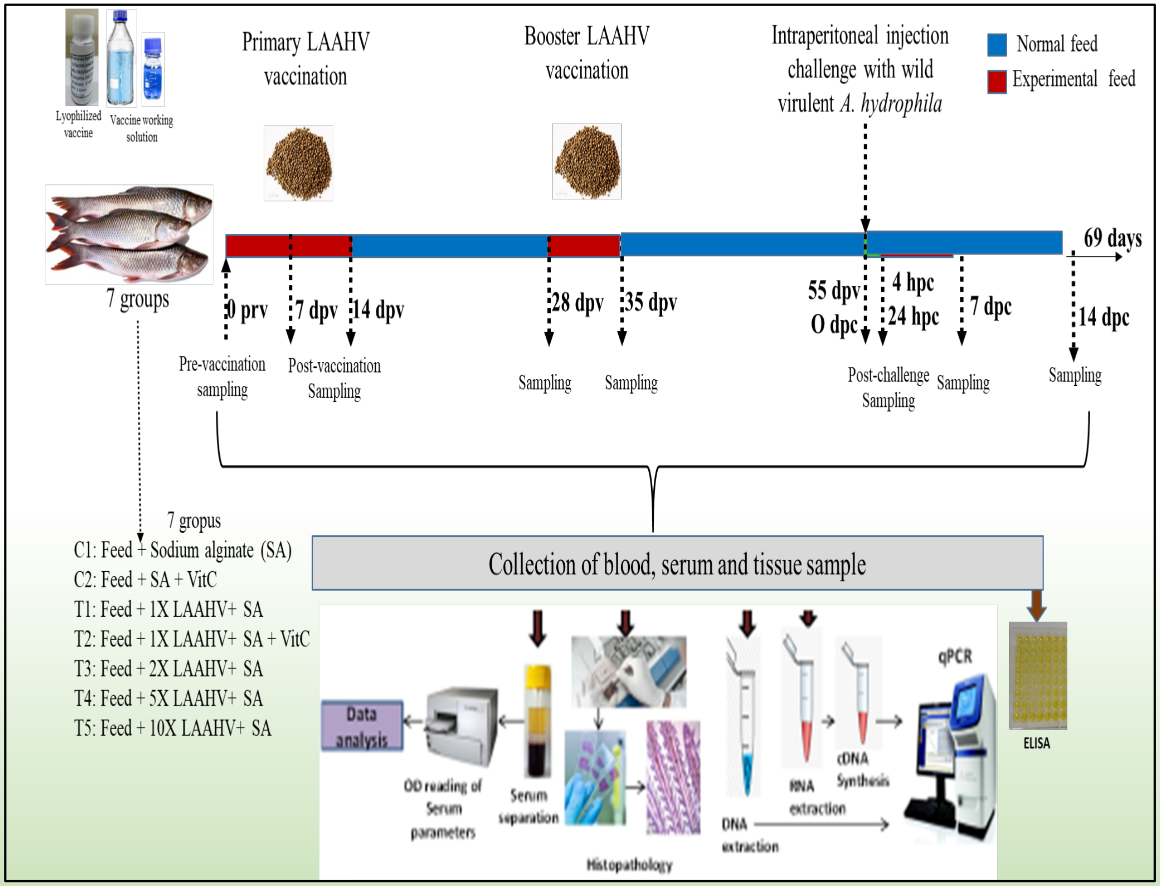


VACCINES

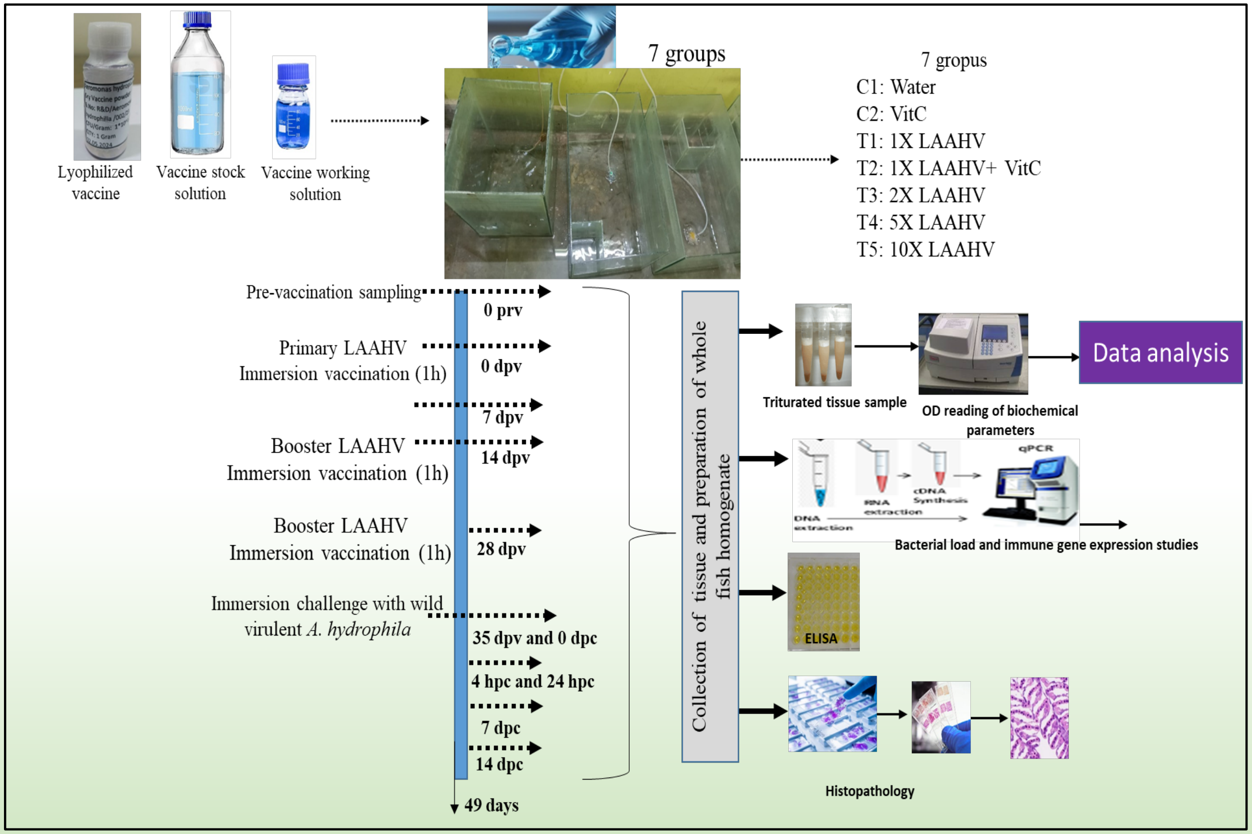
CIFA-Vaccine against *A. hydrophila*

Manufactured and marketed by Indian Immunologicals Limited (IIL), Hyderabad

Schematic diagram of oral feed based vaccination and challenge schedule in rohu juveniles. Timeline of vaccination and challenge are indicated by arrows below. Pre-vaccine sampling was done on the day 0 before first feeding of LAAHV-feed or normal feed. Abbreviations: prv: pre- vaccination, dpv: days post primary vaccination; hpc: hours post challenge dpc: days post challenge. Vaccine doses: 1X: $2X10^6$, 2X: $4X10^6$, 5X: $1X10^7$, 10X: $2X10^7$

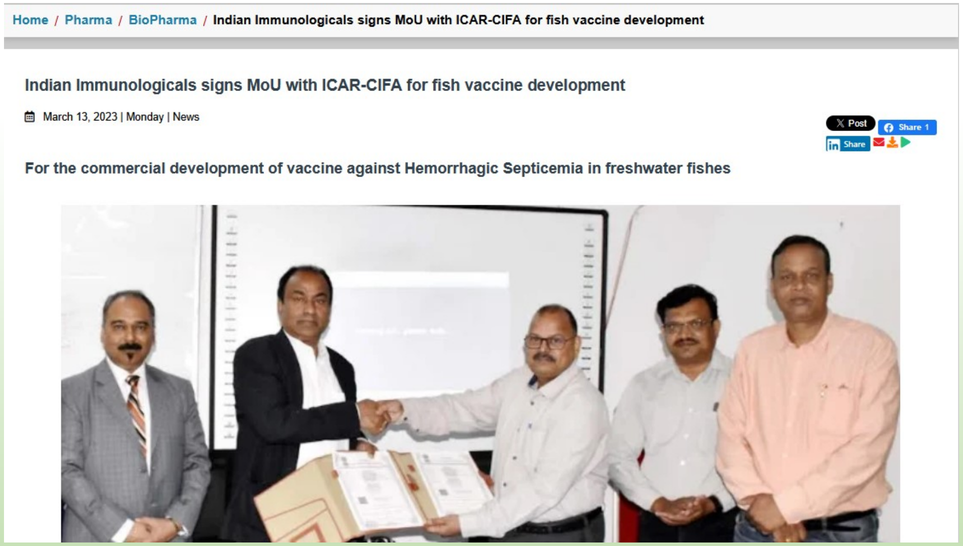


Schematic diagram of immersion vaccination and challenge schedule in rohu fry. Timeline of vaccination and challenge are indicated by arrows below. Pre-vaccine sampling was done on the day 0 before immersion vaccination. Vaccine doses: 1X: $2X10^5$, 2X: $4X10^5$, 5X: $1X10^6$, 10X: $2X10^6$



Other vaccines commercialized

- ICAR-Central Institute of Fisheries Education, Mumbai
- Vaccine against Columnaris caused by *Flavobacterium columnare*
- Edwardsillosis caused by *Edwardsiella tarda*
- ICAR-Central Institute of Brackishwater Aquaculture, Chennai
- "Nodavac-R" against viral nervous necrosis in finfishes


Home / Pharma / BioPharma / Indian Immunologicals signs MoU with ICAR-CIFA for fish vaccine development

Indian Immunologicals signs MoU with ICAR-CIFA for fish vaccine development

March 13, 2023 | Monday | News

For the commercial development of vaccine against Hemorrhagic Septicemia in freshwater fishes

BACTERIOCINS

Bioactive proteins or peptides produced by bacteria.
Low molecular weight : 20-60 amino acids in length

Ribosomally synthesized and kill closely related bacteria

Mechanisms:

- inhibiting cell wall synthesis,
- permeabilizing the target cell membrane,
- inhibiting RNase or DNase activity

Gram positive bacteria (nisin, lacticin, pediocin, lysostaphin, enterocin),

Gram negative bacteria (colicin, microcin, pyocin, klebicin, alveicin, glynericin, enterocoliticin)

Archae bacteria (halocin, sulfolobicin).

NISIN

Discovered in 1928 and has been widely used as food preservative.

pPolycyclic peptide produced by *Lactococcus lactis* ssp. lactis.

Molecular weight of 3,510 Da and the main variants are A, Z and Q of which Z is relatively more soluble at neutral pH.

- Phospholipids in the cytoplasmic membrane are the primary targets for nisin.

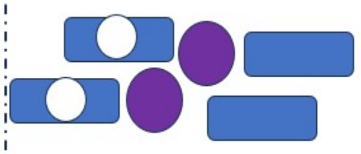
- Forms pores in the membrane, leads to leakage of ions and metabolites causing functional losses and biosynthesis.

Nisin also exhibits activity against endospores as it prevents the post-germination swelling and outgrowth of bacterial spores

Nisin was confirmed as GRAS in 1988 by U.S. Food and Drug Administration

Friendly Microbes / Microbial Products

PROBIOTICS

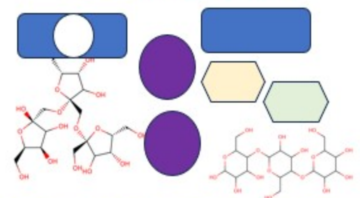


Living microorganisms that provide health benefits for the host

PREBIOTICS

Oligosaccharides that act as substrates for bacterial fermentation within the gut

SYNBIOTICS

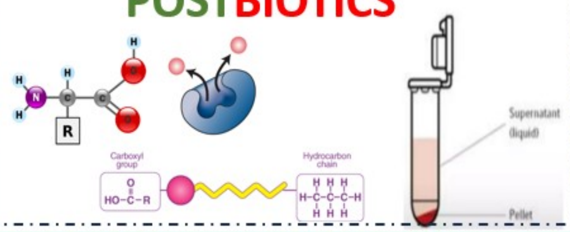


Co-administration of probiotics and prebiotics, to confer synergistic benefits

PARABIOTICS

Non-viable counterparts of probiotics

POSTBIOTICS



Soluble factors or metabolic by-products secreted by bacteria

Mechanism

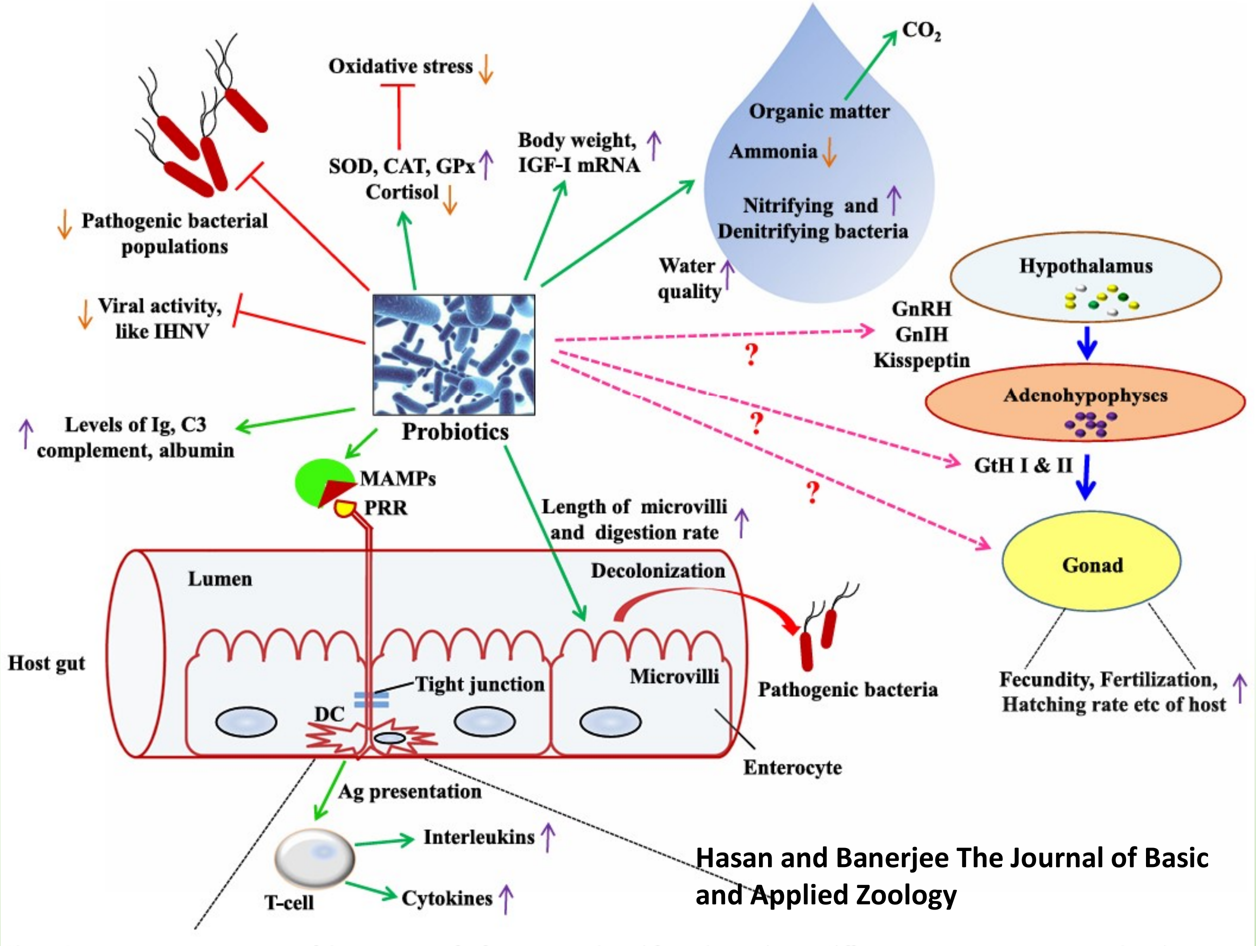
- Modulate gut microbiota.
- Stimulate immune function.
- Strengthen antioxidant defense.
- Modulate gene expression.
- Increase resistance to disease.

- Enhance **SURVIVAL**
- Enhance **GROWTH**

Probiotics

- Probiotics improve body weight, digestion rate, surface area of microvilli, antioxidative enzymes, stress tolerance, immune response, fecundity, fertilization of the host, as well as water quality of the aquatic environment.

- It also downregulates (indicated by downwards arrow) pathogenic bacteria and their enteric colonization, viral activity, cortisol level in host organisms.



Hasan and Banerjee The Journal of Basic and Applied Zoology

Selection of Probiotic Strains

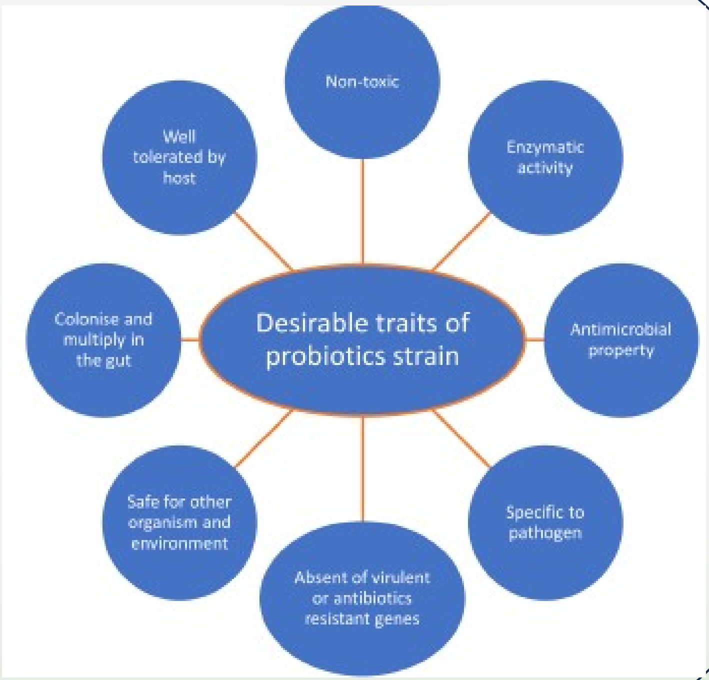
- **Select appropriate probiotic strains that are native to the aquaculture environment**
- **Select the optimum level of use (dose, frequency)**
- **Undertake regular monitoring of the ecosystem to check potential impacts of probiotics.**

CHECKS:

❑ Efficacy

❑ Bacterial Composition & Numbers

❑ AMR potential



Goh et al., (2022)

Probiotics

Name of the probiotics	Beneficial effects
Lactobacillus rhamnosus	Enhance immunity and reduce disease susceptibility
Lactobacillus plantarum	Enhance stress tolerance
Lactobacillus rhamnosus	Improve blood quality
Streptococcus sp.	Improve feeding efficiency and growth rate
Bacillus subtilis	Enhance cellular immunity
Bacillus subtilis + *Lactococcus lactis* + *Saccharomyces cerevisiae*	Enhance survival rate, foster metabolism, enhance weight
Bacillus amyloliquefaciens	Enhance antibody concentration, reduce stre
Bacillus subtilis + *Lactobacillus rhamnosus*	Enhance the food digestibility
Lactobacillus sp.	Reduce pathogen load, provide protection against *Aeromonas hydrophilla*
Bacillus cereu	Protect from *Aeromonas hydrophilla* infection
Different species of *Bacillus*, *Arthrobacter*, *Paracoccus*, *Acidovorax etc*	Reduce pathogen load and provide nutrients
Alcaligenes sp. AFG22	Enhance volatile short chain fatty acids

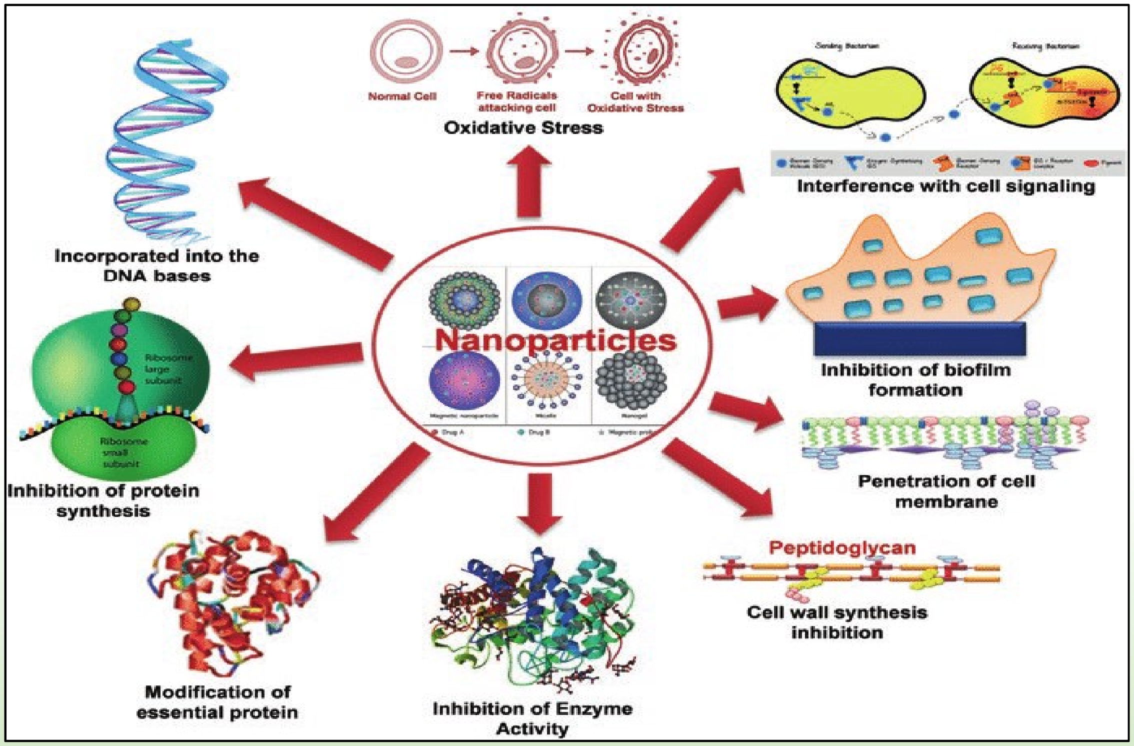
Name of the probiotics	Beneficial effects
Bacillus spp.	Reduces the load of ammonia and nitrite
Enterococcus faecium ZJ4	Improves water quality and enhances immunity
Lactobacillus acidophilus	Improves water quality
Bacillus NL110, *Vibrio* NE1	Reducesammonia and nitrite concentration
Nitrosomonas sp.,*Nitrobacters* sp.	Reduces the concentration of ammonia, phosphates and nitrite in culture pond
Rhodopseudomonas palustris, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae*	Reduces nitrate load, maintain water pH and enhances dissolve oxygen concentration
Paenibacillus polymyxa	Enhances immunity and reduces pathogenic stress
Lactobacillus rhamnosus	Reduces pathogen load in culture tank
Pseudomonas sp.	Enhances transcription rate of anti-microbial peptide
Bacillus spp	Promotes the growth of beneficial algae and reduces the growth of harmful algae
Nitrosomonas sp., *Nitrobacters* sp.	Reduces pathogen load in culture pond and increases dissolved oxygen content

Hasan and Banerjee, The Journal of Basic and Applied Zoology, 2020

Nanobiotics

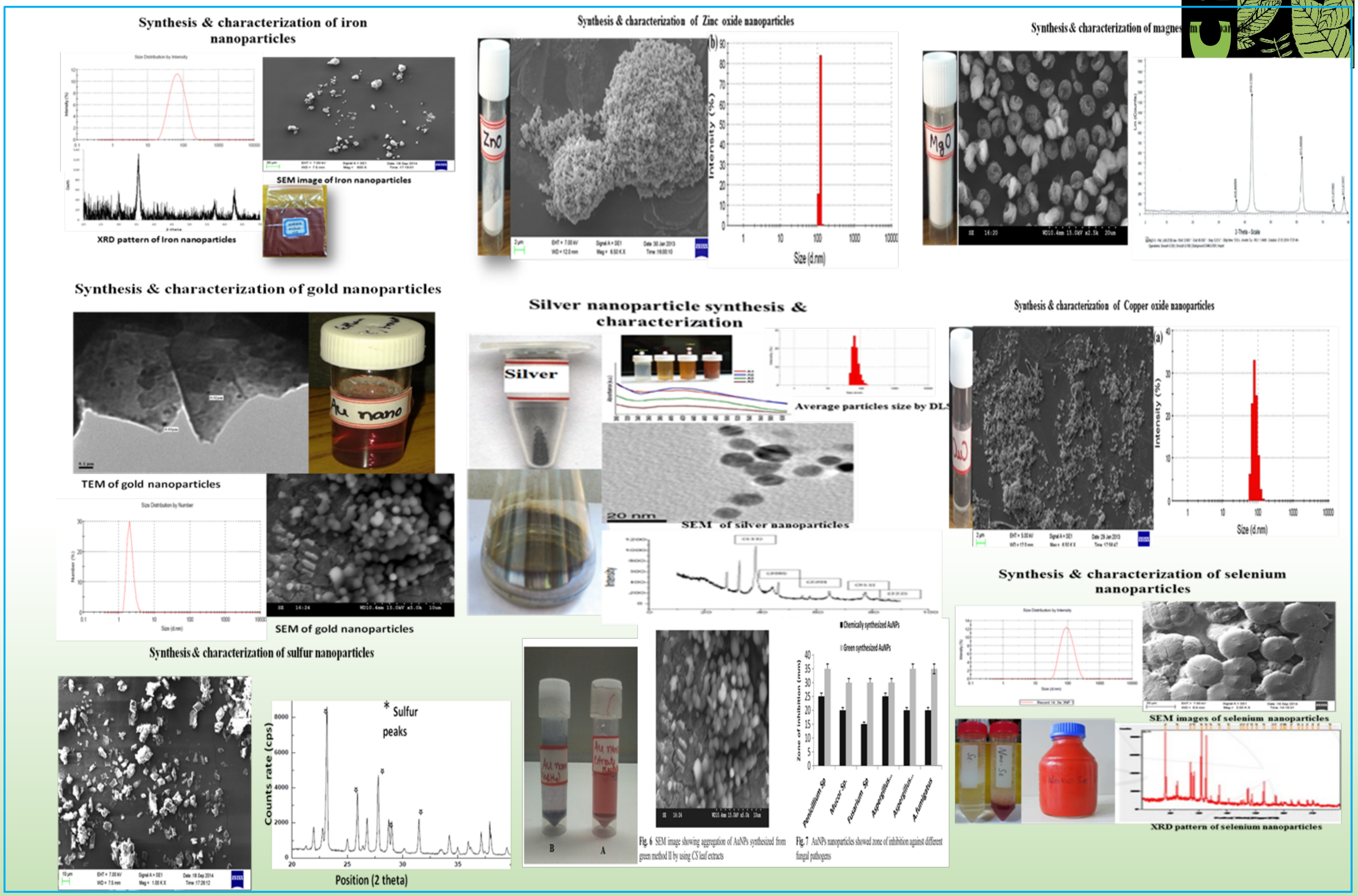
- Certain types of nanoparticles, like silver nanoparticles, zinc oxide nanoparticles, Copper nanoparticle, and titanium dioxide nanoparticles, can act as antimicrobials

- These nanoparticles have antimicrobial properties against important fish pathogens namely, *Aeromonas, Vibrio, Streptococcus, Pseudomonas, Flavobacterium* sps and several fungal pathogens viz. *Saprolegnia, Aphanomyces, Candida* and *Branchiomyces* sps. and different algae.



Green synthesis of nanoparticles

1. Iron nanoparticle
2. Zinc oxide nanoparticle
3. Magnesium nanoparticle
4. Gold nanoparticle
5. Silver nanoparticle
6. Copper oxide nanoparticle
7. Sulfur nanoparticle
8. Selenium nanoparticle
9. Gold nanoparticle



Metal nanoparticles enhance hatching and survival of fish larvae and fry

- **CuO and ZnO exhibited highest percentage of hatching in both direct addition (78.0 ± 3.1% and 78.05 ± 4.2%, respectively) and coating onto tanks (58.6 ± 2.1% and 61.2 ± 2.7%, respectively) at 1 mg ml^{-1}**

- **Highest survival of *L. rohita* fry (50.13 ± 2.2%) was observed after 15 days post hatching in CuO coated tanks followed by ZnO coated tanks (38.6 ± 2.8%)**

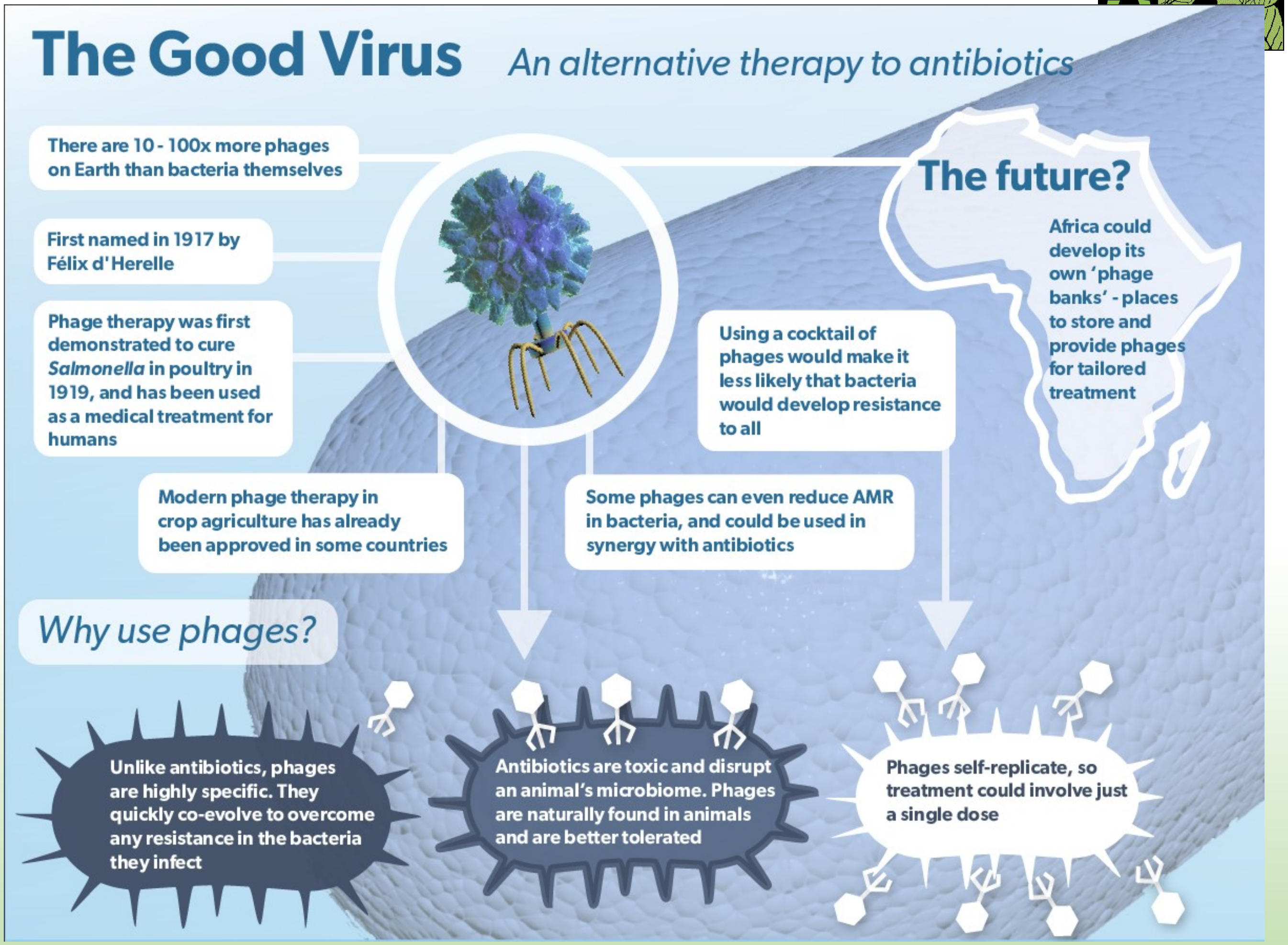
- **Lower microbial load of water was recorded in CuO nanoparticles coated tanks (1.5 9 1010 CFU L $^{-1}$) as compared to uncoated control tanks (1.1 9 1016 CFU L^{-1}) without affecting water quality parameters.**

- **On the other hand, in Ag-TiO2 coated tanks, significantly lower microbial load (1.0 9 106 CFU L^{-1}) as compared to uncoated control tanks at 15 days post hatching**

Coating of metal nanoparticles (Zn, Cu, TiO2) on plastic bioballs, ceramic rings, tanks, jars, aquarium walls for improves water quality

Phage Therapy

- **Phage therapy represents a promising alternative to antibiotics when it comes to bacterial disease control in aquaculture .**
- **The use of phages against pathogenic bacteria in aquaculture was first introduced experimentally in Japan against *Lactococcus garvieae* in 1999, and it has since been a topic of great interest for the aquaculture-related scientific community.**

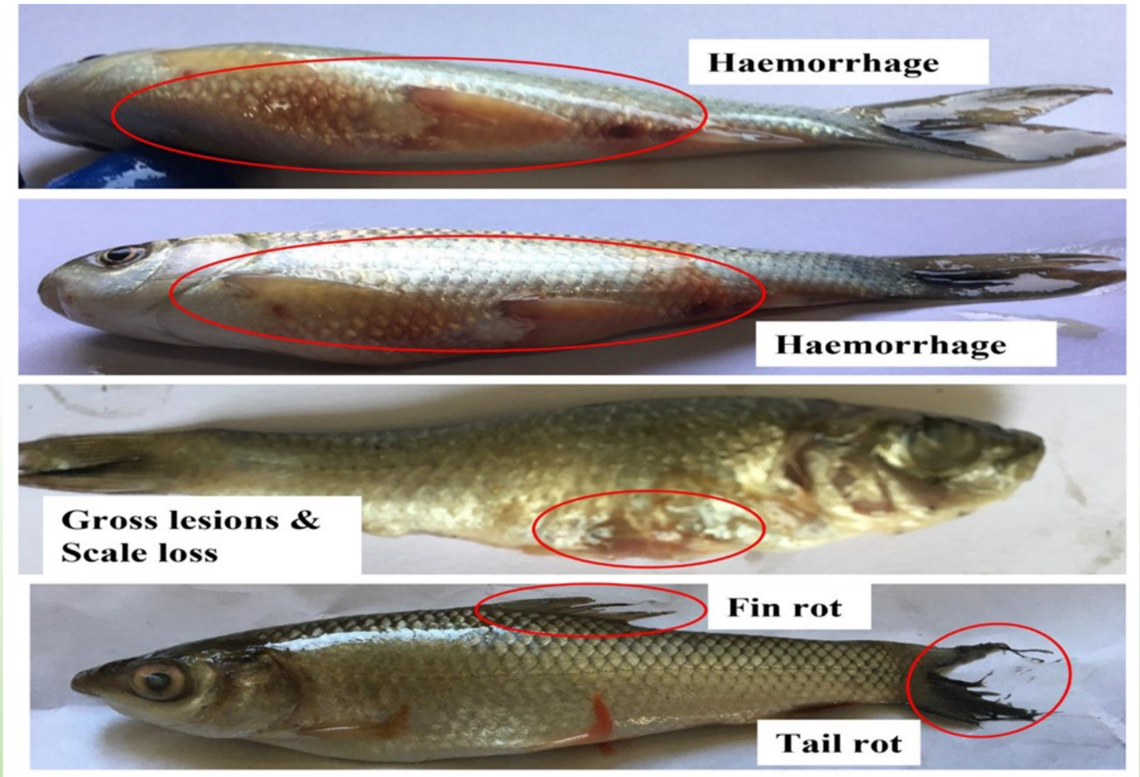


Bacteriophages for control of bacterial infections in fish and shellfish

Fish/ Shellfish on which experiments were performed	Bacteria targeted by bacteriophage	Result of the bacteriophage experiments
Shrimp (*Penaeus monodon*) and Rock lobster (*Panulirus ornatus*)	***Vibrio harveyi***	Survival of shrimp larvae was higher (>85%) in phage treated tanks compared to oxytetracycline and kanamycin treated tanks. Bacteriophage killed *V. harveyi* cells within a short period of 2 hours.
Brine shrimp (*Artemia franciscana*) and Oysters	***Vibrio parahaemolyticus***	Oysters treated with bacteriophage showed a decrease in *V. parahaemolyticus* counts by 2.35 to 2.76 log cfu/g within 36 hours. A cocktail of 2 to 3 bacteriophages was relatively more efficient in reducing *V. parahaemolyticus* counts than using a single bacteriophage.
Oysters	***Vibrio vulnificus***	Bacteriophages used synergistically with oyster extract successfully reduced *V. vulnificus* counts.

Catfish (*Clarias gariepinus*)	***Pseudomonas aeruginosa***	Ulcerative lesions of the infected fish were effectively cured by phage therapy in 8-10 days.
Catfish (*Clarias batrachus*)	***Flavobacterium columnare***	100% of the fish challenged with the bacteria after bacteriophage treatment survived and the load of *Flavobacterium columnare* reduced after 6 hours of phage treatment in the fish sera, gill, liver and kidney.
Japanese eel (*Anguilla japonica*)	***Aeromonas hydrophila***	*A. hydrophila* counts reduced (250 fold reduction) with an increase in phage count (10^6 pfu/ml) in water
Japanese flounder (*Paralichthys olivaceus*)	***Streptococcus iniae***	Lower mortality was observed in fish treated with bacteriophages compared to the control fish.

Oral feed-based administration of phage cocktail protects rohu (*Labeo rohita*) against *A. hydrophila* infection



(Rai et al., 2024)

Phage Therapy Against *Vibrio* spp.

Disease: Vibriosis, caused by *Vibrio* spp., is a major issue in shrimp, grouper, and seabass aquaculture, leading to severe economic losses.

Phages: Phages such as vB_VpS_BA3, Vp5, and Vp6 have been isolated and shown efficacy in reducing *Vibrio* populations in both laboratory and field trials.

A study by Wang et al. demonstrated the efficacy of phage therapy against *Vibrio harveyi*, a pathogen affecting abalone (*Haliotis laevigata*).

The application of two isolated phages significantly improved the survival rates of infected abalone, indicating that phage therapy can effectively treat vibriosis in shellfish.

Mechanism: These phages target specific Vibrio strains, reducing bacterial numbers and improving survival rates in infected animals.

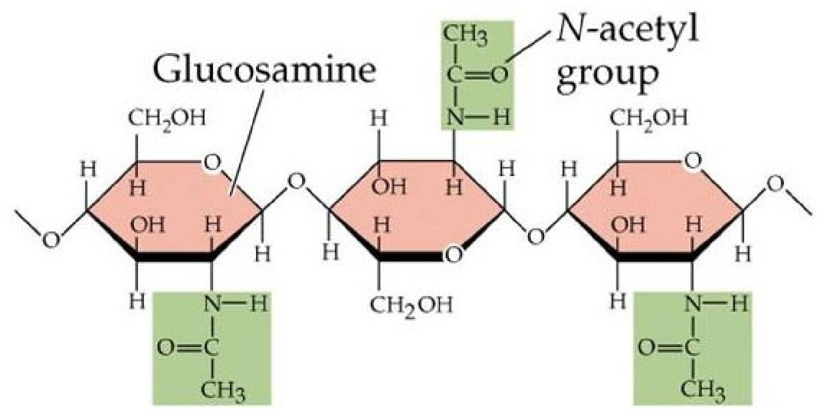
CHITOSAN

Chitosan is a poly-cationic linear polymer of β-(1-4)-D-glucosamine and is produced commercially by deacetylation of chitin.

Low molecular weight chitosans (<10 kDa) and highly deacetylated chiotosans (>95%) have more antibacterial activity

Chitosan has a broad spectrum of antimicrobial activity against gram positive bacteria, gram negative bacteria and yeasts and moulds.

- **Cross linkage between the polycations of chitosan and the anions on the bacterial surfaces that changes the membrane permeability.**
- **Binding of chitosan to teichoic acid present in bacterial cell wall triggers destabilization followed by disruption of cell membrane leading to leakage of cellular components**



Bacteria: Chitosan (95-98% DA) 50 and 200 ppm, *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Vibrio cholerae* and *V. parahaemolyticus*.

Fungi: 200ppm and 500 ppm *Candida albicans* and *Fusarium oxysporum*.

PLANT ESSENTIAL OILS

Essential oils (EO) : aromatic, volatile compounds produced by plants as a defense mechanism against microbes or during adverse conditions such as stress.

Essential oils show broad spectrum antimicrobial activity against different bacteria and fungi

- **Thymol in thyme oil,**
- **Cinnamic aldehyde from cinnamon,**
- **Sesamol in sesame oil,**
- **Eugenol in clove buds,**
- **Terpene in sage and rosemary**

ESSENTIAL OILS

Lipoteichoic acid present in the cell membrane of gram positive bacteria facilitates the penetration of hydrophobic compounds of essential oils and makes them susceptible to essential oils.

In gram negative bacteria the presence of an outer membrane restricts the diffusion of essential oils into the bacterial cell.

Essential oils accumulate in the bilipid layer and disrupt proton motive force, electron flow, active transport and cause coagulation of cell contents.

Plant Extracts: Garlic, turmeric, ginger
(Flavonoids, Alkaloids, Phenolic acid, Terpenoids, Saponins)

Seaweed extracts: Sargassum (brown seaweed)
Asparagopsis (red seaweed)

Herbal Therapy/CIFAs' Role

- CIFA L-Check
- CIFA M-Check
- CIFA Paraherb
- Multiherb therapy for parasites and bacterial infections

Conclusion

- Treatments where necessary, should be done only after accurate diagnosis of fish diseases, with proper doses and frequency under expert's supervision.
- The farmers and State departments should use the approved drugs for aquaculture use.
- Better management practices need to be adopted by the farmers at each stage of fish farming.
- The farming practices are to be done under Biosecurity to prevent occurrence and spread of disease.
- Aquaculture remains as a victim for AMR, since all outlets ends in water
- Use of Natural farming, Nanotechnology, herbal remedies are alternate to reduce AMR
- Mass awareness and regulations.

Our Motto: Healthy Fish Wealthy Farmers

Thank You….