Indian J Anim Health (2022), 61(1): 14-22
DOI: https://doi.org/10.36062/ijah.2022.14821

Review Article

Impact of antimicrobial use and antibiotic resistant pathogens in aquatic products - An Indian perspective

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Abstract

Among the animal food production sector, aquaculture represents a large share of the global antimicrobial consumption as the industry shows an unprecedented growth by means of intensification. The higher production pressure inevitably results in the loss due to overstress and diseases. Thus for limiting morbidity and mortality, practice of indiscriminate use of antibiotics within the aquaculture system became more common. This contributes to the emergence and transmission of drug resistance in both pathogenic and non-pathogenic microorganisms, posing a severe health risk to the human consumer. Moreover, transboundary diffusion of drug-resistant pathogens occurs at greater pace, thatwill seriously impact the seafood trade also. Thus, key action plans are required to control antimicrobial resistance (AMR) by means of proper surveillance of the entire food chain. This review takes a look at the potentially growing contribution of the aquaculture industry to the universal burden of AMR and the important preventive measures.

Key words : AMR surveillance, Antibiotic resistance, Aquaculture, Intensification, Transboundary diffusion

Highlights

- Antibiotic uses are often for the therapeutic, metaphylactic and prophylactic purposes in aquaculture.
- Emergence of AMR is mainly due to the misuse and overuse in clinical, animal and aquaculture settings.
- Aquatic environmental settings act as reservoir and amplification of AMR (hotspots) pathogens.
- Stringent Good Aquaculture Practice, hygiene, biosecurity measures, restricted AMU, use of vaccines, phytochemicals and phage therapy will reduce the emergence and spread of AMR.

Introduction

India experienced an eighteen-fold increase of fish production within the past seven decades with the annual increase of 0.75 million metric tons in 1950-1951 to 14.16 million metric tons in 2019-2020 (Handbook on Fisheries Statistics, 2020). Globally, the country now takes the second position, after China, with regard to annual aquaculture production (FAO, 2020). Indian marine product exports witnessed impressive growth from 37,175 tonnes in 1970 to 12,89,650.90 tonnes in 2019-2020 and the frozen shrimp continued to be the main export value item accounting for a share of 74.31% of the total US $ earnings of which the contribution of cultured shrimp is 90% (http:/www.mpeda.com). Thus the aquaculture industry showed exponential growth by means of super intensive culture practices. But this expansion and intensification of aquaculture farming is causing severe stress and the cultured species are becoming susceptible to many diseases. This is the most common reason for the use of animal drugs in aquaculture. Globally, antimicrobial consumption in aquaculture in 2017 was estimated at 10,259

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tons, with Asia- Pacific region being the largest consumer of antimicrobials, and it is projected to increase 33% by 2030 (Schar *et al*., 2020). Some of the FDA approved drugs used in aquaculture are listed in Table 1 (https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs). Unfortunately antibiotic use is now an integral part of intensive farming and is widely practiced as growth promoter and prophylaxis measure. Some commercially available growth enhancing feedmix containing antibiotics are listed in Table 2 (Bhushan *et al*., 2016). The use of antibiotics to treat the infected fish in the entire farm leads to the development of resistance in the entire microbial population of the

Table 1. List of FDA approved animal drugs used in aquaculture (Grant, 2021)

Sl. No	Drug	Proprietary name	Route of administration	Indication for use
1	Florfenicol	Aquaflor®	Medicated Articles / Feeds	• Warmwater Finfish- control of streptococcal septicemia associated with *Streptococcus iniae*. • Salmonids- control of mortality due to coldwater disease associated with *Flavobacterium psychrophilum* and furunculosis associated with *Aeromonas salmonicida.* • Finfish- control of mortality due to columnaris disease associated with *F. columnare*. • Catfish- control of mortality due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.
2	Oxytetracy-clinedihydrate	Terramycin®	Medicated Articles / Feeds	• Salmonids- control of ulcer disease caused by *Haemophiluspiscium*, furunculosis, bacterial hemorrhagic septicemia caused by *A. hydrophila*, and pseudomonas disease. • Catfish- control of bacterial hemorrhagic septicemia caused by *A. hydrophila* and pseudomonas disease. • Lobster- control of gaffkemia caused by *Aerococcusviridans*. • Pacific Salmon-For marking of skeletal tissue. • Freshwater-reared salmonids, weighing up to 55 gm-For marking the skeletal tissue
3	Sulfamerazine	Sulfamerazine	Medicated Articles / Feeds	• Trout- control of furunculosis
4	Ormetoprim/ Sulfadimethoxine combination	Romet-30®	Medicated Articles / Feeds	• Catfish - control of enteric septicemia • Salmonids- control of furunculosis

aquaculture production system including the beneficial microbes. The term AMR denotes the ability of microbes to resist the effects of drugs, so that either their growth is not stopped or they are not killed or both. The main mechanism of resistance to antimicrobial agents may fall under any one of these categories: changes in the bacterial cell wall permeability or target sites, enzymatic drug modifications or degradation, or and efflux of drugs with the help of membrane bound pumps (Reygaert, 2018).

Another major issue concerned with the imprudent usage of antibiotics is the direct harmful effect of the drug residues in humans causing allergy, cancer etc. For example, chloramphenicol (CAP) residues cause myelosuppression and CAP-induced aplastic anemia (Hanekamp and Bast, 2015), likewise nitrofurans and their metabolites have non-neoplastic effects, genotoxicity and carcinogenicity (EFSA-2015) Panel on Contaminants in the Food Chain (CONTAM, 2015). The aqua farmers also use veterinary drugs since no specific antibiotics are prescribed for aquaculture system. But the problem of veterinary antibiotics in shrimp is a cause of major concern in the Indian shrimp export sector. The 1995 Prevention of Food Adulteration Act & Rules (Part XVIII) regulates amount of antibiotics in aquaculture and residue tolerances in shrimp and fish tissue (Bhawan 2011). The antibiotics and other pharmacologically active substances banned for use in shrimp aquaculture by Coastal Aquaculture Authority (CAA) are chloramphenicol, nitrofurans, neomycin, nalidixic acid, sulfamethoxazole, dimetridazole, metronidazole, ronidazole, ipronidazole, other nitroimidazoles, sulphonamide drugs, fluroquinolones, glycopeptides, clenbuterol, diethylstilbestrol, chloroform, chlorpromazine, colchicine, dapsone and aristolochiasp and preparation thereof (http://caa.gov.in/uploaded/doc/Pharmacologically.pdf). Export Inspection Council of India provides specifications for maximum residual limits (MRLs) for antibiotics in fish and fishery products; 0.1 pmm for tetracycline and OTC, 0.3 ppm for oxolinic acid, 0.05 ppm for trimethoprim and zero tolerance to CAP, furazolidone, neomycin,

Table 2. List of some commercially available growth promoter containing antibiotics (Bhushan *et al.*, 2016)

SSSL Sl. no	Feedmix	Antibiotics present	Dose	Benefits	Produced company
1	Oxy-100-FS	Oxytetracycline	-	• Growth promoter • Prevents and controls all kinds of bacterial diseases • Increases growth and body weights	Neospark Advanced Aqua Biotechnologies
2	DOX-ADD	Doxycycline (2%)	1-2 kg/ton of feed (7-10 days)		
3	ADDCIP-M	Ciprofloxacin(25 gm) Metronidazole (25 gm)			
4	FURZAZ-20	Furazolidone (20%)			
5	OXYTREAT-5	Oxytetracycline hydrochloride (10%)			
6	DOX-KZ	Doxycycline hydrochloride	1-2 kg/ton of feed (7-10 days)	• Prevents and controls all kinds of bacterial diseases • Improves digestion & absorption of nutrients by reducing the bacterial load in the gut • Increases growth and body weights	Kaizen Biosciences
7	OXYTREAT-10	Oxytetracycline			
8	FURA TREAT-20	Furazolidone (20%/ 200 gm)			

nalidixic acid, and sulfamethoxazole. However, for export, the residual levels are fixed by individual countries for specified products (www.eicindia.gov.in).This review discusses how aquaculture is emerging as an AMR hotspot, with rampant use of antibiotics and other chemicals and also the important mitigation strategies to combat this menace.

Evidence of antibiotic residues and bacterial pathogens in fishery products exported to European Union (EU) and United States (US): During 2001 to 2015, a complete of 362 Rapid Alert System for Food and Feed (RASFF) notifications associated with fishery exports from India to EU were notified (https://webgate.ec.europa.eu/rasH-window/portal/event). The major quality issues in the exported fishery products (71% Crustaceans, 15% Cephalopods and 14% Finfish) were veterinary antibiotics (52%), heavy metals (14.4%), and pathogenic microorganisms (12.4%). The residues of veterinary medicines detected in fishery products were furazolidone (AOZ), nitrofurazone (SEM), oxytetracycline (OTC) and CAP and the major pathogenic bacteria included *Vibrio* species (*Vibrio cholerae*/ *V. cholerae non-O1*/ *non-139*, *V. parahaemolyticus*, *V. vulnificus*) and *Salmonella* species (*Salmonella paratyphi B*, and *S. weltevreden*) (Madhusudhana *et al.*, 2017). So, the EU has established a minimum required performance limit (MRPL) of 1 µg/kg (1 ppb) for Nitrofuran metabolites and 0.3 µg/kg for chloramphenicol in aquaculture products. However, the EU has zero tolerance to Nitrofurans which suggests "any confirmed concentration of any of the metabolites may be a non-compliance".

Use of antibiotics in aquaculture and its impact on bacterial pathogens: The majority of the detected bacterial pathogens in aquatic products aren't a native flora of fish. It clearly indicates that the major source of these pathogens is the whole production chain *viz.*, contact of the aquatic products to the environment where they're grown, various implements used, contact surfaces, handlers, water etc. The post-harvest handling process plays a major role in the aqua product contamination with the human pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *V. cholerae*, *V. parahaemolyticus*, *Listeria monocytogenes*, *Shigella* spp, *Aeromonas hydrophila*, *Plesiomonas shigelloides* and viral pathogens such as *hepatitis A* virus etc. (Novoslavskij, 2016). Among these pathogens, *E. coli*, *S. aureus*, *Salmonella* spp., and *Shigella* spp. are non-indigenous to the aquatic environment. Depending on the character of the environment (contaminated water), feeding habits (filter feeders), season of harvest (summer) are considered as crucial factors for the contamination of aquaculture products. In addition, the danger is potentiated not only by the presence of those pathogens but also by the presence of antibiotic resistance in them. Worldwide research deviation is noticed on antibiotic resistant pathogens both from the clinical sector and within the food producing animals.

The major antibiotic-resistant pathogens of clinical importance are Methicillin-resistant *S. aureus* (MRSA), Extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*, carbapenem-resistant *Enterobacteriaceae* (CRE), Vancomycin- resistant *Enterococci* (VRE), *Acinetobacter baumannii* and so on (Kraemer *et al.*, 2019). The link between the use of antibiotics in aquaculture production and the presence of antibiotic-resistant food borne pathogens has been already reported by various researchers. Many reports are available for the presence of virulent MRSA from retail food fishes (Sivaraman *et al.*, 2016; 2017; 2021a; 2021c; Muneeb *et al.*, 2021), seafood and environments (Murugadas and Ezhil, 2017), and shrimp aquaculture farms (Rajan *et al.*, 2021). Likewise, the presence of ESBL *E. coli* and *Klebsiella pneumonia* have been detected from retail food fishes (Sivaraman *et al.*, 2020a; 2020b), and shrimp farms (Sivaraman *et al.*, 2021b). Table 3 shows

Table 3. Antibiotic resistant bacteria associated with fish (reports from India)

I. Resistance detected by phenotypic methods

Antibiotic group	Phenotypic resistance	Bacteria	Source	Reference
Beta lactams	Carbenicillin, Ceftazidime, Cephalothin	*Vibrio* spp.	Retail shellfish samples	Sudha *et al.*, 2014
	Ampicillin, Amoxycillin,	*V. parahaemolyticus*	Shrimp farm	Silvester *et al.*, 2015
	Ampicillin	*V. parahaemolyticus*	Shrimp farm	Devi *et al.*, 2009
	Ampicillin, Penicillin	*Listeria monocytogenes*	Fish and fishery environment	Basha *et al.*, 2019
	Penicillin	*V. harveyi*	Shrimp farm	Stalin and Srinivasan, 2016
Sulphonamides	Sulphamethoxazole	*V. parahaemolyticus*	Shrimp farm	Silvester *et al.*, 2015
	-	*Salmonella* spp.	Shrimp farm	Patel *et al.*, 2020
	Trimethoprim	*S. aureus*	Fish	Saharan *et al.*, 2020
Macrolide	Erythromycin	*V. parahaemolyticus*	Shrimp farm	Silvester *et al.*, 2015
		Listeria monocytogenes	Fish and fishery environment	Basha *et al.*, 2019
Aminoglyco-sides	Streptomycin, Kanamycin, Neomycin	*V. parahaemolyticus*	Shrimp farm	Devi *et al.*, 2009
	Streptomycin	*E. coli and Salmonella* spp.	Fish	Saharan *et al.*, 2020
Polymixins	Polymixin-B,	*V. parahaemolyticus*	Shrimp farm	Devi *et al.*, 2009
Tetracyclines	Tetracycline	*L. monocytogenes*	Fish and fishery environment	Basha *et al.*, 2019
Quinolones	Ciprofloxacin,	*V. harveyi*	Shrimp farm	Stalin and Srinivasan, 2016
II. Resistance genotypes				
Beta lactams	*CTX-M*	*E. coli*, *K. pneumoniae*	Shrimp Farm	Sivaraman *et al.*, 2021a
		E. coli, *Staphylococcus* spp.	Retail Seafood	Naik *et al.*, 2017

Cont. Table 3.

Table 3., Cont. ...

Antibiotic group	Phenotypic resistance	Bacteria	Source	Reference
		E. coli, *K. pneumonia*, *Enterobacter* spp., *Citrobacter* spp., *Salmonella enterica*	Retail seafood	Singh *et al.*, 2017
	TEM	*K. pneumoniae*	Shrimp farm	Sivaraman *et al.*, 2021a
		Vibrio spp.	Shrimp farm and retail sea food	Silvester *et al.*, 2019
		E. coli, K. pneumonia, Enterobacter spp., *Staphylococcus* spp.	Retail seafood	Naik *et al.*, 2017
		E. coli, K. pneumonia, Citrobacter spp.,	Retail seafood	Singh *et al.*, 2017
	SHV	*K. pneumoniae*	Shrimp farm	Sivaraman *et al.*, 2021a
		E. coli, K. pneumonia, Enterobacter spp., *Citrobacter* spp., *Salmonella enterica*	Retail seafood	Singh *et al.*, 2017
	NDM-1	Motile *Aeromonads*	Aquaculture environment	Abraham and Bardhan, 2019
Tetracycline	*tetA, tetB, tetC, tetD* and *tetG*,	*Salmonella*	Retail seafood	Deekshit *et al.*, 2012
	tetA, *tetB, tetC, tet D*, *tetE, tetG, tetH,* and *tetM*	Motile *Aeromonads*	Aquaculture environment	Abraham and Bardhan, 2019
Chloram-phenicol	*catA1*	*Salmonella*	Retail seafood	Deekshit *et al.*, 2012
	catB2, *catB3*, *catB8, floR*	Motile *Aeromonads*	Aquaculture environment	Abraham and Bardhan, 2019
Quinolones	*gyrA*, *parC*	Motile *Aeromonads*	Aquaculture environment	Abraham and Bardhan, 2019
Amino glycosides	*aadA1*, *aadA2*, *aadA1a*, *accA4*, *strA-strB*, *aacA*			
Trimethoprim	*dfrA1/7*, *dfrA12*, *dfr13*, *dhfr2a, dhfr1*			
Sulphonamides	*sul1*, *sul2*			
Streptogramin	*VatE*			
Macrolides	*mefA*, *ermC*, *ermE*, *ermX*, *ermC*			

available reports of antibiotic resistant bacteria associated with fish from India. This perhaps shows the importance of studies on AMR pathogens in food-producing animals with special reference to the development of seafood or aquatic products. In general aquaculture products have the close proximity of getting contaminated to various microbes during the entire production and processing chain. Raw foods in general have the highest culturable bacterial loads, followed by minimally and fully processed foods. The food with acceptable microbiological loads (5 lakh and 1 lakh CFU/g for raw and processed aquaculture products) (Rao *et al.*, 2018) can also function as a sink for the event of antibiotic resistances through bacteria, bacteriophages, bacterial DNA and mobile genetic elements etc. Hence, the food chain ecosystem may be conducive niches for gene transfer, antibiotic selection pressure and persistence of AMR bacteria and this route cannot be disregarded.

Antimicrobial-resistant pathogenic bacteria are released into aquatic environments through waste water and act as potential spread of antibioticresistance genes. Many studies show that the trend of antibiotic resistance is changing depending on the country of origin of the seafood and antibiotic usage in a specific country for aquaculture practices etc., (Murugadas and Ezhil, 2017). Laboratory detection of AMR in bacterial pathogens phenotypically and genotypically is key to monitor the situation and to develop effective action plans. Now-a-days there is a shift in the adoption of methodologies for determination of antibiotics resistances, with genotypic methods being implemented at a high throughput level for better understanding of molecular mechanisms of antibiotic resistance.

Controlling of AMR: Already the US and EU have put a control measure to counteract AMR based on the principle of quality management and process-oriented controls throughout the food chain. Implementation of hygienic practices must be verified and certified by the national authorities. Each and every personnel who are involved in the seafood production chain is responsible to interrupt the chain of contamination and spread of the AMR pathogens. WHO, FAO and OIE have taken a collective tripartite joint venture called 'one health' approach to control AMR spread which is considered as national action plans to each country. India established a national plan on AMR (NAP-AMR; 2017-2021), regarding standardization and guidelines for the use of antibiotics. Food Safety and Standard Authority of India (FSSAI) also launched food safety audits and certification in the Indian meat industry. All these actions may be helpful for the restricted use of antibiotics in the animal food production sector. Other mitigation measures proposed to control AMR include strengthening the surveillance of antibiotic usage and AMR pathogens in healthcare, food producing animals and environment, strengthening the laboratory capacity for surveillance system, formulating regulations for the optimized use of antibiotics in human and animal health, creating awareness and understanding among the general public, effective infection prevention and control programmes, development of alternatives to antibiotics, arranging awareness campaigns in farming areas, monitoring hatchery operations, promoting sustaining aquaculture using natural alternatives, promoting best management practices throughout the culture period, and hazard analysis in each step of the food production and transportation chainetc.

Conclusion

AMR is an increasing global public threat, with rapid emergence of newer resistances and faster spread across countries. This results in prolonged illness, complications in surgical conditions due to infection with resistant organisms, severe fatal forms etc. Antibiotic resistance development is a natural action over an extended time; however, the current situation is happening at an elevated speed due to various reasons such as the misuse and overuse of antibiotics as growth-promoter in food-producing animals, improper surveillance and regulation of the utilization of antibiotics etc. AMR organisms are present in human, animal, food and the environment which make the transmission faster between or within humans and animals. AMR is a complex and interdisciplinary issue, holistic efforts and multisector approach is required to bring down the burden of AMR in public.

Author's contributions: All the authors equally contributed in conceiving the idea, drafting the write up, and technical completion of the write up.

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Received –21.12.2021, Accepted – 19.05.2022, Published – 01.06.2022

Section Editor: Prof. S. K. Das, Editorial Board Member