fish is determined by the method of capture, time taken between the capture and landing and handling practices. Freshness scores were also correlated with the sensory characteristics of the fish. Such tester shall be devised indigenously and distributed at low cost to the fishermen to fix the price based on quality.

SF OR 04

Study of pathogenic potentials of seafood isolates of *Arcobacter butzleri* using human epithelial cell line

R. SRINU, P. PARMANAND, T. GAYATRI, L. MANJUSHA, H. SANATH KUMAR, B.B. NAYAK*

ICAR-Central Institute of Fisheries Education, Off-Yari road, Panch Marg, Versova, Mumbai, Maharashtra, India; **nayakbb@cife.edu.in*

The genus *Arcobacter* comprises of emerging food and waterborne pathogens of which *Arcobacter butzleri* is the predominant pathogen causing gastroenteritis and bacteraemia in humans. The pathogenic potentials of bacterial pathogens are measured by adhesion, invasion and cytotoxicity to the host cells. In this study, a total of 15 *A. butzleri* isolated from fish (5), shellfish (5) and coastal water (5) were tested individually to understand the ability *Arcobacter butzleri* strains to adhere and invade human epithelial cells (HeLa) and their cytotoxicity to Vero cells. Eleven of fifteen *A. butzleri* isolates were able to adhere and invade HeLa cells, while the remaining four isolates (F1, F2, SF5 and CW4) did not show any invasion of HeLa cells. The crude toxin extracted from these strains induced morphological changes in cultured Vero cells. All 15 *A. butzleri* strains showed cytotoxic effects on Vero cell. These results strongly suggest that *A. butzleri* present in seafood have pathogenic characters and hence the environmental isolates of *A. butzleri* should be considered potentially pathogenic to humans.

SF OR 05

Isolation of *Vibrio cholerae* 0139 from freshwater fish and its characterization vis-à-vis virulence genes, genetic heterogeneity and response to antimicrobials

B. MADHUSUDANA RAO[1]*, TOMS C.JOSEPH[2], M.M. PRASAD[2]

[1]Visakhapatnam Research Centre, ICAR-Central Institute of Fisheries Technology, Pandurangapuram, Andhra University, P.O., Visakhapatnam, Andhra Pradesh, India; [2] ICAR-Central Institute of Fisheries Technology, Matsyapuri, P.O., Willington Island, Kochi, Kerala, India; **bmrcift@gmail.com*

Vibrio cholerae belonging to the pathogenic O139 serogroup was isolated from freshwater fish, *Catla catla* procured from fish market in Visakhapatnam, Andhra Pradesh. The *V. cholerae* O139 isolate was identified by employing *V. cholerae* species specific PCR (*sodB*), PCR targeting O139-rfb specific primers and serologically using O139 antiserum. The *V. cholerae* O139 isolate was positive for the presence of virulence genes viz., cholera toxin (*ctx*), toxin co-regulated pilus (*tcp*) and accessory cholera enterotoxin (*ace*) genes but was negative for zonula occludens toxin (*zot*) gene. The heterogeneity of the *V. cholerae* O139 isolate was compared with other cholera toxin gene (*ctx*) positive *V. cholerae* isolates (n=7) from fish and fishery environment. Biochemically, the O139 isolate was similar to other *ctx* positive *V. cholerae* but differed with some isolates in acetoin production. The *V. cholerae* O139 isolate haemolysed human RBC, was positive for gelatinase, caseinase, DNAse, lipase, phosphatase, amylase and chitinase activities. The lipase activity of *V. cholerae*

O139 isolate was relatively higher than other *ctx* positive *V. cholerae*. The *V. cholerae* O139 isolate was resistant to nitrofurantoin (300µg), cefotaxime (30µg) and ampicillin (10µg) but was sensitive to 23 other antibiotics. Plant essential oils inhibited the growth of *V. cholerae* including O139 isolate in the order thyme oil > clove bud oil > clove leaf oil > rosemary oil. ERIC-PCR results showed that *V. cholerae* O139 isolate showed extensive dissimilarity with other *ctx* gene positive *V. cholerae* isolates that belonged to serogroup-O1 and serogroup non-O1/non-O139 but exhibited more similarity with *V. cholerae* O139 type culture.

SF OR 06

Isolation of Shiga toxin-producing *Escherichia coli* (STEC) harbouring putative variant Stx genes from seafood

P. SREEPRIYA, L. MANJUSHA, A.K. BALANGE, B.B. NAYAK, H. SANATH KUMAR*

ICAR-Central Institute of Fisheries Education, Andheri, Versova, Mumbai, Maharashtra, India; **sanathkumar@cife.edu.in*

Shiga toxin-producing *Escherichia coli* (STEC) is an important group of pathogenic *E. coli* capable of causing serious human infections. Several food-borne outbreaks due to STEC have been reported worldwide. Fecal contamination of water and food is responsible for the introduction of STEC. In this study, *E. coli* isolated from seafood in Mumbai were characterized with respect to the virulence genes commonly associated with STEC. Five variant Shiga toxin genes were sequenced and the sequence analysis revealed that 4 sequences were identical with Stx1d subtype of Shiga toxin 1, while one sequence deviated significantly from known Stx1 sequences. In comparison with Stx1d, the new variant of Stx1 identified in this study has 4 amino acid substitutions (Tyr 67Phe, Asp70Asn, Leu255Val, Leu262Ile) in subunit A, while in subunit B, 6 amino acid substitutions (Ile7Lys, Leu19Arg, Leu56Phe, Leu61Ile, Thr69Ala, Glu85Gly) were noted. Partial sequence of a putative variant *stx2* gene was also derived in this study. Further, the presence of *stx2* sequence in a PCR product of size higher than that defined by the primers was detected by Southern hybridization using a biotin-labelled probe. The study reports the occurrence of Shiga toxin 1 variant Stx1d in seafood for the first time. The PCR results, together with Southern hybridization and partial sequencing of a *stx2* gene strongly suggest the occurrence of more, presumably new variants of Stx1 and Stx2 among seafood isolates of STEC.

SF OR 07

PCR-RFLP for authentication of five processed species of snappers targeting d-loop region of mitochondrial DNA

B. SIVARAMAN*, G. JEYASEKARAN, R. JEYA SHAKILA, W. LIDIYA, S. AANAND, D. SUKUMAR

School of Harvest and Post-Harvest Technology, Fisheries College and Research Institute, Thoothukudi,Tamil Nadu, India*; *sivabala17@gmail.com*

This study was undertaken to develop a rapid and reliable method for the identification of processed snapper products. Five different species of snapper viz., *Lutjanus fulvus* (black tail snapper), *L. gibbus* (humpback red snapper), *L. lemniscatus* (yellowstreaked snapper), *L. argentimaculatus* (mangrove red snapper) and *L. rivulatus* (blubber lip snapper) available along the Thoothukudi coast were examined in chilled, frozen, and cooked