Detection of *Shigella* spp. from seafood: Need for protocol harmonization

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***S**higella*, is an enteric pathogen responsible for causing Shigellosis characterized by watery diarrheal like symptoms accompanied by presence of blood in the stool, fever and abdominal pain. Mortality is also seen in case of children with age less than five years (WHO, 2005). The severity of infection is observed more in case of developing countries where frequent outbreaks are occurring due to contamination of food and water. The presence of this bacterium in very low numbers is sufficient to cause an infection in human beings. In the recent years, there is significant increase in consumption of seafood, apart from increased inter-continental mobility which enhanced the chances of spreading this pathogen. There are limited studies available on the presence of *Shigella* in seafood. Hence, a study was conducted to screen seafood from the local markets in and around Cochin, Kerala for *Shigella* spp. A total of 183 seafood samples comprising of fish, shellfish, molluscs, dried fishery products and ice were collected aseptically and brought to the laboratory for analysis. The protocol as described in FDA BAM (2001) was followed for the isolation of *Shigella* spp. Enrichment of samples was carried out in an aerobic jar with gas generating sachet at 44 °C for 24 h in incubator. After enrichment, loop full of broth was streaked on MacConkey agar plates and incubated at 35 °C for 20 h. In addition, Xylose Lysine Desoxycholate and Hektoen Enteric agars were also used to improve the chances of *Shigella* isolation. Presumptive colonies were selected based on colour and size from the respective agar plates and biochemical tests were performed for 1093 isolates. Further confirmation of these isolates were carried out by API 20E and PCR-based method targeting invasion plasmid antigen (*ipaH*) gene specific to *Shigella* spp. None of these isolates were found positive for *Shigella*. Details of the samples used for isolation, identification and confirmation of isolates by PCR are given in Table 1 and Figure 1.

In order to rule out the inhibitory effect of surrounding microflora present in seafood on the growth of *Shigella*, an inoculation study was performed with reference strain of *Shigella*

Table 1. Samples studied for isolation, identification and confirmation of *Shigella* from seafood

Sl. No.	Sample	Number	Presumptive isolates	Suspected isolates	Confirmed isolates by PCR
1	Fish	95	512	45	0
2	Dry fish	18	61	4	0
3	Shellfish	52	276	21	0
4	Ice	18	244	15	0
	Total	**183**	**1093**	**85**	**0**

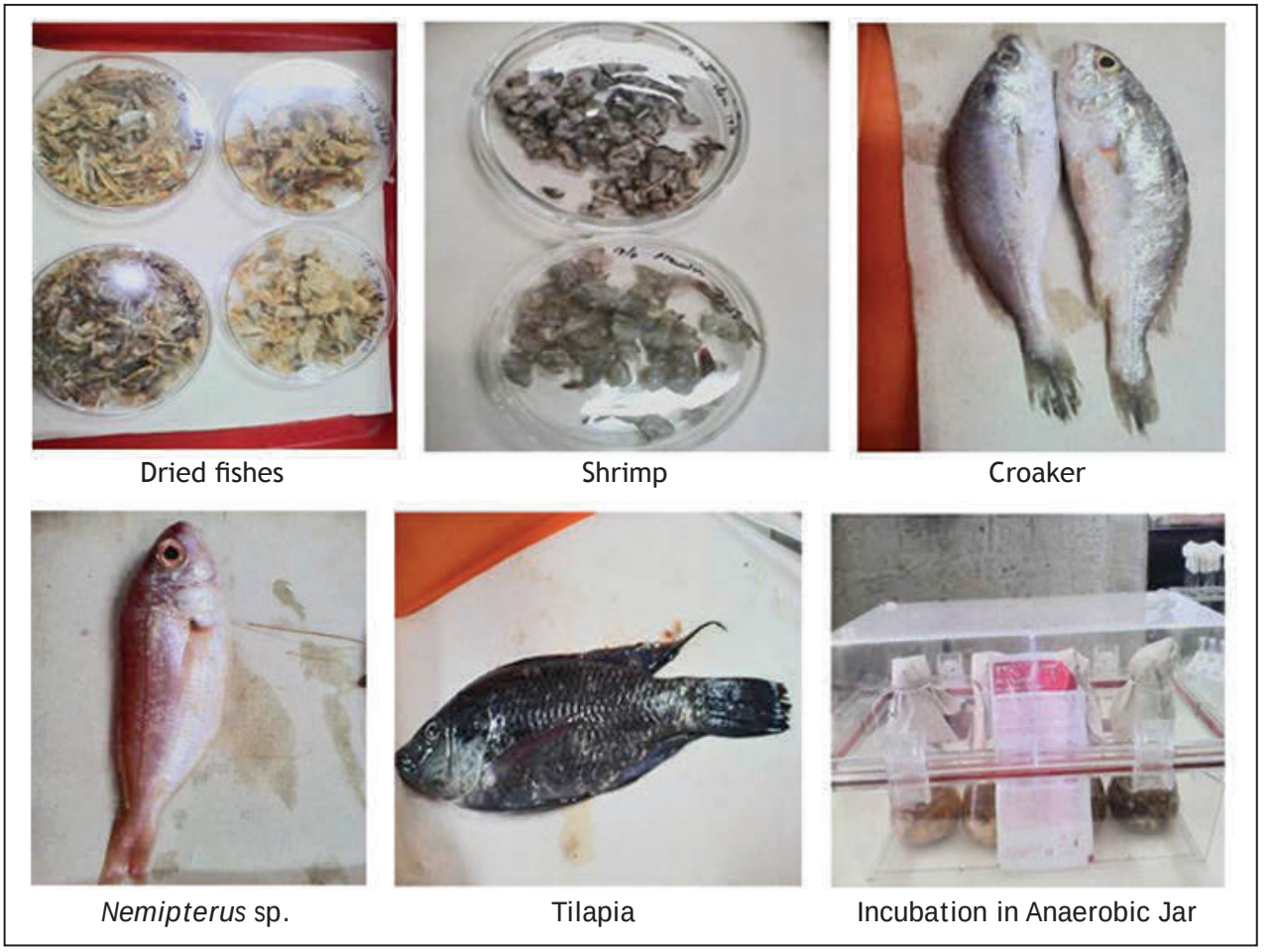

Fig. 1. Samples used for screening of *Shigella* spp. from seafood and incubation in anaerobic conditions using sachet

flexneri separately for raw and sterile fish in triplicate. Fish was sterilized before inculcation. Serial dilutions were performed for isolation, identification and characterization. *S. flexneri* was recovered from 10^2, 10^3 and 10^4 $cfug^{-1}$ from fish only which was sterile using conventional methods of recovery. Contrary, molecular methods was found significant to detect the presence of *S. flexneri* as seen in case of spiked raw fish broth by PCR, although individual colonies were not found on agar methods by conventional isolation method.

The possible reason for not detecting *Shigella* by conventional detection methods is that low numbers of *Shigella* strains are present in samples and the competing ability to grow in presence of other microflora which showed inhibitory effect on the growth and multiplication. It is also understood that *Shigella* is easily overgrown by other members of Enterobacteriaceae such as *E. coli*, *Proteus* spp., *Pleisomonas*, *Citobacter* and *Kliebsiella.* These bacteria also shows similar morphological features with *Shigella* spp. on agar plates and also makes it difficult while picking individual colonies. Therefore, for isolation of *Shigella* from seafood, there is a need of optimized protocol in view of the effect of surrounding microflora on growth of *Shigella.*

References

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Electron Beam Irradiation: A novel approach for shelf stable vacuum packed and chill stored vannamei shrimp

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Microorganisms are key factors for seafood spoilage, particularly a few members of microbial community being responsible for major spoilage processes; referred to as Specific Spoilage Organisms (SSOs). Control of these SSOs would facilitate shelf life extension of the seafood products. Hydrogen sulphide (H_2S) forming bacteria like *Shewanella* sp., *Brochothrix thermosphacta, Pseudomonas* sp. and *Lactobacillus* sp. are among the main SSOs in seafoods. Various researches are being carried out to control these SSOs in fish/shrimp and fishery products. Electron Beam Irradiation (EBI) is a non-thermal processing technique, which is gaining much attention recently by food processors because of its antibacterial activity. Electron Beam (EB) is the flow of electrons with energy, and the energy is obtained as kinetic energy when the electron moves in a high electric field. Even though EBI is an ionizing radiation technique, it is different from the gamma irradiation wherein the latter employs emission of gamma rays from radioactive isotopes such as Cobalt-60 and Caesium-137 for irradiation; which are hazardous to handle and the processing technique is time consuming. The benefit of EB lies in its simplicity and since it is machine source, no hassles of source replenishment and disposal problems arise as well as also require less radiological safety precautions. But, on the other hand it has the disadvantage of poor penetration i.e., 5 MeV machine will penetrate upto 2.5 cms in unit density material and in addition it consumes high electric power and needs proper maintenance. The dose of EBI is measured in KiloGray (kGy). The fast dose delivery by EB machines make it economical to operate at higher throughputs. On account of its potential bactericidal effect, there are sufficient reports available on the reduction of pathogenic and spoilage bacteria in chicken and other meat products. However very scanty literature is available on its application in seafoods.

In the present study, an attempt was made to extend the shelf life of the shrimp by controlling the SSOs by EBI technique. For this, 16/20 grade headless shell-on vannamei shrimp (*Litopenaeus vannamei*) was used. The Electron Beam treatment was carried out using 5Mev, 15 kV machine available with Electron Beam Processing Section of IRAD, BARC at BRIT-BARC Complex, Vashi (Fig. 1). The shrimp samples (3 cms thickness) were given treatment at melting ice temperature with 2.5, 5.0, 7.5 and 10 kGy of EBI. After the treatment, all the sample lots were chill stored at 4 ^{o}C. One lot was untreated and kept as control for comparison.

Analysis of the effect of EBI on SSOs indicated that the *Pseudomonas* count for 2.5 kGy treated