

## **Adverse Effect of Antibiotic Residues in Seafood**

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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 industry.

The processed shrimp exported from India consists of wild shrimp 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.

Use of antibiotics in aquaculture practice is unscientific, unwanted and harmful and has been banned by the Government of India. 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 whitespot 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 not at all proved

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. The chemical, biochemical and gaseous equilibrium in the farm, ie. the water quality, is maintained by the mineralisation process caused by microflora, mainly bacteria. In the shrimp farms, on an average, 60 mg/m<sup>2</sup> of waste is resulted 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 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 notification 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. So, antibiotic residues in food can be dangerous to the consumer. For example, chloramphenicol is known to cause haematotoxic side effects, particularly the chloramphenicol induced aplastic anaemia, which is often fatal. Nitrofurans and their metabolites are genotoxic and carcinogenic. So far, no “doze-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 and antibiotics 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.

**Table 1. Permitted Maximum Residual Level (MRL) of antibiotics in seafood as notified by EU**

Antibiotics	Maximum Residual Level in food/ fish products*
Sarafloxacin	Max. 0.03 ppm Salmonid fishes
Nafcillin	0.3 ppm Meat
Nafcillin	0.03 ppm Milk

\* No other antibiotics permitted

**Table 2. Permitted MRL of antibiotics in seafood as per USFDA 2001**

Antibiotics	MRL	Fish products
Oxytetracycline	2 ppm	In Salmonids, Cat fish & lobster only
Sulfamerazine	Nil	All fishes
Sulphadimethoxine/ Ormetoprim combination	0.1 ppm	In Salmonids and Cat fish only
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)**

Antibiotics	MRL, ppm *
Chloramphenicol	Nil
Furazolidone	Nil
Neomycin	Nil
Tetracycline	0.1
Oxytetracycline	0.1
Oxolinic acid	0.3
Trimethoprim	0.05
Nalidixic acid	Nil
Sulphamethazole	Nil

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

Japan permits no antibiotic residue except tetracyclines, to a maximum of 0.1 ppm. in seafood.

#### Present scenario in India

Antibiotics and antibacterial substances are indiscriminately being used in India in shrimp farms

in Andhra Pradesh, Tamil Nadu, Kerala and Karnataka and maximum use is in Andhra Pradesh. A recent study by Central Institute of Fisheries Technology, Cochin showed that out of the 2086 samples of farmed shrimp (Tiger prawn, White prawn and Freshwater scampi) tetracyclines were detected in 134 samples and Chloramphenicol in 28 samples.

Recently, Chloramphenicol and Nitrofurans were detected in Indian farmed shrimp exported to Spain, Netherlands and UK in ppb levels. All the consignments were confiscated and destroyed. Indian Exporters are reported to have lost nearly Rs.100 crores.

The Government of India, Ministry of Agriculture (GOI, 2002) has banned the use of 24 antibiotics and pharmacologically active substances in aquaculture (Table 4).

**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: Furaladone, Furazolidone, Furfuramide, Nifuratel, Nifuroxime, Nifurprazinc, Nitrofurantoin, Nitrofurazone
3	Neomycin
4	Tetracycline
5	Oxytetracycline
6	Trimethoprim
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

### The EU perception of the residues problem in seafood

The EU has issued Council Regulation 2377/90, updated upto 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 (ie. 0.05 ppm) in most of the cases.

Vide EU legislation 96/23/EC & EEC n°2377/90, the following Antibiotics are banned in seafood (all foods) (EEC-2002).

1. Chloramphenicol
2. Nitrofurans: Nitrofurazone  
Nitrofurantoin  
Furazolidone  
Furaladone and their Metabolites

Further, Maximum Residue Limits (MRL) have been fixed for the antibiotics listed in Table-5.

**Table 5. Maximum residual limits for antibacterial/antibiotics as per EEC, 2002**

Antibacterials/Antibiotics	Maximum Residue Limits (MRL)
Trimethoprim	50 ppb*
Beta lactams (Penicillins)	4 to 300 ppb
Cephalosporins	20 to 1000 ppb
Quinolins	50 to 1500 ppb
Macrolids (Tylosin group)	50 to 200 ppb
Tetracyclines	100 to 600 ppb
Aminoglycosides (Streptomycins)	100 to 1000 ppb

\* 1 ppb = 1 nanogram/gram = 0.001 ppm

### 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 every where. 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. the nitrofurans are metabolized in the tissues. The resulting metabolites are small molecular weight compounds, which have no antibacterial properties, but could cause mutagenesis. They will not be detected by 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 of detection levels at present

being only 100 ppb. Recently HPLC-MS-MS (LC-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. Some of the kits have claimed to have a minimum detection level of 0.025 ppb of chloramphenicol in seafood.

### Difficulties for seafood processors

More than 50% of the processed shrimps including scampi is of aquaculture origin. Testing facilities for detection of very low levels of antibiotic residues in the raw material, are not available in seafood processing establishments. So, seafood processors have to depend on the declarations from the farmers/suppliers that no antibiotics have been used in the farm. When the residues are detected in the importing countries, the seafood processors stand to lose their money and reputation as supplier of safe products.

### How we will redress the problem?

Only earnest and concerted action at the following levels will redress the antibiotic residue problem in our seafood:

1. Action at Government level to ensure that the banned chemicals/antibiotics are not available to farmers and are not used in farms. The following Government Departments may be given the responsibility for this.
  - i) Central - Agriculture, Commerce & Environment Ministries
  - ii) State - Fisheries and Environment Departments
2. Action at Farmers' level – No aquaculture drug to be used in farm.

3. Action at Buyer's level (Seafood processors)  
– To be vigilant on the presence of drug residues in raw materials
  4. Action at Inspection / Enforcement / Promotion/Research Agencies level.
    - i) Export Inspection Council of India (EIC)
    - ii) Marine Products Export Development Authority (MPEDA)
    - iii) Central Institute of Fisheries Technology (CIFT)
- European Union Standards (1990) Annexure III to the EEC Regulation No.2377/90, European Union, Brussels
- GOI (2001) Government of India Notification SO792. The Gazette of India (Extra ordinary) – Part-II, Section-3, Subsection (ii) No.582, dated 17<sup>th</sup> August 2001, New Delhi.
- GOI (2002) Government of India, Ministry of Agriculture – Notification No.33035/4/2003 – Fy(T2) dated July 5, 2002, New Delhi.
- USFDA (2001) Consolidated version of the Annexure-I to IV of Council Regulation is 2377/90, updated on 01.12.2002, Ernakulam, Brussels.

### References

AOAC (1995) *Official Methods of Analysis* 16<sup>th</sup> Edn., Association of Official Analytical Chemists, Washington DC

USFDA (2001) *Fish & Fisheries Products Hazards & Controls Guidance* 3<sup>rd</sup> Edition, June 2001, USFDA