Impact of Antimicrobial resistance (AMR) in aquatic products

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Introduction

India experienced an eleven fold increase of fish production in the past six decades with the annual fisheries and aquaculture production increased from 0.75 million tonnes in 1950-51 to 10.512 million tonnes in 2014-2015 (Handbook on Fisheries Statistics, 2014). Globally the country now takes the second position, after China, with regard to annual fisheries and aquaculture production (FAO, 2014). The aquaculture production system includes the fresh water aquaculture, brackish water aquaculture and marine aquaculture; there is a huge potential to challenge the country's food security. Indian marine product exports witnessed impressive growth from 37,175 tonnes in 1970 to 1051243 tonnes in 2014-15 and the frozen shrimp continued to be the major export value item accounting for a share of 64.12% of the total US$ earnings of which the contribution of cultured shrimp was 73.31% (source: http:/ www.mpeda.com).

The expansion and intensification of aquaculture farming is causing severe stress and become susceptible to many diseases. Occurrence of minor disease results in the loss of the complete aquaculture production system. It is inevitable that higher the production pressure results in the loss due to diseases. Antibiotic use is now an integral part of intensive aquaculture production system and is mainly used for prophylactic and growth enhancing purposes. So as a preventive measures, the aquaculture farmers started using 'antibiotics', and found good results and finally resulted in indiscriminate usage of antibiotics in the aquaculture production system. The use of antibiotics to treat the infected fish in the entire farm leads to the development of resistance in bacteria and also the entire microbial population of the aquaculture production syustem including the beneficial microbes. India was the single largest consumer of antibiotics in the world in 2010, followed by China and the United States (Van Boeckel *et al.*, 2014). The greatest risk associated with the abuse of antibiotics lead to the development of resistant bacteria in aquatic environments. Moreover, aquaculture acts as the medium for antibiotic residues and could spread to the environment. Now a day, antibiotic resistance has emerged as a critical global health priority needing urgent attention.

Antibiotics are commonly used in freshwater farms, brackish-water farms as well as in hatcheries for mass disease control programme and growth promotion purposes. The antibiotic residues are potentially harmful to the consumers like allergy, cancer, chloramphenicol causes aplastic anaemia and nitrofurans are carcinogenic and less responsive to antibiotic treatment of the patient and development of antibiotic resistant bacterial strain. The aqua farmers are bound to use veterinary antibiotics since there is no specific antimicrobial agents are available for aquaculture system.

The problem of veterinary antibiotics in shrimp is plaguing the Indian shrimp export. The 1995 Prevention of Food Adulteration Act & Rules (Part XVIII) bans 20 antimicrobials in aquaculture and sets residue tolerances in shrimp and fish tissue. The antibiotics and other pharmacologically active substances banned for use in shrimp aquaculture by Coastal Aquaculture Authority are Chloramphenicol, Nitrofurans, Neomycin, Nalidixic acid, Sulphamethoxazole, Dimetridazole, Metronidazole, Ronidazole, Ipronidazole, Other nitroimidazoles, Sulphonamide drugs, Fluroquinolones, Glycopeptides, Clenbuterol, Diethylstilbestrol, Choloform, Chlorpromazine, Colchicine, Dapsone and *Aristolochia* sp and preparation thereof.

Evidence of antibiotics residues in fish and fishery products

Antibiotics and bacterial pathogens in fishery products exported to EU: During the last 15 years (2001 to 2015), a total of 362 Rapid Alert System for food and feed (RASFF) notifications related to fishery exports from India to EU were notified. 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 and Chloramphenicol and the pathogenic bacteria includes *Vibrio* species (*V. cholerae/ V. cholerae* non-O1/ non-139, *V. parahemolyticus, V. vulnificus*) and *Salmonella* species (*S. Paratyphi B*, and S. Weltevreden) (Madhusudhana Rao *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 (EU, 2003). However, EU has 'zero tolerance' to Nitrofurans which means 'any confirmed concentration of any of the metabolites is a non-compliance'. It is pertinent to note that Nitrofurans and Chloramphenicol etc., are banned for use in aquaculture in India.

Antibiotic residues in fishery products exported to United States: The antibiotics reported were nitrofurans (47% Furazolidone-AOZ, 37%

Nitrofurazone-SEM, Oxytetracycline and Chloramphenicol). Nitrofurans is being commonly detected in the frozen shrimps exported from India to EU and USA. Nitrofurans and Furazolidone were reported from the frozen shrimp (*M. rosenbergii, P. monodon*, and *L. vannamei*).

Antibiotic residues in cultured frozen shrimp: RASFF notifications due to antibiotics were reported in *Penaeus monodon* (black tiger), *Macrobrachium rosenbergii* (scampi, giant freshwater prawn), *Litopenaeus vannamei* (vannamei shrimp, white leg shrimp), *Parapenaeous stylifera, Metapenaeopsis affinis, Metapenaeus monoceros, Solenocera crassicornis,* Parapaenid shrimp and in some cases the species simply mentioned as frozen shrimp (Madhusudhana Rao *et al.*, 2017).

Use of antibiotics in aquaculture and its impact on bacterial pathogens

Majority of the detected bacterial pathogen in aquatic products are not a native flora of fish. It is clear evidence that the presence of pathogens is mainly from the entire production chain viz., contact of the aquatic products to the environment where they are 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 pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp, *Vibrio cholerae, Vibrio parahemolyticus, Listeria monocyto*genes, *Clostridium botulinum*, Shigella sp, *Aeromonas hydrophila, Plesiomonas shigelloides* and viral pathogens such as hepatitis A virus etc. Among these pathogens, *Escherichia coli, Staphylococcus aureus*, *Salmonella* spp, and *Shigella* spp are non-indigenous to the aquatic environment and others are indigenous to the aquatic environment. Depending on the nature of the environment (contaminated water), feeding habits (filter feeders), season of harvest (summer) are considered as an important factors for the contamination of aquaculture products. In addition, the risk is potentiated not only by the presence of these pathogens but also on the presence of antibiotic resistance. Worldwide research deviation is noticed on antibiotic resistant pathogens both from clinical sector and in the food producing animals. Antibiotic resistant pathogens of aquaculture importance are Methicillin-resistant *Staphylococcus aureus*, Extended spectrum Beta-lactamase producing *Enterobacteriaceae* viz., ESBL *E. coli*, ESBL Salmonella; carbapenamase resistant Enterobacteriaceae viz., *Klebsiella pneumonia*, *E. coli*; Vancomycin resistant Enterococci, *Acinectobacter baumanii* and so on. The link between the use of antimicrobial substances in aquaculture production and the presence of antibiotic resistant food borne pathogens *Salmonella*, pathogenic *E. coli*, *Campylobacter* sp, *Staphylococcus* spp., *Enterococcus* spp. and ESBL has been already reported by various researchers. This perhaps shows the importance of studies on AMR pathogens in the food producing animals with special reference to the seafood or aquatic products development. In general aquaculture products have the close proximity of getting contaminated to various microbes during entire production and processing chain. The 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) may also serve as the sink for the development 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 generally disregarded (Murugadas and Ezhil Nilavan, 2017).

Antibiotic resistance in bacteria: An antibiotic is a drug that kills or stops the growth of bacteria such as penicillin and ciprofloxacin, whereas antimicrobial refers to all microbes viz., bacteria, viruses, fungi, and parasites. Hence, Antibiotic or antimicrobial resistance (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. Mechanism of antibiotic resistance in bacteria. In general main mechanism of resistance to antimicrobial agents in bacteria may falls under any one of these categories, 1. Changes in the bacterial cell wall permeability or target sites 2. enzymatic drug modifications or degradation and 3. Membrane bound efflux pumps- removal of antibiotics through energy dependent.

Trends in antimicrobial resistance among various seafood borne bacterial pathogens, AMR is an increasing global public threat because of their rapid emergence of newer resistances and spread across the various countries and its impact is felt across the globe. This results in prolonged illness; complications in surgical conditions due to infection with resistant organisms, severe fatal forms are also encountered. Antibiotic resistance development is a natural process over a longer time, however the current situation is happening at an elevated speed due to various reasons like misuse, overuse of antibiotics with or without professional oversight, as growth promoting substances in food producing animals, inadequate or inexistent programmes for infection prevention and control (IPC), poor-quality medicines, weak laboratory capacity, inadequate surveillance and insufficient regulation of the use of antimicrobial medicines. AMR organisms are present in human, animal, food, and environment which make the transmission faster than before between or within human and animals.

Possible pathways of AMR spread: *V. parahaemolyticus, V. vulnificus, V. alginolyticus*, and *V. cholerae* are autochthonous Gram-negative bacilli to estuarine and marine environments and found associated with disease through wound infection or through consumption of contaminated seafood especially shellfish. Antimicrobial resistant pathogenic bacteria released into aquatic environments through wastewater and acts as potential spread of antibiotic resistant genes. In general Vibrios sp showed higher resistances towards ampicillin and low to tetracycline. The frequency of resistance reported in aquatic products ranged from 16.6% to 50% level and 10 to 69% of the vibrio strains showed resistance to more than 4 molecules. Common antibiotics showed resistances are teicoplanin, pencillin, oxacillin, vancomycin and low level resistance for cephalosporin groups. Highly resistant to penicillin, ampicillin, tetracycline, and vancomycin was observed in *L. monocytogenes* isolated from seafood and low level less than 10% for tetracycline, enrofloxacin, and ciprofloxacin. The antibiotic resistance pattern and number changes between the serotypes of *L. monocytogenes* isolated from seafood, serotype 1/2a was found to be more resistant than other serotypes. *S. aureus* isolated from fishery products were resistant to penicillin, chloramphenicol and ciprofloxacin and most of them were also resistant to tetracycline. In general to the β-lactams, macrolides, aminoglycosides, ciprofloxacin, co-trimoxazole (4.7%) and tetracycline resistances were observed in most of the studies with varied percentage. Pencillin, macrolides are above 50% and others were less than 50% level. Multidrug resistant strains were also reported in many studies. Salmonella isolated from seafood were in general resistant to the pencillin, erythromycin, tetracycline and other antibiotics were less than 15% level. In a study conducted on imported seafood in to US from 20 countries, *S. enterica* strains of 36 serovar were isolated and twenty isolates showed resistance to at least one antibiotic. Five strains (serovars Bareily, Oslo, Hadar, Weltevreden and Rissen) were resistant to two or more antibiotics. Two *S. enterica* strains (serovars Bareilly and Oslo) from seafood from Vietnam and India were resistant to trimethoprim/sulfamethoxazole, sulfisoxazole, ampicillin, tetracycline and chloramphenicol. Multidrug resistant strains were also observed in Salmonella isolated from seafood. In addition to this, fish are reservoirs for zoonotic pathogens not only infecting the host animal but also humans in contact during aquaculture activity. The infections includes *Aeromonas hydrophilia*, *Mycobacterium marinum*, *Streptococcus iniae, Vibrio vulnificus*, and *Photobacterium damselae* etc are noted few.

All the study demonstrated that there is a change in the trend of antibiotic resistances which depends on the country of origin of the seafood, antibiotic usage in particular country for aquaculture practices etc. Laboratory detection of AMR in bacterial pathogens either qualitatively or quantitatively. Qualitatively antibiotic resistances can be determined by disk diffusion assay for particular antibiotic against the pathogens. Quantitatively antibiotic resistances can be determined by minimum inhibitory concentration (MIC) either in broth dilution or agar dilution. In this the resistances are estimated for concentration from microgram to milligram. MIC can also be performed in microdilution or macrodilution in microtitre plate or tube, respectively. Antibiotic resistances determination can be divided into phenotypic and genotypic. Phenotypic is based on disk diffusion and MIC, whereas genotypic is based on the detection of genes responsible for the antibiotic resistances. Now-a-days there is a shift in the adoption of methodologies for determination of antibiotics resistances. Genotypic methods are implemented in high throughput level for better understanding of molecular mechanism of antibiotic resistance. AMR and seafood trade implications during the last three decades have shown a remarkable increase in World trade of fishery and aquaculture products. 40% of fish producers are now engaged in international trade, majority from Asian countries. In which China gives major shares. Japan, EU and the US are the major importers of seafood for processed products of crustaceans, molluscs and aquatic invertebrates and fish, as well as cured and fresh/chilled fish. If transboundary diffusion of AMR pathogens occurs at greater pace, it may seriously impacts the seafood trade in near future. Already US and EU has put a control measure to counteract this based on the principle of quality management and process oriented controls throughout the entire food chain (from the fishing vessel or aquaculture farm to the consumer's table). Implementation of hygienic practices must be verified and certified by the national authorities. Each and every personnel‘s are responsible who are involved in the seafood production chain to interrupt the chain of contamination and spread of the AMR pathogens (Murugadas and Ezhil Nilavan, 2017).

Controlling of AMR: AMR is a complex and interdisciplinary issue, holistic efforts are required to bring down the burden of AMR in public. WHO, FAO and OIE have taken collective tripartite joint venture called one health approach to control AMR spread which is considered as national action plans to each country. Key action plans proposed to control AMR are

1. Strengthen the surveillance system in healthcare, food producing animals on Antimicrobial usage and antimicrobial drug resistant bugs
2. Emphasis needs to be given to the food and environmental sectors also
3. Strengthening the laboratory capacity for surveillance system
4. Guideline for the optimized use of antibiotics in human and animal health
5. Reduce the infection loss due to AMR pathogens by providing assured quality medicines
6. Awareness and understanding among the general public
7. Effective infection prevention and control programmes

8. Development of alternate to antibiotics protocols
9. Controlling the resistances development in bacteria for medically important antibiotics.

Further reading

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