Rehydration ability and water absorption index of MVD samples were slightly higher to that of HAD samples. HAD sample had more salt-soluble and water soluble protein nitrogen fraction than MVD samples. Higher instrumental hardness values were observed for HAD samples. The study revealed that through microwave vacuum drying, drying time can be reduced to a greater extent compared to hot air drying. It was also realized that microwave vacuum drying can retain the original colour, improve the sensory attributes while maintaining the enhanced physico-chemical qualities. Microwave vacuum drying technique can be adopted by small scale and large scale entrepreneurs to market high quality dried fish products.

Molecular phylogenetic study of *femA* gene sequences of Methicillin Resistant *Staphylococcus aureus* in seafood

Sivaraman G.K., Visnuvinayagam S., Murugadas V. and Prasad M.M.

ICAR-Central Institute of Fisheries Technology, Cochin

Staphylococcus aureus is a common inhabitant of human skin surfaces and anterior nostrils of the healthy people and animals. But it's a well-known opportunistic pathogen that can cause a broad range of infections including mild skin infections, invasive diseases, and toxin-mediated diseases (Kroneberg *et al*., 2011). It is an ubiquitous gram-positive, catalase positive Cocci and facultative anaerobic bacteria and most frequently occurring food-borne pathogen world-wide by the presence of heat stable preformed Staphylococcal enterotoxins (FDA, 2012). Methicillin was introduced as a new β-lactam antibiotic in 1950 to overcome the penicillin resistant *S. aureus*. A decade later in 1961, Methicillin Resistant *Staphylococcus aureus* (MRSA) was reported in United Kingdom (Jevons, 1961) which is resistant to most recent β-lactam antibiotics (Katayama *et al*., 2000). In India, the significance of MRSA had been recognized relatively late and it emerged as a major health problem in the 1980s and 1990s (Mantri Rupali *et al*., 2014). MRSA is a major nosocomial pathogen causing significant morbidity and mortality (Sachdev *et al*., 2003).

Seafood contamination with antibiotic resistant MRSA could be a major threat to public health, as this resistance can be transferred to humans (Diana Gutiérrez *et al*., 2012). No strict guidelines are being practised in India regarding the use of antimicrobials in animal feeds as growth promoters that are used in human medicine (Patrick Butaye *et al*., 2003). The present study was undertaken to know the *femA* (factor essential for methicillin resistance) gene sequence differences in the identified MRSA isolates and its phylogeny in seafood (Sivaraman *et al*., 2017). The fish and fishery products (n= >400) were collected from the retail fish markets and fish processing establishments in Gujarat State, India and 19 number of isolates confirmed as *femA* positive.

A multiplex PCR was carried out with *mec*A gene (293 bp) and Staphylococcus genus specific (597 bp) for the confirmation of MRSA and found that 3.84% of the samples were positive for MRSA. These results made quite interesting to study the gene sequence of the factor essential for methicillin resistant gene A (*femA*) and was amplified by PCR (450 bp) as shown in Figure 1 and the DNA sequencing were out-sourced. 50 µl PCR reaction contains 200 µM dNTPs, 2.5 mM $MgCl_2$,

1X PCR buffer, 0.5U Tag DNA polymerase, 100 µg DNA/ µl with primer concentration of 0.6 pmol 16S rRNA, 0.8 pmol *femA*, 1.0 pmol mecA. The *femA* primer sequences of CGATCCATATTTACCATATCA and ATCACGCTCTTCGTTTAGTT.

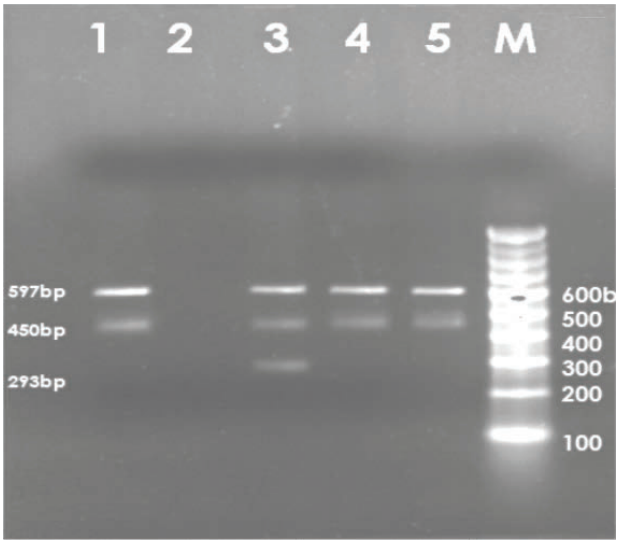

Fig. 1. Multiplex PCR amplification of MRSA isolates with genus specific primers (16S rRNA), femA and mecA gene (Lane 1: ATCC 25293 (MSSA) as reference strain; Lane 2: Negative control (without DNA); Lane 3: ATCC 43300 (MRSA) reference strain; Lane 4: Sample 1; Lane 5: Sample 2; Lane 6: 100 bp DNA ladder)

The phylogenetic tree was constructed based on the *femA* gene sequence differences from these 19 MRSA isolates by Clustal W (Weighted) method with the nucleotide substitutions of 1000 nucleotides and is shown in Figure 2. The DNA sequences were submitted to the ICAR DNA sequence submitting portal: http://webapp.cabgrid.res.in/dnadb/ (ID: 20150530051206, 20150530052426, 20150530052850, 20150530053351, 20150530053719, 20150530054137, 20150530054510, 20150530054804, 20150530055141,

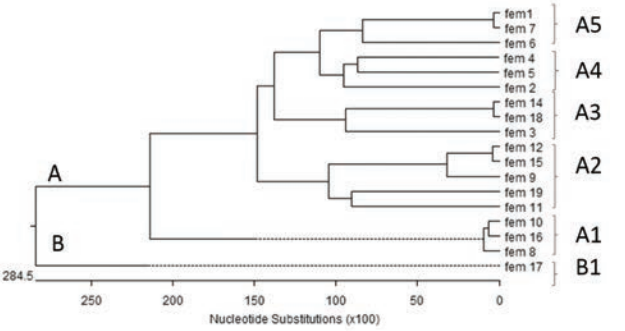

Fig. 2. Phylogenetic tree of the MRSA isolates based on femA gene sequence

20150530055802 and 20150530060133). Two distinct clade is formed based on the sequences and *fem 17* was alone in one clade (A) and the rest is in another clade (B). Whereas, in clade B consisting of two different subclade, the sequences of the isolate 8, 10 and 16 forming in one group (A1) and rest is on the another subclade (A2- A5). The formation of two distinct clades and also with significant differences among the A sub clade indicates the MRSA isolates may be from different source of contamination.

Hence the present study revealed that the presence of MRSA isolates in seafood is from the different sources i.e. possibly from infected fish handlers and processing and unhygienic environment of the fish source. Hence, all fish handlers should be made aware on the importance of personal hygiene and hygienic handling practices at all stages of processing, maintaining cold chain, adequate cleaning and disinfection of equipment and prevention of cross-contamination for ensuring the supply of safe seafood. This study highlights the need for continuous monitoring of antibiotic resistant pathogen MRSA in seafood with a view to prevent the source of contamination.