

Chapter 28

Antimicrobial resistance (AMR) in aquatic products

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Fisheries sector plays an important role in the food security and fish is now traded internationally. Shift in the trading policies (import and export) of seafood/aquatic products are happening at a rapid pace and consumption of seafood increased globally in substantial quantum. It is estimated that aquatic products export from Asian countries outcompetes earlier contributions to their importing partners year after year. Aquatic products includes chordates, molluscs and arthropods from freshwater, brackish and marine system. They are nutrient rich diet and perishable too in nature and this prompted the industry to process the seafood in to different forms such as frozen, canned, cured and dried to extend its shelf life and recently value addition step being followed to improve the customer satisfaction. Nevertheless the risk associated with the transboundary exchange of pathogens of seafood importance and its antibiotic resistance are generally cannot be disregarded. Majority of the pathogens are not a native flora of fish. Each step in the aquatic products production chain either in the captured or cultured fisheries involves the contact of the seafood to the environment where they are grown, various implements used, contact surfaces, handlers, water etc. This post harvest handling makes the seafood contaminated with the pathogens of seafood importance's such as *Escherichia coli*, *Salmonella* spp, *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Shigella* sp, *Aeromonas hydrophila*, *Plesiomonas shigelloides* and viral pathogens such as hepatitis A virus etc. Among these pathogens, *Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus* 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 source), feeding habits(filter feeders), season of harvest (summer season) are very crucial factors which cause seafood inherently contaminated in nature.

In the present scenario, the risk is potentiated not only by the presence of these pathogens but also on the antibiotic resistances they possess. Worldwide research deviation is noticed on antibiotic resistant pathogens both from clinical sector and in the food producing animals. Antibiotic resistant pathogens of seafood importance are Methicillin-

resistant *Staphylococcus aureus*, Extended spectrum Beta-lactamase producing Enterobacteriaceae viz., ESBL *E. coli*, ESBL *Salmonella*; carbapenemase resistant Enterobacteriaceae viz., *Klebsiella*, *E. coli*; Vancomycin resistant Enterococci and so on. The link between the use of antimicrobial substances in food production and the presence of antibiotic resistant foodborne pathogens *Salmonella*, pathogenic *E.coli*, *Campylobacter*, *Staphylococcus* spp., *Enterococcus* spp. and extended-spectrum beta-lactamase (ESBL) has been already proved 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 to exception of commercially sterile and other pro, pre and synbiotics food products, food have the proximity of getting contaminated to various microbes during entire production and processing chain. The raw food in general have the highest culturable bacterial concentrations, followed by minimally and fully processed foods. Minimally or fully processed food including ready-to-eat food contamination depends on the level of sanitary hygiene followed during the processing and preservation steps.

The food with acceptable microbiological quality range may also serve as the sink for the development of antibiotic resistances through bacteria, bacteriophages, bacterial DNA and mobile genetic elements, some of which may include AMR genes. Hence, the food chain ecosystem may be conducive niches for gene transfer, selection and persistence of AMR bacteria and this route cannot be generally disregarded.

Antibiotic resistance in bacteria: definition

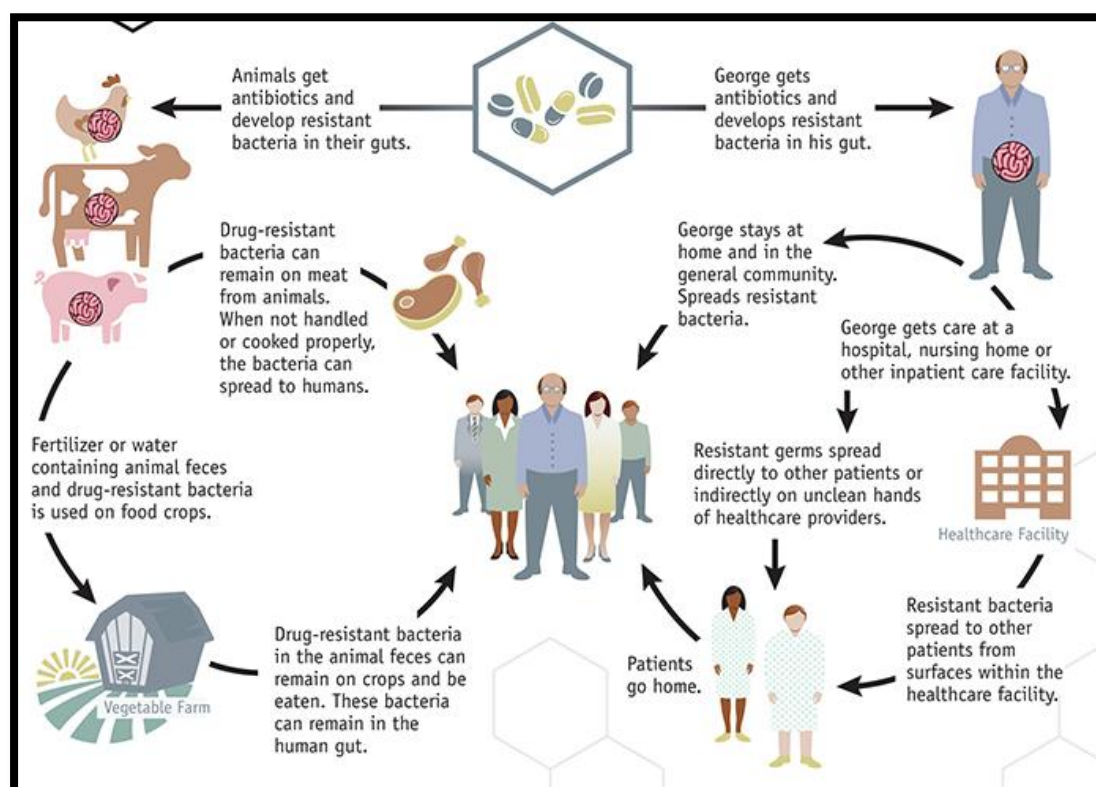
An antibiotic is a drug that kills or stops the growth of bacteria like penicillin and ciprofloxacin, whereas antimicrobial refers to all microbes viz., bacteria, viruses, fungi, and parasites. Hence, Antibiotic or antimicrobial resistance (AMR) denotes the ability of microbes to resist the effects of 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 fall 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 in various facets of healthcare system because of their rapid emergence of newer resistances and spread across the various countries. 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 occurring during due to change in genetic makeup of microbes in a longer time, however the current situation is happening at an elevated speed due various reasons such as 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 organism are present in human, animal, food, and environment which makes the transmission more 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 acts as potential spread of antibiotic resistant genes spread. In general *Vibrios* sp showed higher resistances towards Ampicillin and low tetracycline resistances. 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 serovarwere isolated and Twenty isolates showedresistance to at least one antibiotics. Five strains(serovars Bareily, Oslo, Hadar, Weltevreden and Rissen) were resistant to two or more antibiotics. Two *S. enterica* strains (serovars Bareily and Oslo) from seafood from Vietnam and India were resistant to trimethoprim/sulfamethoxazole, sulfisoxazole, ampicillin, tetracyclineand 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 alsohumans in contact during aquaculture activity. The infections includes *Aeromonas hydrophilia*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus*, and *Photobacterium damsela* 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 diagnosis of AMR in bacterial pathogens

Antimicrobial resistance can be detected 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 shown by these pathogens.

AMR and seafood trade implications

Last three decades has 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.

Controlling of AMR

AMR is a complex and interdisciplinary issue, coherent efforts are required to bring down the burden of AMR among public. WHO, FAO and OIE have taken collective tripartite joint venture called one health approach to control AMR spread which are considered as national action plans to each countries. 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 need to be given to the food and environmental sectors also
3. Strengthening the laboratory capacity for surveillance system
4. Guideline for the optimised 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



The four pillars of the FAO plan of action to support the food and agriculture sector in addressing AMR

Further reading

<https://www.cdc.gov/drugresistance/about.html>

<http://www.who.int/antimicrobial-resistance/publications/situationanalysis/en/>

Sarah M. Cahill, Patricia Desmarchelier, Vittorio Fattori, Annamaria Bruno and Andrew Cannavan. 2017 Global Perspectives on Antimicrobial Resistance in the Food Chain. Food Protection Trends, Vol 37, No. 5, p. 353–360

Amagliani, G., Brandi, G.F. Schiavano. 2012. Incidence and role of Salmonella in seafood safety. Food Research International 45 (2012) 780–788