is of high concern in terms of consumer health. Therefore, awareness is to be created among the food handlers about the cleanliness and the measures to be taken to reduce the prevalence of this hazardous bacterial pathogen.

References

Bennett, R.W. and Lancette, G.A. (2016) - *Staphylococcus aureus*, In: Bacteriological Analytical Manual, USFDA.

Bergey's Manual of Systemic Bacteriology (2005) - The Proteobacteria, Part B: The Gammaproteobacteria. (Eds.) Garrity, G., Brenner, D.J., Krieg, N.R. and Staley, J.R., 1106 p.

Clinical and Laboratory Standards Institute (CLSI) (2012)-Performance standards for antimicrobial susceptibility testing. 25th supplement. Clinical and Laboratory Standards Institute, Wayne.

Murugadas, V., Toms C. Joseph and Lalitha, K.V. (2016) - Antibiotic resistance pattern of *Staphylococcus aureus* isolated from seafood, *Fish. Technol.*, **53:** 232- 237.

Evaluation of dry rehydratable film (3M™ Petrifilm™) method for microbial enumeration in fish samples

Femeena Hassan and Nija K.V.

ICAR-Central Institute of Fisheries Technology, Cochin

Petrifilms eliminate the time for media preparation and sterilization.The 3M™ Petrifilm™ is an all-in-one plating system. Rather than a petridish, 3M™ Petrifilm™ makes use of a thin plastic film as carrier of the culture medium. 3M™ Petrifilm™ comprises a cold-water-soluble gelling agent, nutrients and indicators for activity and enumeration (Jasson *et al.*, 2010). Day by day developing food testing sector provides alternatives to existing standard methods. Before adopting novel techniques it is important to evaluate the effectiveness of the method. In this backdrop, a study was carried out to evaluate the applicability of the petrifilm method for enumeration of aerobic microorganisms, Enterobacteriaceae, *S.aureus*, *E. coli* and Coliforms by comparing the results of petrifilm method with that of standard enumerating techniques. Evaluation of dry rehydratable film (3M™ Petrifilm™) method for microbial enumeration in seafood samples is depicted in Fig.1.

Seven seafood samples were analyzed for microbial parameters like Aerobic Plate Count, Enterobacteriaceae, *Escherichia coli*, Total Coliforms and *Staphylococcus aureus*. Analysis were performed using petrifilms and standard conventional agar plates in triplicate (n=3).The fish *Elagatis bipinnulata*, *Caranx* spp., *Scomberomorus commerson* and *Lethrinus lentjan* were collected from Cochin Fisheries Harbour. *Fenneropenaeus indicus* (headless), *Thunnus albacores* (loin) and *Octopus vulgaris* (Baby octopus) were obtained from a processing plant in Cochin.

Petrifilms were developed in the early 1980s for enumerating aerobic bacteria in food samples. Petrifilm™ Aerobic count plates have an indicator dye and built in grid allows for fast and accurate identification of colonies, within 48 hours over a growth area of approximately 20 cm^2. The comparison of mesophilic count of fish samples with Petrifilm method against the pour plate method showed a significant correlation ($r=0.991$, $p < 0.05$). The reduction of the dye in the upper film of the plates gives red colour to aerobic bacterial colonies. Linton *et al.* (1997) reported that in conventional media certain mesophilic

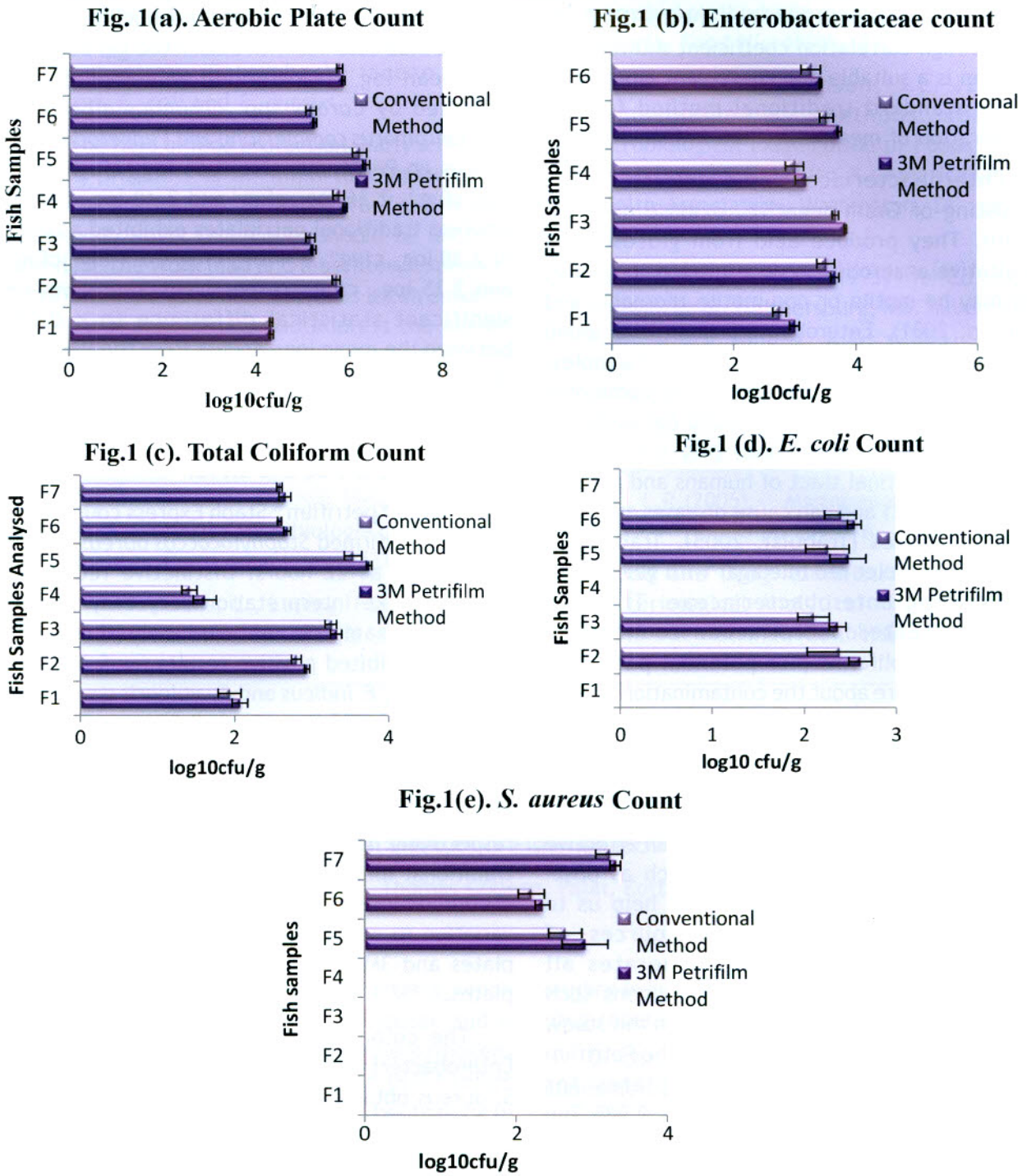

Fig. 1. Evaluation of dry rehydratable film (3M™ Petrifilm™) method for microbial enumeration in fish samples

F1 - *Elagatis bipinnulata*, F2 - *Caranx* spp., F3 - *Scomberomorus commersonii*, F4 - *Lethrinus lentjan*, F5 - *Fenneropenaeus indicus* (headless), F6 - *Thunnus albacores* (loin), F7 - *Octopus vulgaris* (Baby octopus)

bacteria may be better recovered due to an optimal water activity and oxidation/reduction potential. In the present study, these differences did not affect the microbial counts. The mean log values of Aerobic Plate Count given by two methods are shown in Fig.1(a). The lack of

significant differences between means ($P< 0.05$) and the high correlation coefficient showed that Petrifilm is a suitable and convenient alternative to the standard traditional method for the enumeration of mesophilic flora in fish samples.

Enterobacteriaceae is a diverse family consisting of Gram-negative, oxidase negative bacilli. They produce acid from glucose; are facultative anaerobes; reduce nitrate to nitrite; and may be motile or non-motile (Kornacki and Johnson, 2001). Enterobacteriaceae were found to be a part of spoilage microflora in fish samples. Members of Enterobacteriaceae are commonly used as indicator microorganisms for assessing food safety and hygiene, since they are found in the gastrointestinal tract of humans and animals (Forsythe, 2002) and can cause diseases and even economic losses (Trabulsi, 2005). Traditional methods use violet red bile agar with glucose for enumerating Enterobacteriaceae. The 3M™ Petrifilm™ Enterobacteriaceae count plate enumerates Coliforms plus potential pathogens and give a picture about the contamination of food samples in as quick as 22 hours. The product consists of a medium optimized for the growth of Enterobacteriaceae, yet inhibitory to the growth of Gram-positive bacteria. The 3M™ Petrifilm™ Enterobacteriaceae Count Plates are an effective method to assess environments such as post-process food contact surfaces, and help us to quickly determine potential sources of contamination. This plate enumerates all Coliforms as well as potential pathogens such as *Salmonella*, *Shigella* and *Yersinia*. In this study, the correlation coefficient between the Petrifilm method and traditional VRBG plates for Enterobacteriaceae enumeration was 0.985.The counts given by both methods were not significantly different ($p < 0.05$).Therefore, the Petrifilm EB count plate method can be adopted for the enumeration of Enterobacteriaceae in fish samples.

The 3M™ petrifilm™ *E. coli*/Coliform count plate provides a confirmed result in 24 to 48 hours. Omitting the confirmation steps increase productivity and reduce overall lab costs. Fast, accurate, results will be obtained in 24 hours. In 3M™ petrifilm™ confirmed Coliform colonies are red and blue with associated gas bubbles and *E. coli* are blue colonies with associated gas bubbles. The mean $\log_{10}$ transformed values of *E. coli* exhibited by *Caranx* spp., *Thunnus albacares*, *Scomberomorus commersonii* and *Fenneropenaeus indicus* on Petrifilms were 2.61 $\log_{10}$ cfug^{-1}, 2.55 $\log_{10}$ cfug^{-1}, 2.36 $\log_{10}$ cfug^{-1} and 2.48 $\log_{10}$ cfug^{-1}, whereas traditional petriplates exhibited a count of 2.38 $\log_{10}$ cfug^{-1}, 2.4 $\log_{10}$ cfug^{-1}, 2.1 $\log_{10}$ cfug^{-1} and 2.25 $\log_{10}$ $cfug_1$, respectively. There was no significant statistical difference ($p < 0.05$) between the mean $\log_{10}$ counts from the Petrifilm plate procedure and those with the conventional T_7 agar plates and VRBA plates. The corresponding correlation coefficients of *E. coli* and total Coliforms were 0.88 and 0.995.

The 3M™ petrifilm™ Staph Express count plate provides confirmed *Staphylococcus aureus* results in as quick as 22 hours. Distinctive red-violet colonies make interpretation very easy. Of the seven fish samples analyzed only three fish samples exhibited positive results for *S. aureus*. *T. albacares*, *F. indicus* and *O. vulgaris* gave mean $\log_{10}$ value of 2.35 $\log_{10}$ cfug^{-1}, 2.91 $\log_{10}$ cfug^{-1} and 3.31 $\log_{10}$ cfug^{-1} on Petrifilms whereas *S. aureus* count on Baird parker petriplates were 2.2 $\log_{10}$ cfug^{-1}, 2.65 $\log_{10}$ cfug^{-1}, and 3.2 $\log_{10}$ cfugm^{-1}, respectively. The correlation coefficient between traditional method and Petrifilm method for *S. aureus* enumeration was 0.928. No significant variation ($p < 0.05$) was noted between BP agar plates and 3M™ petrifilm™ Staph Express count plates.

The colony counts of *E. coli*, Coliforms, Enterobacteriaceae, aerobic microorganisms and *S. aureus* obtained by Petrifilm method are well correlated with the counts obtained by conventional standard plate techniques. The $\log_{10}$ counts of the Petrifilm plate procedure were slightly higher than those of the traditional methods. Traditional methods have great possibility of errors during media preparation, sterilization etc. which in turn reflects in the test result. Compared to traditional methods, chances of contamination is less with petrifilms. Petrifilms are compact and space savers in incubators. Therefore, the Petrifilm technique can effectively be applied for routine microbiological analysis of

food samples as a convenient alternative to conventional method for enumeration of aerobic microorganisms, Enterobacteriaceae, Total Coliforms, *E. coli* and *S. aureus*.

In the field of food protection, early screening of food products is an important measure to prevent epidemics relating to food-borne pathogens. Novel techniques should be adopted to reduce the work load and laboratory expenses. Petrifilms are a good alternative as it can enhance accuracy of test results and address the overall concerns facing the laboratory today: job satisfaction, decreased length of stay and safety. Financial savings can also be realized as a result of labor reduction.

References

Forsythe, S.J. (2002) - Microbiologia da Segurança Alimentar. Porto Alegre: ARTMED, 1-378.

Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A. and Uyttendaele, M. (2010) - Alternative microbial methods: An overview and selection criteria, *Food Microbiol.*, **27:** 710-730.

Kornacki, J.L. and Johnson, J.L. (2001) - Compendium of Methods for the Microbiological Examination of Foods, (Eds.) Pouch Downes, F. and Ito, K., American Public Health Association, Washington, DC, pp 69-82.

Official Methods of Analysis (1995) - 16th Ed., AOAC International, Gaithersburg, MD. Silbernagel, K.M. and Lindberg, K.G. (2003) - 3M™ Petrifilm™ Enterobacteriaceae count plate method for enumeration of Enterobacteriaceae in selected foods: Collaborative study, *J. AOAC Intl.*, **86(4):** 802-815.

Trabulsi, L.R.(2005) - Microbiologia, 4th Ed. São Paulo: ATHENEU pp 269-276.

U.S. Food and Drug Administration (1995) - Bacteriological Analytical Manual, 8th Ed., AOAC International, Gaithersburg, MD.

Multi-drug resistant *Salmonella* in seafood

Greeshma S.S., [1] Navami Krishna, Toms C. Joseph, Murugadas V. and Prasad M.M.

ICAR-Central Institute of Fisheries Technology, Cochin

[1]St. Thomas College, Palai, Kottayam

Salmonella is a gram negative bacteria which belongs to the family Enterobacteriaceae and is one of the most important food-borne pathogens globally. Non typhoidal *Salmonella* (NTS) refers to a range of *Salmonella* serotypes other than typhi and paratyphi. They mainly cause diseases ranging from mild gastroenteritis to life threatening illness and is of great public health concern world-wide. Due to indiscriminative use of antibiotics in animal and human disease treatment, there is an increasing incidence of antibiotic resistance in non-typhoidal *Salmonella* and it is quite alarming too. Along with this, disposal of untreated organic wastes also adds significantly to the development and wide spreading of Multi-drug resistant (MDR) *Salmonella* strains recently. There are only limited studies in India regarding the prevalence of multi-drug resistance in seafood *Salmonella*.

In this context, the present study was conducted to estimate the antibiotic sensitivity pattern of *Salmonella* isolates collected from seafood. *Salmonella* isolates (n=157) from seafood of Cochin local markets were tested for antibiotic susceptibility using Kirby-Bauer disc diffusion method as per CLSI standards. The test was performed on Mueller-Hinton agar (Difco, USA) using *Escherichia coli* (ATCC 25922) as a reference organism for quality control. The isolates were tested for the 20 antibiotics using ICOSA G-II minus disc (Himedia, India). These antibiotics belongs to different classes *viz.*, quinolones, aminoglycosides, carbapenems, cephalosporins,