Antibiotic Residues in Fish and Fishery Products

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Introduction

Antibiotic residues in processed seafood have become a serious health hazard to the consumer and an economic hazard to the exporters. This is a major quality problem being faced by the Indian seafood processors and exporters. Our fishery exports consists mainly of processed shrimp, cephalopods and certain varieties of finfishes. Of these, the frozen shrimp constitutes 80% of value and 50% of the quantity. The problem of antibiotic residues has been associated mainly with processed shrimps.

The processed shrimp exported from India consists of wild shrimps from the sea and brackish water and farmed tiger prawn (*Penaeus monodon*) and scampi (*Macrobrachium rosenbergii*). Wild shrimps are mainly marine, only a very small share comes from open brackish water areas. Marine ecosystem is totally free from antibiotics; hence there is no chance for the occurrence of antibiotic residues in marine shrimps. But shrimps from aquaculture farms used to have antibiotic residues as a consequence of aquaculture practices.

The use of antibiotics in aquaculture practice is unscientific, unwanted and harmful and has been banned by the Government of India (GOI 2001, 2002). However, antibiotics are being used in aquaculture for (i) therapeutic (ii) prophylactic and/or (iii) growth promoting purposes. Also, some manufactures are incorporating certain antibiotics in shrimp feed as a feed preservative. The devastating shrimp diseases like white spot syndrome disease and yellow head disease are caused by viruses. Antibiotics have no therapeutic value against viruses at all. Still many of our shrimp farmers are dumping antibiotic formulations in their farms against viral diseases. Further, even bacterial diseases cannot be treated with antibiotics, since in the aquaculture environment, effectiveness of antibiotic therapy is doubtful.

The use of antibiotics is banned in aquaculture because of the following reasons.

i) Effect on environmental microflora

The aquaculture environment is a very dynamic system. Chemical, biochemical and gaseous equilibrium in the farm, ie. the water quality, is maintained by the mineralisation process caused by microflora, mainly bactreria. In the shrimp farms, on an average, 60 mg/m^2 of waste is generated for every kilogram of shrimp produced. On this basis, in every hectare, nearly 7-8 tonnes of waste is generated per crop. These organic wastes are mineralized and farm environment kept clean and healthy by the farm/soil microorganisms.

When we use antibiotics/antibacterial chemicals in the farm either as therapeutical, prophylactic or growth promoters, they destroy the environmental

microflora. The scavenging action by microflora is stopped. So waste accumulates in the farm, resulting in favourable environment for disease.

ii) Development of drug resistant bacteria, including pathogens.

Use of antibiotics results in the emergence of drug resistant bacteria, some of which are human pathogens, like *Salmonella.* Consequently, antibiotics will not be effective in combating the diseases caused by these resistant pathogens.

iii) Retention of drug residue in farmed shrimps

The antibiotics used in aquaculture will accumulate in the shrimp tissue and exoskeleton. The drug so accumulated has to be eliminated from the tissue by a biological process called detoxification. In the case of aquatic animals, the biochemical process of drug elimination is very slow compared with land animals. The drug

Table-1. Permitted Maximum Residual Level (MRL) of antibiotics in seafood as notified by European Union

Sl.No.	EU standards	Maximum Residual Level of antibiotics permitted in food/fish products*	
1	Sarafloxacin	Max. 0.03 ppm	Salmonid fishes
2	Nafcillin	0.3 ppm	Meat
3	Nafcillin	0.03 ppm	Milk

* No other antibiotics are permitted in food products.

Table-2. Permitted MRL of antibiotics in seafood as per USFDA (2001)

1	Oxytetracycline	2 ppm	In Salmonids, Cat fish & lobster only
2	Sulfamerazine	Nil	All fishes
3	Sulphadimethoxine/ Ormetoprim combination	0.1 ppm	In Salmonids and Cat fish only
4	Other antibiotics	-	Not permitted in fish/fishery products

Table-3. Maximum Residual Level (MRL) of permitted antibiotics by Government of India in fishery products (GOI, 2001)

S.No.	Antibiotics	Max. Residual level in ppm *
1	Chloramphenicol	Nil
2	Furazolidone	Nil
3	Neomycin	Nil
4	Tetracycline	0.1
5	Oxytetracycline	0.1
6	Oxolinic acid	0.3
7	Trimethoprim	0.05
8	Nalidixic acid	Nil
9	Sulphamethazole	Nil

* For export to EU, USA & Japan, the MRL fixed by individual countries are to be complied with.

Table-4. Antibiotics and other Pharmacologically Active Substances* banned in aquaculture practice in India

Sl.No.	Antibiotics and other Pharmacologically Active Substances
1	Chloramphenicol
2	Nitrofurans including: Furaltadone, Furazolidone, Furylfuramide, Nifuratel, Nifuroxime, Nifurprazinc, Nitrofurantoin, Nitrofurazone
3	Neomycin
4	Tetracycline
5	Oxytetracycline
6	Trimethoprin
7	Oxolinic acid
8	Nalidixic acid
9	Sulphamethoxazole
10	Aristolochia spp and preparations thereof
11	Chloroform
12	Chlorpromazine
13	Colchicine
14	Dapsone
15	Dimetridazole
16	Metronidazole
17	Ronidazole
18	Ipronidazole
19	Other nitroimidazoles
20	Clenbuterol
21	Diethylstilbestrol (DES)
22	Sulfonamide drugs (except approved sulfadimethoxine, Sulfabromomethazine and Sulfaethoxypyridazine)
23	Fluroquinolones
24	Glycopeptides

* The list is proposed to be included in the amendment to the Notification SO 792 (E) dated August 17, 2001 of the Ministry of Commerce and Industries

withdrawal period before harvesting, usually 2-3 weeks will not eliminate the accumulated residues from the tissues, and particularly from exoskeleton. So, the accumulated antibiotic residues will remain in tissues and exoskeleton, which will be detected in the testing laboratory. The European Union, US FDA and Japan have notified that residues of the antibiotic listed in their notifications should not be present in the shrimp imported to those nations.

iv) Health hazard to the consumers

Antibiotics are used as therapeutics in humans to fight microbial diseases. But, certain antibiotics have been shown to cause serious health hazards in susceptible individuals. So, antibiotic residues in food can be dangerous to the consumer. For example, chloramphenicol is known to cause haematoxic side effects, particularly the chloramphenicol induced aplastic

Table-5 Maximum Residual Limits of antibiotics in fish

S.No.	Antibacterials/Antibiotics	Maximum Residue Limits (MRL)
i)	Trimethoprim	50 ppb*
ii)	Beta lactams (Penicillins)	4 to 300 ppb
iii)	Cephalosporins	20 to 1000 ppb
iv)	Quinolins	50 to 1500 ppb
v)	Macrolids (Tylosin group)	50 to 200 ppb
vi)	Tetracyclines	100 to 600 ppb
vii)	Aminoglycosides (Streptomycins)	100 to 1000 ppb

* 1 ppb = 1 nanogram/gram = 0.001 ppm

anaemia, which is often fatal. Nitrofurans and their metabolites are genotoxic and carcinogenic. So far, no "dose-effect" relation could be established in man for these two groups of antibiotics. So, they are declared as zero tolerant antibiotics.

European Union Standards (EEC 1990) stipulates that anti-infection agents, antibiotics and quinolones SHOULD NOT BE PRESENT in fish/meat products imported to the EU Countries except those mentioned in Table-1. Permitted maximum residue levels of notified antibiotics as per USFDA (2001) are given in Table-2 and those by the Government of India (GOI, 2001), in Table-3. Japan permits no antibiotic residue except tetracyclines, to a maximum of 0.1 ppm. in seafood.

The Present Scenario in India

Antibiotics and antibacterial substances are indiscriminately being used in India in shrimp farms in Andhra Pradesh, Tamil Nadu, Kerala & Karnataka and the practice is exceptionally high in Andhra Pradesh. A recent study in Central Institute of Fisheries Technology, Cochin showed that out of the 2086 samples of farmed shrimps (Tiger prawn, white prawn and freshwater scampi), tetracyclines were detected in 134 samples and chloramphenicol in 28 samples. The Government of India, Ministry of Agriculture (GOI, 2002) have banned the use of 24 antibiotics and pharmacologically active substances in aquaculture (Table-4).

The EU perception of the Residues problem in Seafood

The EU has issued Council Regulation 2377/90, updated up to 1-12-2000 enlisting nearly 300 pharmacologically active substances/antibacterials/antibiotics/ sulphonamides, which are either declared zero tolerant or for which maximum residue limits (MRL) have been fixed. But, the MRLs are less than 50 ppb (i.e. 0.05 ppm) in most of the cases. Vide EU legislation 96/23/EC & EEC No.2377/90, chloramphenicol and nitrofurans (nitrofurazone, nitrofurantoin, furazolidone, furaltadone) and their metabolites (Semicarbazide (SEM), 1-amino hydantoin (AHD), 3-amino 2-oxazolidinone (AOZ), 3-amino 5-morpholino methyl 2 – oxazolidinone (AMOZ)) are banned in seafood (all foods) (EEC-2002). Further, Maximum Residue Limits (MRL) have been fixed for the antibiotics listed in Table-5.

Bacterial inhibitors

Recently, bacterial inhibitors have become another problem in seafood. Bacterial inhibitors include the residues of antibiotics, food preservatives like Cl_2, hypochlorites, citric acid, sodium tri-polyphosphate (STPP), potassium sorbate etc. Some of those preservatives are approved for use in seafood by EU/USFDA/ FAO. Bacterial inhibitors are detected by Four Plate Technique (FPT). A positive test for bacterial inhibiters could be due to antibiotic residues or some other chemical / biological compounds. So, further analysis by specific methods for antibiotic residues like ELISA or LCMS-MS is required to confirm the presence of antibiotic residues.

Difficulties in analytical field

The assay of antibiotics in tissues is posing great difficulties in analytical laboratories. So far, the microbiological assay as per AOAC (1995) methods, using specific sensitive bacterial cultures has been in vogue everywhere. But the microbiological assay has the limitation of detection level. At present, under the best conditions and using the most sensitive strains, the lowest limit of detection is only 100 ppb (ie. 0.1 ppm) at 95% confidence level. Hence the microbiological assay will not detect zero tolerant antibiotics, if less than 100 ppb. In the case of nitrofurans, within 6 hrs, they are metabolized in the tissues. The resulting metabolites are small molecular weight compounds, which have no antibacterial properties, but could cause mutagenesis. They cannot be detected by usual microbiological methods; but advanced instrumental analysis will detect them. Metabolites of nitrofurans are also banned in seafood by the EU countries.

Instrumental methods like HPLC and GC have also the limitation, the detection levels at present being only 100 ppb. Recently HPLC-MS-MS and GC-MS have come to the scene, which could detect in ppb levels. However, the cost involved in the analytical set up is enormous. Now, ELISA kits are available for the detection of certain antibiotics like chloramphenicol, tetracyclines and sulphonamides. Recently, ELISA kits have also been brought out to detect AOZ and AMOZ.

Testing methods for Antibiotics and Bacterial inhibitors

1. ***Microbiological Assay – AOAC Method***

   Requirements: -

   a) A sensitive bacteria – *Bacillus cereus*

   b) Standard antibiotics – Tetracycline, Chloramphenicol, Penicillin etc.

   c) Extraction buffer – Citrate Phosphate buffers.

   d) Assay medium – pH 6.2. 7.0, 8.0

   e) Assay plates - 90mm diameter.

   f) A well trained Technical personnel- Graduate in Science with experience in Microbiology lab.

2. ***Four Plate Technique***

   Requirements:-

   i) Two Sensitive cultures: *Bacillus subtilis* (ATCC 6633)

   Micrococcus luteus (ATCC 9341)

   ii) Assay media pH 6 (Merck 10663)

   pH 7.2 (Merck 15787)

   pH 8 (Merck 10664)

iii) Reference antibiotics:- Trimethoprim
Penicillin
Sulfamethazine
Streptomycin
Erythromycin

iii) Assay discs: 6mm dia.

iv) Assay plates - 90mm dia

v) Specially trained technical personnel

3. ELISA

ELISA kits for certain antibiotics and nitrofuran derivatives are available in India. Test will detect upto 0.02 ppb of Chloramphenicol and 0.2 ppb levels of AOZ and AMOZ. It makes use of the principle of antigen – antibody reaction The ELISA kits for antibiotics works on the principle of competitive ELISA, where the antibiotics residue in the sample extract and the antibiotic-enzyme conjugate compete for the limited number of specific binding sites on the antibody coated wells on ELISA plate.

Requirements :-

i) ELISA unit consisting of plate or strip washer and plate or strip reader

ii) Specific ELISA test kits.

References

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