

Review on Bacteria Affecting Pisciculture Leading to AMR Strains, Alternatively Herbals Used to Combat These Bacteria Strains

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To cite this article:

Rupal Mishra, Rupinder Kaur, Gaganjyot Kaur. Review on Bacteria Affecting Pisciculture Leading to AMR Strains, Alternatively Herbals Used to Combat These Bacteria Strains. *Advances in Applied Sciences*. Vol. 8, No. 2, 2023, pp. 36-43. doi: 10.11648/j.aas.20230802.11

Received: January 16, 2023; **Accepted:** February 15, 2023; **Published:** April 18, 2023

Abstract: Pisciculture contributes a big part in global food basket. Fishes are reliable source of nutrients & proteins which are cheap comparatively. Production in pisciculture gets significantly affected (around 60%) by infectious diseases caused by fungi, bacteria, viruses and parasites. Bacteria possess the ability to survive even without the host, which makes them a greater threat in pisciculture environment. More than twenty different bacterial infections are recognized which are caused by various species of bacteria in pisciculture. Method of drug administration differs for diverse types of drugs being used according to their route of administration, stability, effective dosage, etc. which makes them costlier than antibiotics. Use of antibiotics is prohibited as bacterial species gets resistant to the drugs and more & more drug resistant bacteria are emerging. These bacteria end up in our food chain and sometimes lead to transfer of drug resistant genes. The residual antibiotics can even lead to other health problems like hypersensitivity reactions, Carcinogenicity, etc. Herbal extracts or phytobiotics are in focus as antibiotic alternatives as they are environment friendly and there are extremely less chances of drug resistance possible. This review discusses about the need of alternative for the current treatments against drug resistant bacteria which is eventually leading to AMR diseases.

Keywords: Aquaculture, Food Chain Antibiotics Accumulation, Pisciculture, Bacterial Diseases, Antibacterial Drugs, AMR Strains, Antibiotic Alternatives, Phytobiotics

1. Introduction

The world population is expected to reach nine billion by the year 2050 which is currently more than seven billion [88]. Pisciculture a major sector of Aquaculture is a sector that is economically profitable and one of the rapidly growing and blooming sectors worldwide which can help meet the food demand of increasing population [36, 48, 52, 54, 112]. Fishes contribute a big part in global food basket; are cheap and a reliable source of protein & nutrients [88]. Aquaculture can surpass total capture fish production by the 2025 which is estimated to reach 196 million tons [113]. In past 2 decades from 1997 to 2017 the production is estimated to be increased by 78 metric tons and from that 90% production comes from Asia [27, 70, 94]. The economic losses are majorly due to overcrowding, temperature changes, culture

intensification, disease outbreaks and spread of pathogens [9, 21, 48, 59, 63, 73]. Infectious diseases caused by fungi, bacteria, viruses and parasites are reason for around 60% of production loss and consumers are much more aware and concerned of the food safety so good practices should be adopted to ensure quality and safety [5, 20, 25, 29, 38, 46, 47, 59, 78, 90].

2. Problem of Bacterial Diseases in Pisciculture

Bacteria can survive independently of their hosts therefore bacterial diseases are one of the major threats as in aquatic environment [59]. Fishes cannot be not confined to a single location which makes controlling a disease a vital task in

aquaculture as their motions make individual diagnosis and treatment difficult [90]. Bacterial genera belonging to gram-negative bacteria such as *Aeromonas*, *Edwardsiella*, *Flavobacterium*, *Francisella*, *Photobacterium*, *Piscirickettsia*, *Pseudomonas*, *Tenacibaculum*, *Vibrio* & *Yersinia*; and gram-positive bacteria such as *Lactococcus*, *Renibacterium* and *Streptococcus* are most pathogenic to aquatic animals [59]. Foodborne diseases are common due to bacterial pathogens;

many countries reject the fish lots after investigating the microbial pathogens leading to major economic losses [39]. Although there is no common mechanism of action of these pathogens but different paths and virulence factors have been studied [102, 103]. Co-infection is also a major concern where these pathogens can infect the host fish with three-strain co-infection or two-strain co-infection and the intensity of infection depends on the infecting strains [49].

Table 1. Common bacterial diseases and causative agents.

Sr. no.	Bacterial Disease	Symptoms	Organism	Gram stain
1	Columnaris Disease [7, 14]	Haemorrhagic and ulcerative lesions on fins, head, back, which may look yellow to orange due to bacterial growth and pigmentation.	<i>Flavobacterium columnare</i> (<i>Flexibacter</i> / <i>Cytophaga columnaris</i>)	-ve
2	False columnaris [81]	Frayed fins, Lesions on the back & White or greyish spots on the head, fins, or gills	<i>Flavobacterium johnsonae</i>	-ve
3	Tail rot and Fin rot [2]	Erosions, discoloration and disintegration of fins and tails.	<i>A. hydrophila</i> , <i>Pseudomonas spp.</i> <i>Cytophaga spp.</i> , <i>Haemophilus</i>	-ve
4	Bacterial gill disease or Gill rot or Environmental disease [80]	Gasping, lethargic, gills look discoloured with trapped materials, secondary fungal infection	<i>Flavobacterium branchiophilum</i> , <i>Cytophaga species</i> , <i>Flexibacter species</i>	-ve
5	Eye disease	Cataract of eyes, affect cornea, eyeball gets putrefied	<i>Aeromonas liquefaciens</i> , <i>Staphylococcus aureus</i> , various other bacteria	-ve
6	Enteric Red Mouth Disease (ERM) / Yersiniosis [51, 106]	Haemorrhagic lesions on skin around mouth, fins, tail. Internal haemorrhages	<i>Yersinia ruckeri</i>	-ve
7	Winter ulcer disease [50, 95]	Ulcers, pale gills and fin rot	<i>Moritella viscosa</i>	-ve
8	Pasteurellosis or Photobacteriosis [43, 93]	Prominent white granules in internal organs such as spleen, kidney or liver. slight darkening of the skin and abdominal distension, hemorrhagic areas on the head and gills	<i>Photobacterium species</i> (formerly <i>Pasteurella species</i>)	-ve
9	Piscirickettsiosis or rickettsial septicaemia [53, 64, 71]	Skin lesions, darkening of skin and pale gills, swollen abdomen, grey, swollen spleen and kidney	<i>Piscirickettsia salmon</i> and <i>Piscirickettsia</i> -like organism	-ve
10	Tenacibaculosis [22, 55, 98]	Ulcers, hemorrhagic and necrotic lesions (skin, fins & tail), hemorrhagic stomatitis and corrosion of the tail & fins	<i>Tenacibaculum maritimum</i>	-ve
11	Aeromoniasis/ Motile <i>Aeromonas</i> septicaemia / Furunculosis [45, 79, 89, 99, 107]	Haemorrhagic and ulcerative lesions on skin fins, head, exophthalmia	<i>Aeromonas hydrophila</i> , <i>A. veronii</i> ssp. <i>Sobria</i> , <i>A. sobria</i> Motile <i>Aeromonas</i> species.: <i>Aeromonas caviae</i> , <i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. veronii</i> , <i>A. jandaei</i> ; <i>A. salmonicida</i>	-ve
12	Edwardsiellosis/ <i>Edwardsiella</i> septicaemia / putrefactive disease [15, 57, 76, 104]	Ulcerative abscesses in internal organs, haemorrhagic ulcers on skin, fins and body, rectal protrusion	<i>Edwardsiella tarda</i> , <i>Edwardsiella anguillarum</i> , <i>E. ictaluri</i> , <i>E. piscicida</i> , <i>Yersinia ruckeri</i>	-ve
13	Vibriosis / Septicaemia/ Ulcerative disease/ Intestinal necrosis/enteritis/ Ascites disease/ Eye disease, necrotising enteritis, vasculitis, Granuloma/ Eye disease [31, 65]	Ulcerative abscesses in internal organs, haemorrhagic ulcers on skin, fins and body	<i>Vibrio anguillarum</i> , <i>V. parahaemolyticus</i> , <i>V. alginolyticus</i> , <i>V. harveyi</i> , <i>V. anguillarum</i> , <i>V. harveyi</i> clade, <i>V. parahaemolyticus</i> , <i>Aliivibrio salmonicida</i> (<i>V. salmonicida</i>), <i>V. vulnificus</i> , <i>Photobacterium damsela</i> , <i>Vibrio aestuarianus</i> , <i>V. ichthyenteri</i> , <i>V. mimicus</i> , <i>V. ordalii</i> , <i>V. ponticus</i> , <i>V. scophthalmi</i> , <i>V. splendidus</i> , <i>V. vulnificus</i>	-ve
14	Pseudomoniasis/ <i>Pseudomonas</i> septicaemia/ red spot disease	Haemorrhagic lesions on skin, fins, tail	<i>Pseudomonas sp.</i> <i>Pseudomonas fluorescens</i> <i>Pseudomonas anguilliseptica</i> , <i>P. fluorescens</i>	-ve
15	Flavobacteriosis/ rainbow trout fry syndrome [1, 110]	Bacterial gill disease	<i>Flavobacterium branchiophilum</i> , <i>F. columnare</i> , <i>F. psychrophilum</i> , <i>Tenacibaculum maritimum</i>	-ve
16	Chryseobacteriosis [62, 108]	White patches on the gills, excessive mucus secretion, caudal peduncle lesions, haemorrhagic eye and opercula, scale loss, skin discoloration, skin peeling, emaciation, pale kidneys and black spleen	<i>Chryseobacterium species</i>	-ve
17	Francisellosis [32, 97, 109]	Erratic swimming, spiralling, buoyancy control problems, anorexia, lethargy, exophthalmia, anaemia, petechiation, darkening, necrotic gills with red and white patchiness	<i>Francisella species</i>	-ve
18	Mycobacteriosis [6, 37, 74]	Skin lesions; wasting exophthalmia; weakness, color changes	<i>Mycobacterium fortuitum</i> , <i>M. marinum</i> , <i>Nocardia asteroides</i> , <i>N. crassostreae</i> (<i>ostreae</i>), <i>N. seriolae</i>	+ve
19	Streptococcosis [8, 44, 101]	Erratic swimming, loss of buoyancy control, lethargy,	<i>Streptococcus agalactiae</i> , <i>S. iniae</i> ,	+ve

Sr. no.	Bacterial Disease	Symptoms	Organism	Gram stain
		darkening, exophthalmia, corneal opacity, haemorrhage, ascites and ulcerations.	<i>Lactococcus garvieae</i> , <i>Aerococcus viridans</i> , <i>Streptococcus ictaluri</i> , <i>Streptococcus dysgalactiae</i> , <i>Streptococcus parauberis</i> , <i>Streptococcus phocae</i> , <i>Vagococcus salmoninarum</i>	
20	Renibacteriosis/ Bacterial kidney disease [75]	Lethargy, skin darkening, protruding eyes (exophthalmia), anaemia, distended abdomens, blood-filled blisters on the flanks and bruising (haemorrhaging) around the vent, presence of fluid in the abdominal cavity, swollen kidneys and diffuse white membranes over the internal organs.	<i>Renibacterium salmoninarum</i>	+ve
21	Lactococcosis [19, 28, 69]	Anorexic, darkening of skin color, sluggish movement, erratic swimming. Swollen abdomen, anal prolapsus, exophthalmia, cataracts, congestion of the internal organs, spleen and liver enlargement etc	<i>Lactococcus garvieae</i> (formerly <i>Enterococcus seriolicida</i>)	+ve
22	Nocardiosis [60, 100]	Nodules in gills, spleen, kidney and liver, with or without multiple skin ulcers and nodules.	<i>Nocardia</i> species	+ve
23	Haemorrhagic septicemia [4, 58, 111]	Haemorrhaging (bleeding), Bulging eyes, Anaemia, Bloating abdomens, Erratic swimming	<i>Weissella</i> species	+ve

3. Antibacterial Drugs Used in Pisciculture

Treating a diseased fish requires a few factors to be considered like diagnosis & identification of aetiological agent, species of fish, whether it is a systemic or localized disease, culture environment, heterogeneity, appetite and nature of drug required [10, 68]. Different drugs tend to have

distinct spectrum of activity. Some act better on gram positive bacteria well some act better on gram negative bacteria. The most effective drug should be taken ahead after evaluating the susceptibility against target pathogen. Guidelines for Disc diffusion and broth dilution methods of susceptibility testing should be followed according to rules provided by Clinical and Laboratory Standards Institute (CLSI) [16].

Table 2. Mechanisms of Action (MOA) of Antibacterial Drug [18, 33].

Sr. No.	Drug	Target	MOA	Spectrum	For Gram Character
1	Tetracycline	protein synthesis inhibitors	Inhibit the binding of aminoacyl-tRNA to the mRNA – ribosome complex	Broad	Both –ve and +ve
2	Penicillin	bacterial cell wall	Inhibits the cross-linking of peptidoglycan in the bacterial cell wall	Narrow	+ve
3	Macrolide	protein synthesis inhibitors	Prevent peptidyl transferase from adding the peptidyl attached to tRNA to the next amino acid	Intermediate	+ve
4	Quinolone	DNA gyrase	Inhibit the bacterial enzyme DNA gyrase	Broad	-ve
5	Sulfonamide	Prevents Folic acid synthesis	Binds and inhibits enzyme dihydropteroate synthase (DHPS)	Broad	Both –ve and +ve
6	Nitrofurantoin	reduced by bacterial flavoproteins to reactive intermediates	Inhibit bacterial ribosomes and other macromolecules	Broad	Both –ve and +ve
7	Chloramphenicol	protein synthesis inhibitor	Inhibits the peptidyl transferase activity of bacterial ribosomes	Broad	Both –ve and +ve
8	Florfenicol	protein synthesis inhibitor	Inhibits protein synthesis by binding to ribosomal subunits	Broad	Both –ve and +ve

4. Harmful Effects of Antibiotics

The residual or accumulated antibiotics can stimulate carcinogenicity, hypersensitivity reaction, disruption of normal intestinal flora and exchange of genetic information between the mobilome of aquatic & terrestrial bacteria [41, 98]. Antimicrobial drugs used in pisciculture globally in the year 2017 were approximately 10,000 tons and which is expected to increase up to 13,000 tons by year 2030. Figure 1 shows the intensity of antimicrobials consumption on varied species in pisciculture [27].

Studies have shown some shared genetic elements and resistance determinants between aquatic and human pathogens which originated in aquatic bacteria. Quinolones, tetracyclines and beta-lactamases are known to have resistance in both aquatic and human pathogens. This shows that antibiotics are negatively affecting both aquatic and terrestrial worlds and their use should be controlled because the antibiotic resistant pathogens are increasing in number [17, 40, 41, 114]. For major resistant bacteria's; more than 256 microgram antibiotics is the MIC (minimum inhibitory concentration) which is very high in amount [82]. Few plants have shown high efficiency as anti-microbial in fish farms

and also possess the ability to boost immune response; their use can reduce the usage of antibiotics [12, 92]. Antibiotics can control bacterial infections but their excessive use and residues are the major problem for environment [72]. Some well-known side effects of antibiotics are nephrotoxicity and immuno-modulation. Antibiotics are mostly mixed with the feed and then fed to fishes or are added to water directly. This creates an unfavourable environment and pressure on the fishes where bacteria can take advantage of mobilome and exchange the genetic elements [66]. While the exchange of genetic elements i.e. itinerant genes; genes for antibiotic resistance are also shared via plasmids and transposable elements [11, 23]. Bacteria in such environment ends up

acquiring genes encoding enzymes, genes for activating enzymatic reactions, genes for a metabolic pathway and mutations that limit the access of antimicrobial agents to the intracellular target site via the down regulation of porin genes [56, 66]. Multidrug resistance in bacteria is another major concern which can take place via two mechanisms i.e. accumulation of different genes offering resistance to different bacteria or by increased expression of genes that code for multidrug efflux pumps that excrete a wide range of drugs [66]. Another major reason for multi-drug resistance bacteria is usage of water contaminated by sewage waste containing different human pathogenic bacteria [24].

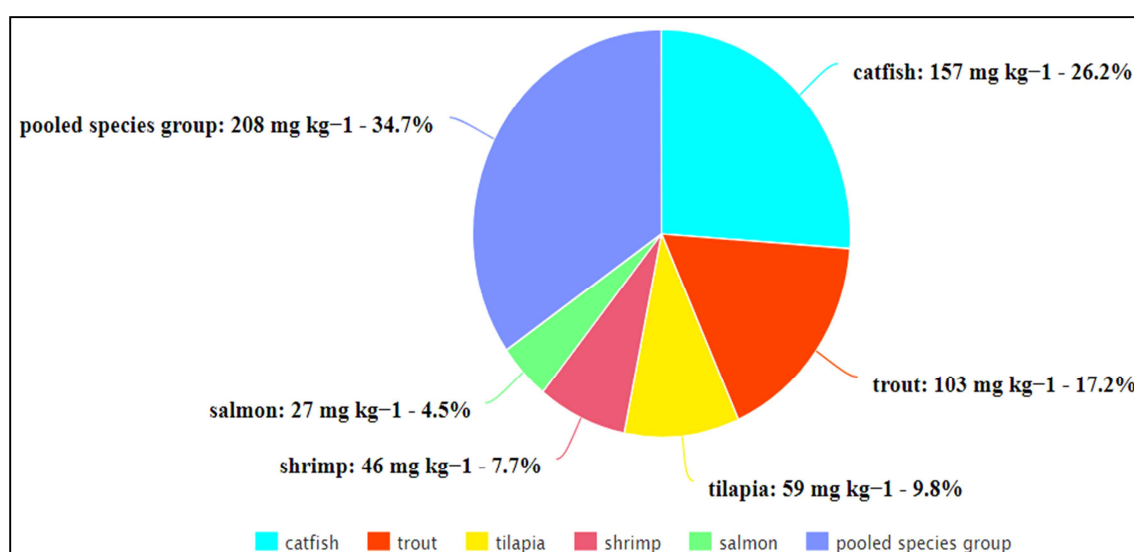


Figure 1. Consumption of Antimicrobials per species group [26].

5. Alternatives to Antibiotics

For a sustainable further development of the aquaculture industry it has become a necessity to find an alternative novel strategy to control bacterial infections with compounds that has anti-bacterial properties and is also environment friendly [34].

- i. **Phytochemicals** - Phytochemicals have the potential to replace antibiotics as many plants are known to possess phytochemicals with anti-bacterial property while some phytochemicals can even effect quorum sensing activity in bacteria. Phytochemicals are also known to increase fish immunity, help growth and treat many different disorders [34].
- ii. **Vaccination** -Vaccination assures long term protection by creating immunological memory. The outer membrane proteins are conserved and function as potential and efficient vaccine targets whereas antigens differ even in same bacterial species which acts as obstacle to create vaccine [67].
- iii. **Immunostimulants**-Immunostimulants are compounds that have the capability to activate or boost the immune system of aquatic animals and provide the

animal with higher resistance power to protect them from bacteria, fungus, virus and parasite [30].

- iv. **Probiotics** -Probiotics act in the digestive tract of animal and inhibits the colonization of potential pathogens by diverse ways and are known to inhibit fish pathogens. They aid in digestion by breaking down indigestible components, stimulating appetite, improving nutrition, detoxifying diet compounds etc. Non-pathogenic bacteria are suggested to be used as probiotics to control antibiotic resistance [13].
- v. **Prebiotics** – They are naturally present in diet and are non-digestible. They improve growth, disease resistance, nutrient digestion and promote growth of gut microbes to improve host health providing the immunity to fight against pathogenic microbes [115].
- vi. **Bacteriophage** -Being natural enemies of bacteria; bacteriophages are great candidates to replace antibiotics. They stop the replication of bacterial cell by interfering with antitoxin expression and thus blocking the process of translation in bacteria [86].
- vii. **Prophenoloxidase (proPo)** - Innate immune response consists of proPO system which has several proteins and activation of proPO cascade leads to melanization that is incredibly important in

invertebrate immune response. Activation of proPO enzyme results in formation of melanin by converting phenols into quinines [42].

- viii. Antimicrobial peptides - AMPs have immunomodulatory and anti-microbial activity and function as first line of defense. It has cationic or anionic property, amphiphatic structure, length less than 150–200 amino acid residues and are produced by crustacean and stored in its hemocyte cells.

6. Conclusion

More than twenty distinct bacterial infections disrupt the pisciculture, which leads to fish deteriorating in massive quantities and significant economic loss. These diseases are caused by many different bacterial species. The pressure to boost fisheries productivity has resulted in antibiotic overuse, which has contributed to antibiotic resistance and an influx of antibiotic-resistant bacteria in the food chain.

Antibiotic alternatives are actively being researched, but no thorough analysis has been done to determine which is best for pisciculture. Alternatives can only be trusted when comparison studies are done to determine whether option is more cost-effective, quick, practical, and produces superior results.

Currently antibiotics are favoured to prevent or treat diseases. In addition to being non-ecofriendly, these substances are causing bacterial resistance and AMR strains to grow in number. There are a variety of eco friendly drug options that can avoid antibiotic resistance while also being environmentally friendly.

As there are many undiscovered phytochemicals that may be investigated, phytotherapy may be the greatest choice. Numerous researches have been done on new plant-derived chemicals but there are still issues with their practical application is limited looking at problems like phytochemicals stability, production in large quantities, appropriate method of extraction and development. The antibacterial effects of individual phytochemicals and their combined effects can be compared in investigations. Additionally, they can be compared to examine the antimicrobial effects of plants growing in various environments, such as on land, in fresh water, salt water, and at various altitudes.

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