

# BACTERIA OF PUBLIC HEALTH SIGNIFICANCE AND LABORATORY METHODS FOR THE DETECTION OF BACTERIA IN FISH / SHRIMP MEAT

**Madhusudana Rao, B. and Ahamed Basha, K.**

## **Food Safety**

Food safety means that the food produced / processed is 'safe' for human consumption. Hazard is any biological, chemical or physical agent that may cause an unacceptable health risk to consumers if present in the product. Safe food means that the food is free from biological hazards (eg.harmful bacteria such as *Salmonella*, *Listeria monocytogenes*, *Vibrio cholerae*, viruses, worms, protozoa etc.), chemical hazards (harmful chemicals such as antibiotic residues, pesticides, heavy metals, dyes, cleaning compounds, food colours and additives other than permitted ones etc.)and physical hazards (foreign objects such as metal pieces, bolts, nuts, glass pieces, filth etc.).

Food safety regulations have been developed by all countries to ensure that the fish / shrimp that is available for human consumption is safe to eat. Non-compliance to standards leads to import refusals, detention, or destruction at the entry points of the importing country. Unsafe fish /shrimp products containing biological, chemical or physical hazards is not permitted to be sold in the markets.The food safety regulations for export of fish/ shrimp from India are governed by the Export Inspection Council of India [Order S.O. 729 (E), dated 21 Aug, 1995 subsequently amended vide orders 792(E), 722(E), 464(E), 1227(E) (EIC, 1995). However, if specific food safety standards are prescribed by the importing countries, then the standards of the importing countries must be invariably followed.

Fish and shrimp products that are meant for sale in India for human consumption are governed by the Food Safety and Standards Regulations (FSSR) of the Food Safety and Standards Authority of India (FSSAI), Government of India.

**Table: Microbiological Requirements for Raw-Frozen Crustaceans (FSSAI, 2017)**

	Sampling Plan		Limits (cfu/g)		Action in case of Unsatisfactory results
	n	c	m	M	
<b>Hygiene Indicator Organisms</b>					
Aerobic Plate Count (cfu /g)	5	3	1x10 <sup>6</sup>	1x10 <sup>7</sup>	Improvement in hygiene; Time-Temperature Control along value chain
<b>Safety Indicator Organisms</b>					
<i>Escherichia coli</i>	5	3	11 MPN/g	500 MPN/g	
<i>Salmonella</i>	5	0	Absent/25g		
<i>Vibrio cholerae</i> (O1 and O139)	5	0	Absent/25g		

Where, n: Number of units comprising the sample

c: Maximum allowable number of defective sample units

m: Acceptable level in a sample

M: Specified level when exceeded in one or more samples would cause the lot to be rejected

Bacteria are broadly categorized as Gram-positive (blue/purple colour) and Gram-negative (orange/red colour) based on their colour observed under microscope after being subjected to Gram staining. The important Gram-negative pathogens are *Salmonella*, *Shigella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, pathogenic *Escherichia coli* such as Enterotoxigenic *Escherichia coli* (ETEC), Enteropathogenic *Escherichia coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC) etc. The important Gram-positive pathogens

are *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum*, *Bacillus cereus* etc.

**Table: Bacteria of public health significance**

Gram negative pathogens	Gram positive pathogens
<ul style="list-style-type: none"> <li>• <i>Salmonella</i></li> <li>• <i>Shigella</i></li> <li>• <i>Vibrio cholerae</i> serogroups O1 and O139</li> <li>• <i>Vibrio parahaemolyticus</i></li> <li>• <i>Vibrio vulnificus</i></li> <li>• <i>Aeromonas hydrophila</i></li> <li>• <i>Brucella</i></li> <li>• <i>Yersinia enterocolitica</i></li> <li>• <i>Campylobacter jejuni</i></li> <li>• <i>Cronobacter (Enterobacter sakazakii)</i> spp.</li> <li>• <i>Coxiella burnetii</i></li> <li>• <i>Plesiomonas shigelloides</i></li> <li>• <i>Francisella tularensis</i></li> <li>• Pathogenic <i>Escherichia coli</i> Group</li> <li>• Enterotoxigenic <i>Escherichia coli</i> (ETEC)</li> <li>• Enteropathogenic <i>Escherichia coli</i> (EPEC)</li> <li>• Enterohemorrhagic <i>Escherichia coli</i> (EHEC)</li> <li>• Enteroinvasive <i>Escherichia coli</i> (EIEC)</li> </ul>	<ul style="list-style-type: none"> <li>• <i>Staphylococcus aureus</i></li> <li>• <i>Listeria monocytogenes</i></li> <li>• <i>Clostridium perfringens</i></li> <li>• <i>Clostridium botulinum</i></li> <li>• <i>Bacillus cereus</i></li> <li>• <i>Streptococcus</i></li> <li>• <i>Mycobacterium bovis</i></li> <li>• <i>Enterococcus</i></li> </ul>

**a) Salmonella:** *Salmonella* is a motile, non-spore forming, Gram negative, rod-shaped bacterium. The genus *Salmonella* is divided into two species that can cause illness in humans viz., *S. enterica* and *S. bongori*. *Salmonella enterica*, which is of the greatest public health concern, is comprised of six subspecies: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), *S. enterica* subsp. *indica* (VI). *Salmonella* is further subdivided into serotypes, based on the Kaufmann-

White typing scheme which differentiates *Salmonella* strains by their surface (O) and flagellar (H) antigenic properties. *S. enterica* subsp. *enterica* is divided into numerous serotypes. Salmonella causes typhoid disease which is characterized by high fever, diarrhoea, aches, headache, and lethargy (drowsiness or sluggishness) and up to 10% of people who don't get treatment may die. Typhoid fever is caused by serotypes *S. Typhi* and *S. Paratyphi*. Salmonella spreads through the fecal-oral route.

**Sources of Salmonella:** Intestinal tracts of vertebrates, including livestock, wildlife, domestic pets, and humans, and may also live in environments such as pond-water sediment, farm-irrigation water, soil and insects, factory equipment, hands, and kitchen surfaces and utensils. Cross contamination of fish / shrimp when a food handler does not adequately clean utensils, surfaces, equipment, and hands after they have come into contact with contaminated products. Cross contamination may occur at any point in the fish / shrimp processing unit.

**b) *Vibrio cholerae* :** *V. cholerae* is Gram negative, non-spore forming, comma shaped bacterium. It is the etiological agent of the dreaded disease 'cholera'. *V.cholerae* causes profuse watery diarrhoea, vomiting, and muscle cramps. The diarrhoea has a characteristic "rice water" appearance. The disease results in 30 to 40% mortality if rehydration therapy is not given. Virulence of *V. cholerae* serogroups O1 and O139 is due to the production of an enterotoxin called cholerae toxin (CT) and the toxin co-regulated pilus (TCP). Serogroups O1 and O139 were responsible for all the epidemic and pandemic cholera outbreaks. *V. cholerae* is excreted in great numbers in the faeces of cholera patients and convalescents. The disease is transmitted primarily by the faecal-oral route, indirectly through contaminated water supplies.

**Sources of *V. cholerae*:** Cholera infections have been associated with wide variety of seafoods such as oysters, mussels, clams, crab, lobster, shrimp, squid and finfish. Raw, improperly cooked seafood, or cross contaminated by a raw product. Although cooking kills these bacteria, serogroups O1 and O139 can grow in

fish and shellfish that have been contaminated after cooking. In endemic areas infections occur through ingestion of water, ice, unwashed and contaminated food.

**c) *Vibrio parahaemolyticus*:** Gram-negative, curve-shaped rod frequently isolated from the estuarine and marine environments. It is a halophilic bacterium and requires at least 0.5% NaCl in all media, and 2% NaCl is optimal. *V. parahaemolyticus* is highly susceptible to low pH, freezing, and cooking. *V. parahaemolyticus* isolates from the environment are usually non-pathogenic. The pathogenic strains of *V. parahaemolyticus* are identified by the presence of one or both of the hemolysins TDH (thermostable direct hemolysin) and TRH (thermostable-related hemolysin). Diarrhea caused by *V. parahaemolyticus* is usually self-limiting. **Sources:** Consumption of raw or improperly cooked oysters, fin-fish, squid, octopus, lobster, shrimp, crab and clams.

**d) *Vibrio vulnificus*:** *V. vulnificus* is associated with various marine species, such as plankton, shellfish, crustaceans, and finfish. It is a halophilic bacterium that requires at least 0.5% NaCl in all growth media, and 2% NaCl is optimal. Death occurs in an average of 35% of septicemia cases (and 20% of wound infection cases). In healthy people, ingestion of this organism can cause gastroenteritis that generally remains localized and is self-limiting. Among susceptible people, the organism may cause primary septicemia (septic shock). Susceptible people include those with a predisposing condition; for example, those who are immunocompromised or have high serum iron levels (usually due to liver disease). *V. vulnificus* causes wound infections directly, either through wounds incurred while handling fish, crustaceans, or shellfish, or when a pre-existing wound is exposed to marine or estuarine waters harboring the organism. More than 90% of *V. vulnificus* illnesses in the U.S. are associated with consumption of raw Gulf Coast oysters. Ingestion of clams and shrimp also has been associated with disease.

**e) *Listeria monocytogenes*:** *L. monocytogenes* is a Gram-positive, rod-shaped, facultative bacterium, motile and salt-tolerant bacterium. *L. monocytogenes* can survive below 1 p C and can grow at low temperatures (less than 3 p C) making it

an important organism in seafood industry. *L. monocytogenes* can be found in moist environment and is persistent in food-manufacturing environments. The genus *Listeria* contains 6 species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* but only *L. monocytogenes* is commonly associated with human listeriosis. Listeriosis associated infection by *L. ivanovii*, and *L. seeligeri* is extremely rare in humans. *L. monocytogenes* bacterium causes two forms of disease. The first form manifests as non-invasive gastrointestinal illness and the infected persons suffer from nausea, fever, diarrhoea and is self-limiting. The second form manifests as a serious invasive illness that causes septicaemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. **Sources:** Food workers, food contact surfaces, incoming air, raw materials, and food -processing environment.

**f) *Staphylococcus aureus:*** *S. aureus* is a Gram-positive, nonmotile, catalase-positive, small, spherical bacteria (cocci), which, on microscopic examination, appear in pairs, short chains, or bunched in grape-like clusters. *S. aureus* is a versatile human pathogen capable of causing staphylococcal food poisoning, toxic shock syndrome, pneumonia, postoperative wound infection, and nosocomial bacteremia. **Sources:** Staphylococci are widely distributed in the environment. They can be found in the air, dust, sewage, water, milk, and food, or on food equipment, environmental surfaces, humans, and animals. Foods that require considerable handling during preparation and are kept slightly above proper refrigeration temperatures for an extended period after preparation are frequently involved in staphylococcal food poisoning. Unless heat processes are applied, staphylococci are expected to exist in any and all foods that are handled directly by humans or are of animal origin. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50% or more of healthy individuals. Contamination may be introduced into foods by direct contact with workers with hand or arm lesions caused by *S. aureus*, or by coughing and sneezing, which is common during

respiratory infections. Food handlers are frequently the source of food contamination in staphylococcal outbreaks.

### Suggested Reading:

Bacteriological Analytical Manual (2022). US Food and Drug Administration. Bacteriological Analytical Manual (USFDA-BAM). <https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>

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