STUDIES ON COAGULASE - POSITIVE STAPHYLOCOCCI AND *VIBRIO PARAHAEMOLYTICUS* IN SELECTED ITEMS OF FISH, CRUSTACEANS AND FISHERY PRODUCTS

THESIS
SUBMITTED TO
COCHIN UNIVERSITY OF
SCIENCE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

By

SANJEEV, S., M. Sc.

FEBRUARY - 1990

CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)
COCHIN - 682 029

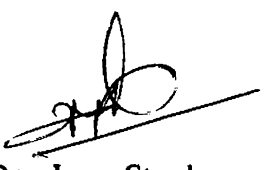
CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
Matsyapuri P.O., Cochin - 682 029

Dr. Jose Stephen, M.Sc., Ph.D., A.R.S.
Scientist (Selection Grade)
Biochemistry, Nutrition & Microbiology
Division.

This is to certify that this thesis entitled "Studies on coagulase-positive staphylococci and Vibrio parahaemolyticus in selected items of fish, crustaceans and fishery products" embodies the results of original work conducted by Shri. Sanjeev. S. under my supervision and guidance from 6.8.1983 to 5.2.1990. I further certify that no part of this thesis has previously been formed the basis of the award of any degree, diploma, fellowship or other similar titles of this or any other University or Society. He has also passed the Ph.D. qualifying examination of the Cochin University of Science and Technology held in July, 1987.

Cochin-29

5th February, 1990



Dr. Jose Stephen

DECLARATION

I hereby declare that this thesis entitled "Studies on coagulase-positive staphylococci and Vibrio parahaemolyticus in selected items of fish, crustaceans and fishery products" is a record of bonafide research carried out by me under the supervision of Dr. Jose Stephen, M.Sc., Ph.D., A.R.S., Scientist (Selection Grade) and it has not formed the basis for award of any degree, diploma, fellowship or other similar titles from this or any other University or Society.

Cochin-29.

5th February, 1990

SANJEEV,S.

ACKNOWLEDGEMENTS

The author expresses his deep sense of gratitude to Dr. Jose Stephen, Scientist (Selection Grade), Biochemistry, Nutrition and Microbiology Division, Central Institute of Fisheries Technology, Cochin-29 for his guidance and valuable suggestions during the entire course of his investigations. Grateful thanks are also due to Dr. C.C. Panduranga Rao, the previous Director, Mr. M.R. Nair the present Director of C.I.F.T. and to Mr. K. Mahadeva Iyer former Head of the Division of Biochemistry, Nutrition and Microbiology of C.I.F.T. for encouragements, valuable suggestions and for providing facilities for this work.

My sincere thanks are also due to Dr. M.S. Bergdoll, Food Research Institute, University of Wisconsin, U.S.A. for supply of reference enterotoxins, antisera, OSP template etc., to Dr. D.S. Agarwal, Prof. and Head of Microbiology Department, Maulana Azad Medical College, New Delhi for providing facilities to carry out phage typing, to Dr. N.K. Gopinath, Superintendent, General Hospital, Ernakulam for providing blood and to Dr. I. Karunasagar, Associate Professor of Fishery Microbiology, College of Fisheries, Mangalore for the supply of bacterial cultures.

The author is very much grateful to Dr. A.K. Kesavan Nair, Principal Scientist, C.I.F.T. for the help rendered in carrying out the statistical analysis of the data, to Dr.T.S. Gopalakrishna Iyer and to Shri. Cyriac Mathen, Principal Scientists, C.I.F.T. for the help extended by them while carrying out this investigation.

Technical assistance given by Shri. T.K. Sayed Ali, N.M. Vasu, K.V. Rajan, V.V. Johni, V.K. Ibrahim, P.S. Raman Nampoothiri, P.K. Raghu, K.K. Radhakrishnan, Smt. K.S. Mythri and C. Ammini is gratefully acknowledged.

Sincere thanks are due to my parents for all their blessings and to my wife and daughter for the co-operation rendered to me throughout the study.

CONTENTS

CHAPTER - I

INTRODUCTION

INTRODUCTION

The world's challenging task of today is to find out ways and means to irradicate hunger and to provide enough protein rich food for man. The rate at which human population increases, makes one believe that even the expected multifold increase in the production of food from the land is unlikely to meet the requirements of mankind. Therefore, man has to depend to a great extent on aquatic resources for food.

India, with a coastline of 5700 kilometers and shelf area of more than 2,50,000 square kilometers into which numerous large and perennial rivers discharge their silt-laden waters and with a number of small gulfs and bays all along the coasts, offers great scope for the development of fisheries. The scientific exploitation of the fishery resource is not only important for meeting the acute shortage of protein foods in India, but also necessary for raising the socio-economic status of the fishermen who constitute one of the low income groups.

India is a major fish producing country ranking eighth among the world nations. Out of the total fish production of about 3 million tonnes, less than 5 percent is exported to other countries, while the rest is sold in domestic markets for direct human consumption and only a negligible quantity is utilized for reduction purpose (Iyer et al., 1986). The fish and fishery products meant for export are subjected to rigorous quality tests at different stages of production and storage by individual entrepreneurs as well as by Government agencies to ensure high quality finished product. As the existing fish inspection and quality control measures are exclusively applied to export products, thus

leaving out of their perview more than 95 percent of the total production for domestic consumption. Therefore steps are needed to introduce and enforce quality control measures in this sector as well.

In India much work has been done on different aspects of quality of processed fish and fishery products (Pillai et al., 1965; Iyer and Chaudhuri, 1966; Iyer et al., 1966; Sreenivasan and Joseph, 1966; Pillai and Rao, 1969; Mathen et al., 1975; Valsan et al., 1985) and standards have been formulated for almost all the fishery products. Quality standards for fresh fish cover only a few fish species available in the markets. For the formulation and recommendation of quality standards for fish and fishery products for domestic trade detailed background informations are necessary. Khot et al., (1982), Valsan et al., (1985) and Iyer et al. (1986) have reported on the bacterial flora of sea foods at retail level in Bombay. Cochin is a major fish landing centre of the south west coast of India, and an average of 25,000 tonnes of fish/shell fish are landed annually at this harbour which is about 10 percent of the total catch of fish in Kerala (Lakshmanan et al., 1984). All the varieties of marine, brackish water and fresh water fishes are available in Cochin throughout the year for catering to its cosmopolitan population and informations regarding their bacteriological quality are scanty. Hence the present study was undertaken to investigate the bacteriological quality of fish and fishery products available in the markets and cold storages situated in and around Cochin meant for internal consumption.

The work reported here is aimed at studying the total bacterial count and incidence of organisms of public health significance mainly coagulase-positive staphylococci and Vibrio parahaemolyticus in fish and fishery products

collected from the markets and cold storages situated in and around Cochin meant for internal consumption. The isolates of coagulase-positive staphylococci and V. parahaemolyticus were subjected to detailed studies on their characteristics, seasonal variations, survival, antibiotic sensitivity and its enteropathogenicity. Studies were also carried out to find out the source of contamination of fish and fishery products with Staphylococcus aureus. Staph. aureus isolated from fishery products and fish processing factory workers were phage typed. V. parahaemolyticus has been considered as a normal flora of marine fish (Cann, 1977; Liston, 1980; Mossel, 1982 and Hobbs, 1982).

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A. General bacterial flora of fresh fish and shell fish

It is generally accepted that the flesh of newly caught healthy fish is sterile, but bacteria are found in variable numbers in three sites on the fish: the slime coat, the gills and the intestine. Reay and Shewan (1949) have reviewed the work done during the period from 1920 to 1945 on the bacterial flora of fish and their involvement in spoilage. The composition of the microflora of different species of fish tested has mostly been reported to be dominated by Gram-negative bacteria usually identified as Achromobacter, Pseudomonas, Flavobacterium and less frequently Vibrios. However, there are a few reports of isolation of Gram-positive bacteria also. Since in many studies, fishes were obtained from commercial sources, the presence of Micrococcus is not unexpected. The bacterial load on different parts of fish has been found to vary according to the seasonal changes in the environment in which the fish lives (Shewan, 1961; Shrivastava and Floodgate, 1966; Karthiayani and Iyer, 1967; Hobbs and Hodgkiss, 1982). The method of fishing has also been found to influence the bacterial flora of fish (Shewan, 1949; 1977). The qualitative and quantitative variation in the bacterial flora of fish are found to be closely associated with the feeding habits of fish (Shewan, 1961; Shrivastava and Floodgate, 1966; Hobbs, 1982).

Shewan (1977) has concluded that the bacteria on the fishes, from temperate waters are psychrophilic in nature, whereas those in the fishes from tropical areas are mesophilic. Hobbs (1982) has also upheld Shewan's (1977) conclusion. It is therefore clear that the normal microflora of fish from

warm waters are quite different from those occurring in fishes from cold waters. Achromobacter was found to predominate in some species of fishes caught from tropical waters (Liston and Colwell, 1963; Karthiayani and Iyer, 1967). On the other hand, marine fishes caught from the warmer coastal waters of Australia have the predominance of Gram-positive organisms especially Micrococcus (Wood, 1952, 1953; Shewan, 1977). In India, Jadhav and Magar (1970) recorded the predominance of Bacillus and Achromobacter in white pomfret and seer fish. Banik et al., (1976) reported Proteus, Aeromonas, Achromobacter and Micrococcus to be the dominant flora in white pomfret. Studies of Surendran and Iyer (1976) revealed the presence of Vibrios on fresh mackerel to the extent of 37 percent of the total bacterial load. Anand and Shetty (1977) isolated Achromobacter, Vibrio and Alkaligenes from six different species of Indian fishes. These observations seem to support Shewan's hypothesis that bacterial population is affected more by the environmental factors than by the species of fish. This view has also been concurred by Cann (1977) and Liston (1980).

The microflora of crustaceans seems to vary with the temperature of water in which they live (Cann, 1977; Liston, 1980). Gram-negative bacteria belonging to Achromobacter and Pseudomonas dominate the microflora of most crustaceans from cold waters while warm water species carry mostly Gram-positive bacteria belonging to Coryneforms or Micrococcus. Crustaceans from temperate zones may also carry a high proportion of Coryneform bacteria (Hobbs et al., 1971; Lee and Pfeifer, 1975). The bacterial flora of iced Pacific shrimps (Pandalus jordani) in the order of predominance, has been shown to comprise Moraxella, Pseudomonas, Acinetobacter, Arthrobacter, Micrococcus and Flavobacterium cytophaga (Lee and Pfeifer, 1977). Lee and Pfeifer (1975)

found the same group of organisms in fresh crab meat. The bacterial count of shrimps and other bottom-dwelling creatures is difficult to evaluate because these creatures tend to be contaminated with sediment material (Liston, 1980).

B. Coagulase-positive staphylococci

Staphylococcus aureus was discovered by R. Koch (1878) and later isolated from furuncle pus by L. Pasteur (1880). It has been described as the causative agent of numerous suppurative processes by A. Ogston (1881) and has been studied in detail by P.J. Rosenbach (1884). Taxonomically the staphylococci have been placed in the family Micrococcaceae. These organisms are asporogenous, non motile and gram-positive. Colonies may appear white, yellow to orange as a result of water insoluble pigments. The genus Staphylococcus consists of three species Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus saprophyticus. They are differentiated primarily on the basis of the ability to produce coagulase, ferment mannitol (both aerobically and anaerobically), produce heat-stable endonuclease and by the cell wall composition (Baird Parker, 1974). The species of public health significance is Staphylococcus aureus; pigmented and non pigmented varieties. This species is distinguished from the other species in the genus viz. Staphylococcus epidermidis, by its ability to ferment mannitol and to coagulate citrated rabbit or human plasma (coagulase-positive). At present this genus includes organisms formerly known as Staphylococcus pyogenes, Staph. albus, Micrococcus pyogenes Var. aureus and Var. albus and Staph. citreus as well as Staph. aureus (Frank L. Bryan, 1968). Demonstration of heat stable nuclease that was first detected by Chesbro and Auborn (1967) is an additional test recommended for Staph. aureus.

The Staphylococci typically occur as cluster like a bunch of grapes, approximately 0.8 to 1.0 μ in diameter. The spatial arrangement of cells in clusters is more frequently observed on solid media than in liquid media, and the occurrence in broth cultures of pairs, short chains and clusters of few cells is not uncommon. Capsules may be formed in very young cultures, but this disappear within a few hours (Bergdoll, 1979). They are Gram-positive in a young culture (18 to 24 hours) but tend to gram variable as the culture ages.

It is generally agreed that all strains classified as Staph. aureus are potential pathogens. There is no particular characteristic or substance produced by staphylococci that can be associated with pathogenicity. These organisms produce many substances, a number of which are toxic to one animal or another. Among these are the hemolysins (α, β, δ), fibrinolysin, hyaluronidase, exfoliation, leucocidin, phosphatase, penicillinase, proteases, lipase, catalase, lysozymelactic dehydrogenase, deoxyribonuclease, coagulase and the last but not of least important is the enterotoxin. Much is known about the enterotoxin because of their clear cut involvement in staphylococcal food poisoning. Many attempts have been made to correlate enterotoxin production to some other substances produced by staphylococci, but so far no correlation has been established. There is some evidence that only coagulase-positive and DNase-positive staphylococci can produce enterotoxins. However, not all coagulase-positive staphylococci or DNase-positive staphylococci are capable of causing food poisoning. Food borne outbreaks due to coagulase-negative staphylococci have also been seldom reported (Omori et al., 1960; Breckinridge & Bergdoll, 1971). At present it is not possible to test all strains for enterotoxigenicity, a selection could be made on the basis of coagulase or DNase production.

Shewan (1962) has reviewed the work carried out by various authors on the incidence of Staph. aureus in fish and fishery products upto 1962. In his review, Shewan has stated that 10-30% of fish handled onboard ship, filleted onshore or purchased over counter in the fresh or frozen state contained Staph. aureus. Further, he has also reported the incidence of the organism in Atlantic cod and North Sea fish caught during summer and expressed the opinion that incidence of Staph. aureus in freshly caught fish was less significant compared to the subsequent contamination during handling and processing onboard vessels and onshore. Studies carried out in subsequent years have further substantiated Shewan's above observations. Lovell & Barkate (1969) in their studies on freshwater cray fish from 22 sources representing the major commercial cray fishing areas in Lousisiana could isolate Staph. aureus only from 3% of the samples. Similarly in the recent work of Ridley and Slabyj (1978) on shrimps (Pandalus borealis) from Maine, has reported the absence of this organism in the sample collected onboard, but it was present to the extent of 4 organisms per gram in the material handled by workers.

The incidence of Staph. aureus has been found to be comparatively higher in cooked and prepared fishery products evidently due to the additional human handling after cooking and the inherent behaviour of Staph. aureus to grow comparatively faster in substrates containing minimum number of competing microorganisms. Thus, 40% of the frozen fish cakes and 60% of the smoked fish tested by Appleman et al. (1964) were found to contain Staph. aureus. Surkiewiez et al. (1967) have isolated the organism from 20% of the samples of frozen raw-breaded fish processed under "good" and "poor" conditions of sanitation. Carroll et al. (1968) isolated Staph. aureus from 87% of the samples

of frozen raw breaded shrimps examined by them and the average count recorded was 1.9 x 10^4 per gram. About 70% of the cooked frozen shrimps imported by Canada before 1968 carried more than 1000 Staph. aureus per gram, whereas the organism exceeded 100 per gram in only 16.7% of the breaded raw shrimps (Neufeld, 1971). Virgilo et al. (1970b) have reported that 70 out of 392 samples of pre-cooked frozen shrimps from the Chilean manufacturers contained more than 100 cells of Staph. aureus per gram.

Almost 50 percent of the frozen breaded raw shrimps of Gulf coast was found to contain Staph. aureus (Vanderzant et al., 1973). In U.K. Gilbert (1974) isolated Staph. aureus from 110 of the 3023 samples of imported cooked peeled and frozen shrimps. In each of the positive samples, the organism was more than 100 per gram. According to Cann (1977) pre-cooked frozen shrimps imported to U.K. contained 50 to 1.9x10^4 Staph. aureus per gram.

No general conclusion can be drawn on the occurrence of Staph. aureus in shrimps processed in tropical countries. Shrimps processed in Thailand were free from Staph. aureus (Cann, 1974) and only 1% of the cooked frozen shrimps processed and exported from Malaysia had Staph. aureus count of more than 1000 per gram (Hobbs, 1976). According to Beckers et al., (1981), 34% of the frozen pre-cooked and peeled shrimps from the South East Asian countries had Staph. aureus exceeding 2.0 x 10^3 per gram. In a recent study of process hygiene in Sri Lanka prawn industry, the Staph. aureus count of all the samples of fresh shrimps tested were in excess of 100 per gram whereas in frozen shrimps, only 32% of the head less (HL), 35% of Peeled and deveined (PD) and 37% of the cooked shrimps carried this (Sumner et al., 1982). Such differences in results are not surprising as the levels of hygiene maintained in the different tropical countries are not uniform.

A few studies have indicated that, in picked crab meat, the Staph. aureus count was related to the hygienic conditions maintained in the processing units. Thus all the samples of cooked and picked crab meat collected from factories along the Atlantic and Gulf coasts showed incidence of Staph. aureus. In "good" factories the count was between 3 and 37 per gram, while in poorly maintained factories, the count varied between 3 and 1070 per gram (Phillips & Peeler, 1972). In a study conducted by Olson & Shelton (1973) in 46 crab meat processing factories, the log average MPN coagulase-positive staphylococci per gram of the "lump" and special crab meat was 38 and 29 respectively in the processing factories having "good" hygienic status. On the contrary the corresponding figures in respect of the processing plant with "poor" hygiene were 70 and 450 respectively.

Though a few studies have been made in our country on the occurrence of Staph. aureus in fish and fishery products meant for export (Iyer, 1985) no such information is available in respect of fresh fish, frozen and dried fishery products meant for internal consumption. Studies of that nature has to be made to assess the potential health hazards to the consumers from staphylococcal intoxications through the consumption of fish and fishery products available in market and cold storage.

Foods, whether fish products or not, that are usually involved in staphylococcal food poisoning are protein containing cooked products contaminated by workers during preparation and thus allowed to incubate for several hours. Fish products are high in protein and thus support the growth of Staph. aureus. The foods involved in outbreaks are cooked or heat processed which destroys organisms that would normally compete with

staphylococci and perhaps out grow and overhelm them, or the foods contain high levels of salt, which inhibits the growth of competing organisms (Bryan, 1973).

Staphylococci especially enterotoxigenic staphylococci do not form part of the normal bacterial flora of fresh marine fish but they get contaminated either from the handlers or from the surfaces with which they come in contact. It is also known that Staph. aureus can grow vigorously in fish, if conditions are favourable (Matches & Liston, 1968; Bryan, 1973; Liston, 1980) and produce toxin.

Since 1930, it is known that contamination of food with Staph. aureus could cause gastroenteritis of sudden onset because the organism growing in food, secretes an exotoxin (Jay, 1978). This toxin is generally termed as enterotoxin, because it causes gastroenteritis or inflamation of the lining of the stomach and intestine.

Staph. aureus can grow in media containing upto 18% salt. Production of enterotoxin B in the favourable pH range 4.8-8.0 is inhibited by about 10% salt (Genigeorgis, 1974), but enterotoxin A can be produced in much higher concentration of salt (Lotter & Leistner, 1978). Most cured meats contain only 3-6% salt and in fish in the water phase, an environment suitable for toxigenosis by Staph. aureus, if sufficient oxygen is available. Fortunately, Staph. aureus is readily killed by heat. Even in uncooked products such as fermented sausages, the anaerobic interior presents an ecosystem that is inhibitory to the growth and toxigenosis, despite the frequency of Staph. aureus in such products (Pullen & Genigeorgis, 1977; Task Force, 1977). Staph. aureus can withstand drying at room temperature (Bryan, 1968).

The temperature range for optimum growth of staphylococci is 35 to 37°C, although growth does occur throughout the range 10 to 45°C with occassional strains growing at slightly lower and higher temperature (Begdoll, 1979). Staph. aureus has been reported to grow at a temperature as low as 5°C (Munch, 1960), but the lower limit for toxin production is somewhat higher. For example enterotoxin has been detected in foods held at 10°C (Genigerogis et al., 1969; Tatini, 1973) but toxin production at temperature below about 20°C is slow. One strain producing enterotoxin B required 158 hours at 13°C to produce detectable levels of toxin (1 µg/ml). Another strain grew at 13°C but failed to produce toxin below 19°C (Scheusner et al., 1973).

The temperature for enterotoxin production that has been used most frequently is 37°C. Experiments in the Food Research Institute in USA showed that toxin can be produced at 25 and 30°C, but in lesser amounts than at 37°C (Bergdoll, 1979). McLean et al., (1968) reported that only small amounts of enterotoxin B were produced at 16 and 20°C (10-20 µg/ml versus 340 µg/ml at 37°C) even though growth was almost equivalent to that at 37°C. Scheusner et al., (1973) showed that enterotoxin A-D were produced in brain heart infusion broth (BHI) over a range of temperature (19-39°C). All, but enterotoxin B was produced at 45°C, but this was the only one produced at 13°C, however the D producing strains did not grow at this temperature. Vandenbosch et al., (1973) obtained maximum production of enterotoxin B (Strain S-6) and C (FRI-137) at 40°C with somewhat lesser amounts at 35 and 42°C. Small amounts of toxin were produced at 15 and 45°C with none at 10 and 50°C. Thota et al., (1973) obtained maximum enterotoxin E production at 40°C.

Most strains of staphylococci grow at pH values between 4.5 and 9.3 with the optimum being 7.0 to 7.5. The condition for enterotoxin production are somewhat more restricted than for growth, for example entrotoxin production was limited to pH 5.15 to 9.0 (Scheusner et al., 1973). In the case of salt concentration for enterotoxin B and C production, the pH range for production became narrower as the salt concentration was increased (Genigeorgis and Sadler, 1966; Genigeorgis et al., 1971; Thompkin et al., 1973).

Staph. aureus, the most drought tolerant of the pathogenic bacteria, has a lower limit variously reported in different substrata as between 0.86 and 0.83 a_w (Anon, 1980). Enterotoxin production varied from medium to medium, since in some, enterotoxin A was produced at a_w's as low as 0.90 (Troller, 1972). In the case of enterotoxin B, production occurred at a_w 0.93 in potato dough but only at 0.97 or above in other cases (Troller, 1971). Enterotoxin production depends upon the system involved, as under some conditions it is possible to get good growth without subsequent enterotoxin production.

Staphylococci can grow both under anaerobic and aerobic conditions. but growth is much slower anaerobically and even after several days growth does not reach that level attained under aerobic conditions. Enterotoxin production also can occur under anaerobic conditions and has been reported to occur for enterotoxin B in cured meats, the same level of growth was achieved as that under aerobic conditions (Genigeorgis et al., 1969). In experiments with non acidulated sausage, Barber & Deibel (1972) were unable to demonstrate the production of enterotoxin A in the absence of O_2 at growth levels equivalent to those producing enterotoxin in the presence of O_2.

The growth rate of Staph. aureus in different foods has been reviewed by Minor & Marth (1972a). According to the information detailed in this review, the number of staphylococci inoculated on to the surface of chicken increased 10,000 fold within 8 hours at ambient temperature. Further increase in temperature (in the range of 35.5 to 42.5°C) did not appreciably alter the growth rate. However, at 45°C the number of staphylococci increased 1000 fold within 8 hours, but at 47°C, they did not grow at all. Luxurient growth of this organism occurred in canned meat held at 22°C and 37°C for upto 60 days. The above review has also cited that the minimum temperature for the growth of staphylococci in chicken gravy was between 5°C and 10°C and the growth reached maximum stationary phase in 48 hours and 15 days at 20°C and 10°C respectively. There is a paucity of data on the growth pattern of Staph. aureus in cooked, picked crab meat.

Raj & Liston (1961) observed that the organism showed a seven fold reduction in number in fish homogenate during the first 130 days of storage at -17.8°C followed by little change thereafter till the end of the study, which continued upto 393 days. Iyer (1985) observed that there is 8 to 15% reduction in Staph. aureus in cooked shrimp during freezing at -40°C. In raw shrimp but on the other hand the destruction during freezing was between 40 and 50%. He also observed that during frozen storage at -20°C, the organism disappeared from the raw shrimps much earlier (3-4 months) than from the cooked shrimps (5-6 months). Apparently only little work seems to have been done on the behaviour of Staph. aureus at sub-zero temperatures and hence there is a need for detailed study on this important aspect.

The staphylococci are ubiquitous in nature, with man and animals as the primary hosts. They are present in the nasal passages and throats, on the hair and on the skin of probably 50% or more of healthy individuals (Bergdoll, 1979). Surveys have shown that the throat is positive about 4-60% of the time (William, 1963). The incidence of Staph. aureus on human skin is estimated to be between 5 and 40% (Bryan, 1968). Kallander (1953) found these organism on the skin of 30% food handlers and faecal carriage is not uncommon (Allison, 1949; Matthias et al., 1957). These organisms multiply freely in the salt rich secretion of the human nose and exists as symbiots. Showers of droplets from the nose charge the skin and clothing as well as air. Use of handkerchiefs and touching the nose can transmit large number of organisms to hands (Stephen, 1959).

On the basis of fundamental research carried out recently, it may now be assumed that approximately 80% of all strains of Staph. aureus of human origin do possess the capacity of forming one or more staphyloenterotoxins (Evans et al., 1950; Zak et al., 1971; Morris et al., 1972; Untermann, 1972; Hajek & Mursalek, 1973; Muller et al., 1973; Mochmann et al., 1976 and Reali, 1982). When these bacteria grow in foods to levels over approximately 10^5/g (La Chapelle et al., 1966; Baumgrat, 1970; Tatini et al., 1971, Milling, 1971; Weimann et al., 1971; Bergdoll et al., 1972; Gilbert et al., 1972; Tatini et al., 1973 and Niskanen & Nurmi, 1976) corresponding with the formation of µg quantities of enterotoxin (Hallender, 1966; Raj & Bergdoll, 1969; Stojanow & Meeser, 1970; Muller & Stojanow, 1971; Fung, 1972; Minor and Marth, 1972; Reiser et al., 1974 and Van et al., 1979) the risk of staphyloenterotoxicosis is imminent. Jay (1970)

Hobbs (1973) Gilbert & Wieneke (1973) Lee & Pfeifer (1975) and Hobbs (1982) maintained that food handlers are the main source of contamination of the processed product with Staph. aureus. Therefore, this organism while itself a food poisoning organism is a useful indicator of the hygiene in a process involving human handling (Shelton et al., 1962; Cann, 1977; ICMSF 1978; Liston, 1980, Connel & Shewan, 1980; Hobbs, 1982b). According to Hobbs (1983) the presence of Staph. aureus even in small numbers is an excellent indicator of human hygiene.

No information is available on the carrier status of enterotoxigenic staphylococci among food/fish handlers, although unpublished data are available on the carriage of enterotoxigenic staphylococci among the general population (Pandurange Rao, unpublished data).

The number of Staph. aureus in foods at the time of contamination may be very low but when handled unhygenically and exposed to ambient temperatures it may multiply rapidly as this organism can grow exponentially between 6.7^{o}C and 45.5^{o}C (Angelotti et al., 1961, Michener & Elliot, 1964; Bryan, 1973) and produce toxin (Gilbert, 1974; ICMSF, 1978). Any food touched by hand are subject to contamination by the infected person. Hodge (1960) reported that 99% of staphylococcal outbreak that he surveyed were caused by cooked high protein foods. Fish and fishery products are good substrata since they are rich in protein. The foods involved are usually those which have been cooked or heat processed or treated with high levels of salt (cured fish). The heat or high salt content destroys or retards the growth of competing saprophytes and then leaves a free field for staphylococci which happens to contaminate the food at the time of handling and processing.

Thus the presence of staphylococci especially in the absence or among small number of other saprophytes on a protein rich substratum can cause food poisoning hazard under improper storage condition. The toxin once formed will withstand boiling for 20 to 60 minutes or even autoclaving, although it gradually losses its potency during such heating (Frazier, 1976). Therefore, the normal cooking temperature, though sufficient to kill Staph. aureus could leave the toxin unaffected. It is thus evident that once sufficient quantity of toxin is formed in a food material before its consumption food poisoning can follow even though the material is cooked. Large number of Staph. aureus usually more than one million organisms per gram of food must be present or must have been present at a time to produce the symptoms of food poisoning. But in the study of Hobbs (1960), the counts obtained in the incriminated foods were usually less than $5x10^5$ per gram. Counts of Staph. aureus in 39 incidents of food poisoning varied between $7.5x10^5$ and $9.0x10^9$ per gram (Gilbert et al., 1972). Almost a similar observation has been reported by Gilbert (1974) Hobbs (1974) and Mossel (1982). Bergdoll (1973) has stated that less than 1 microgram of enterotoxin is sufficient to cause illness in a sensitive individual. Ingestion of food containing sufficient quantity of enterotoxin results in the onset of symptoms within 2 to 4 hours, although in certain cases it takes only 30 minutes (Bryan, 1968). The most common human symptoms are salivation, then nausea, vomiting, retching, abdominal cramping of varying severity and diarrhoea. Blood and mucus may be found in stools and vomitus in severe cases. Headache, muscular cramping, swetting, chills, prostration, weak pulse, shock and shallow respiration may occur. Usually a subnormal body temperature is found rather than fever.

Recovery usually takes place within 24 hours, but may sometimes takes several days (Frazier, 1976). Although there is high morbidity, the rate of mortality is low (Gilbert, 1974).

Staphylococcal food poisonings, are caused by antigenically distinct polypeptides which function as emetic toxins known as enterotoxins. Five specific enterotoxins called enterotoxin A, B, C, D and E have been identified by Bergdoll (1970) and Gilbert (1974), that differ in toxicity. Recently Mossel (1982) has reported the identification of eight enterotoxins designated as A, B, C_1, C_2, D, E, F and G. Enterotoxin A was reported to be the most potent and most common in causing food poisoning followed by D, C and B (Casman & Bennett, 1965; Frazier, 1976). Enterotoxin C was produced by majority of the isolates, recovered from food poisoning cases in India (Rajalakshmi & Rajyalakshmi, 1982). Food implicated in Staphylococcal food poisoning may contain 0.01 mcg or more of enter toxin per gram of the food (Casman & Bennett, 1965; Bergdoll, 1972).

Though a few studies have been carried out in recent years on the prevalence of enterotoxigenic Staphylococci in milk and meats in this country (Ghosh, 1970; Rao, 1976; 1977a, 1977b) no such information in respect of fish and fishery products are available.

Until recently, the methods for the detection of staphylococcal enterotoxin involved intraperitonial or intravenous injection of cats and kitten and feeding young rhesus monkeys. As these techniques are expensive and not entirely reliable, they have been largely replaced by serological techniques • Several methods for the laboratory production of enterotoxin

have been comparatively evaluated by Simkovicova and Gilbert (1971). The cellophane sac-culture dialysis methods of Casman & Bennett (1963) and Donnelly et al., (1967) have been used successfully in a number of laboratories in Europe and North America. The method of Casman & Bennett produce large amounts of toxin, but that of Donnelly et al., (1978) (ICMSF, 1978) is easy to perform. The cellophane-over-agar-method (Hallender, 1965; Robbins et al., 1974) has also been used successfully. A number of methods which employ specific antibodies have been used in various ways for the serological detection and measurement of the enterotoxins. These includes the Duchterlony plate technique, the fluorescent antibody test, reversed passive hemagglutination and radio immuno assay (ICMSF, 1978).

It is known that ingestion of as little as 1 µg of staphylococcal enterotoxin in a portion of food can cause an intoxication. For safety, therefore, it should be possible to detect at least 0.1 µg/100 g of food. Both the optimum sensitivity plate (OSP) method and the microslide technique fail to detect such small amounts of toxin. Therefore other techniques are being developed to provide increased sensitivity. One of the most promising technique in this respect is the enzyme linked immunosorbent assay (ELISA), originally described by Engvall & Perlmann (1971). Reversed passive Latex Agglutination (RPLA) has got sensitivity of upto 1 ng/g of food matrix (Shingaki et al., 1981; Oda et al., 1979; Park & Szabo, 1986).

The increase in the number of antibiotic resistant strains of Staph. aureus is of serious concern when viewed from thereapeutic and epidemilogical angles. During the early years of antibiotic era, a few

penicillin resistant strains were isolated. In Poland Kurylowiez & Slopek (1946) studied 200 strains of Staph. aureus and found only 1% of the resistant strains. With the introduction of newer antibiotics there is change in the antibiotic resistance pattern of staphylococci (Barber et al., 1949; Gupta & Chakaravarthi, 1954; Trivedi & Sarkar, 1954; Beaven & Burry, 1956; Myers & Acharya, 1956; Barua et al., 1959; Goyal & Madhavan, 1961; Pal & Ghosh Ray, 1962; James, 1962; Wasek et al., 1963; Agarwal et al., 1963; Chatterjee & Aikat, 1964; Rao et al., 1966; Bhaskaran & Jayakar, 1969; Bhujwala & Mohapatra, 1972; Nanu & Soman, 1980; Vijayalakshmi & Bhaskaran, 1981). The most important problem in dealing with the staphylococcal infection has been progressive emergence of the organism resistant to various antibiotics and chemotherapeutic agents due to the widespread and indiscriminate use of antibiotics. Resistance may appear rapidly or slowly depending on the organism concerned, the volume and type of drug used and the method of application. Information concerning the drug resistant pattern of the prevailing pathogenic bacteria and the appearance of new resistant characteristics is of utmost value for a proper selection of antimicrobial agent for therapeutic purpose (Sundaram et al., 1982).

Phage typing has been used for strain characterization and discrimination in Staph. aureus for more than 35 years. The method has proved extremely useful for tracing the source of outbreaks of food poisoning as it is most successfully applied to incidents of 'simultaneous' infection in which relatively small numbers of strains are isolated and there is less possibility of variation in the phage-sensitivity of the strains. Because many

strains of Staph. aureus are enterotoxigenic, tests for the production of enterotoxin may not be sufficient alone to identify the strain responsible for an outbreak. Most staphylococci implicated in food poisoning are lysed by phages of group III or mixed group of I and III (Gilbert and Antonetee, 1973). The information regarding the phage pattern of Staph. aureus strains isolated from fish, fishery products and fish processing factory workers are scanty.

Reports on food poisoning caused due to consumption of various fishery products including staphylococcal food poisoning due to canned shrimps canned smoked cod, sparts in oil, North African sardines in oil, Moroccan sardines in oil, kippers fish sausage, fish pudding, boiled salmon, light salted smoked mackerel, weakly salted herring, frozen fish sticks and fish cakes have been reviewed by Shewan (1962). In many cases, the contamination during handling and processing coupled with favourable time - temperature condition in the factory before processing has been shown to be the cause of staphylococcal food poisoning. Generally, subsequent faulty heat processing has been found to increase the chances of food poisoning. In most cases, where canned fish were incriminated in food poisoning, the cans were not bulged as Staph. aureus neither produced gaseous products nor did it produce any abnormalities in appearance, odour or taste of the product (Shewan, 1962). Out of 175 outbreaks of staphylococcal food poisoning in England and Wales, 17 were due to consumption of fish (Anon, 1963; 1964). Canned fishes, canned prawns and frozen shrimps were the main products incriminated in these food poisoning outbreaks. In a review on the food poisoning outbreaks for the period 1967 to 1969, Bryan (1973) stated that fish and

shellfish products were responsible for 7.2% of the reported outbreaks of staphylococcal food poisoning in the United States. The food products for most of these outbreaks were tuna, shrimps and other unspecified salads. Sutton (1973) has reported a case of food poisoning in Australia due to the consumption of cooked peeled frozen shrimps.

Staphylococcal food poisoning has been one of the major types of food borne illnesses even in countries with good environmental sanitation. In India, where a proper system of reporting food-borne illnesses is non-existent, there have been reports of staphylococcal food poisoning and all were attributed to milk products (Saha & Ganguly, 1957; Ghosh & Chattoraj, 1963; D'Souza et al., 1965; Pal, 1972; Narayan & Sharma 1979; Rajalakshmi & Rajyalakshmi, 1982). In none of these reports, except that of Rajalakshmi & Rajyalakshmi (1982) enterotoxigenicity of the staphylococcal isolates were determined even though the only method of ascertaining the food poisoning potentialities of staphylococcal isolates has been the test for enterotoxigenicity.

Sufficient information on the incidence and source of contamination of Staph. aureus in fish has been collected in cold countries. Unfortunately, there is a lack of similar information from tropical countries were the high ambient temperature usually favours multiplication of Staph. aureus. Even though India is the 8th largest fish producing country of the world, there is a paucity of information on fish and fishery products meant for internal consumption.

C. Vibrio parahaemolyticus

Vibrio parahaemolyticus as it is known today was first isolated by Fujino et al. in 1951 and named as Pasteurella parahaemolytica. This organism caused gastroenteritis in 272 persons resulting in 20 deaths in Osaka, Japan. The persons involved in this food poisoning had consumed "Shirasu" which was contaminated with V. parahaemolyticus. Because of its bipolar staining and fastidious nature on ordinary media, the organism was placed in the genus Pasteurella (Fujino et al., 1951).

Salted chopped cucumber (Asazuke) was incriminated in the outbreak of gastroenteritis involving 120 persons in 1955. The causative organism was classified as Pseudomonas enteritis by Takikawa (1958). He observed that the characteristics of the isolates were similar to the cultures isolated by Fujino et al. (1951) but changed the genus name to Pseudomonas based on biochemical properties and growth in media containing salt. This was the first indication of halophilic nature of this organism.

Based on serological differences between this organism and Pseudomonas Miyamoto et al. (1961) proposed the generic name Oceanomonas. Sakazaki et al. (1963) proposed the generic name as Pasteurella based on its halophilism. Attempts to clarify the taxonomical position of this organism, extensive studies of many of the halophilic organisms were carried out. Among those studied were the strains of Fujino et al. (1951) and of Takikawa (1958) and reported that these organisms were closely related to the genus Vibrio. Based on the differences of growth in peptone water containing 7 and 10% sodium

chloride, Voges Proskauer reaction and fermentation of sucrose, arabinose and cellobiose, they recognized three subgroups: Sub group 1 and 2 were designated as Vibrio parahaemolyticus and sub group 3 resembled Vibrio anguillarum, which did not grow in 7 or 10% sodium chloride. Sub group 2 grew in 7 and 10% sodium chloride, whereas sub group 1 grew in 7% sodium chloride only (Sakazaki et al., 1963).

Zen-Yoji et al. (1965) confirmed the difference between sub group 1 and 2 and reported the differences in pathogenicity between the two groups. Sub group 1 was isolated frequently from patients with unidentifiable enteritis. The occurrence of sub group 2 in gastroenteritis patient was not significantly higher than the patients without the illness and they concluded that sub group 2 was not pathogenic to man.

Sakazaki (1968a) re-examined 100 cultures of each sub groups and confirmed the results reported by Zen-Yoji et al. (1965). Cultures of sub group 2 grew in 10% sodium chloride, fermented surcrose and produced acetoin whereas those of sub group 1 did not. Because of these differences he proposed the specific name Vibrio alginolyticus for sub group 2 (biotype 2). The organism of sub group 1 (biotype 1) continued to be classified as Vibrio parahaemolyticus.

Since V. parahaemolyticus was isolated from cases of gastroenteritis in 1951, this vibrio has been recognized as a potential enteropathogen all over the world. V. parahaemolyticus inhabits the marine and brackish water environments and it is therefore, associated with fishes harvested from these environments. Although this halophilic organism was first isolated more than 35 years ago, it has remained practically unknown

elsewhere for sometime. At first it was thought to be limited to Japan and Far - East. But during the last 15-20 years it has been isolated from many species of fish, shell fish and marine environments such as bottom sediments and plankton. This has been reported from many countries viz. U.S.A. (Liston, 1973) U.K. (Barrow, 1974) Philippines, Taiwan, Hong Kong, Singapore (Sakazaki, 1969), Panama, West Africa, Indonesia (Beuchat, 1977), Malaysia (Cann et al., 1981) and in India (Chatterjee et al., 1970; Chatterjee & Neogy, 1972; Nair et al., 1975; Victer & Freda, 1976; Natarajan et al., 1979a,b; Lall et al., 1979; Nair et al., 1980; Karunasagar & Mohan, 1980; Pradeep & Lakshmanaperumalsamy, 1984). The earlier work on V. parahaemolyticus has been extensively reviewed (Sakazaki, 1969; Lee, 1973; Liston, 1973; Sakazaki, 1973b; Barrow, 1974; Joseph et al., 1982). In 1973 an international conference on V. parahaemolyticus was held in Tokyo (Anon, 1974).

V. parahaemolyticus is of marine origin and can be found in sea water, sediments, plankton, fishes and shell fishes of coastal and estuarine environments in warm water areas throughout the year. But its prevalence in temperate region appears to be seasonal and restricted to warmer inshore waters, particularly where organic content is high (Baross & Liston, 1970; Cann, 1977; Liston, 1980). It rarely occurs in north sea although it has been found in sea mud and shell fishes off south-west of England (Cann, 1977). Shewan (1977) has reported the incidence of V. parahaemolyticus in the Baltic, Adriatic, Meditarranean and Indian Ocean and off the east and west coasts of North America.

De et al. (1977b) showed the incidence of V. parahaemolyticus in marine fishes of Calcutta to be 35.2%. Natarajan et al. (1979b) reported 36.8% occurrence in fishes from brackish water environments. Karunasagar & Mohan (1980) found that the incidence varied from 8.33 to 33.3%. The studies of Nair et al. (1980) revealed that 35.6% of the freshly harvested fishes from the estuarine waters, 40.6% fishes of mangrooves, 37.5% of freshly caught brackish water fishes and 44% fishes of market samples showed the incidence of V. parahaemolyticus. In our country very little information is available on the occurrence of V. parahaemolyticus in dried fishery products and frozen fishery products meant for internal consumption.

Liston (1973) reported isolation of V. parahaemolyticus from breaded shrimps, frozen breaded oysters, packaged crab meats, fresh clams and shucked oysters. However, Vanderzant et al. (1973) could not isolate this organism from breaded shrimps collected from processing units. The incidence of V. parahaemolyticus has been reported in 15% of clams 19% of mussels and 38% of oysters from eight sampling areas in the Canadian Maritime Provinces (Thomson & Thacker, 1972). Several surveys have shown that this organism is frequently present in normal shell fish caught from Canadian and the U.S. waters, although the number of cells in fresh material is low i.e., 100 or less per gram (Thomson & Pivnick, 1972). In Netherlands, 2.4% of mussels were known to carry this organism (Kampelmacher et al., 1972). Wide variation in the incidence of the organism have been reported in the market samples, the incidence being 6% in Korea and 13% in Japan during Summer (Liston, 1973). In India very little work is reported on the occurrence of this organism in cooked

shucked clams, cooked picked and frozen crab meat and its seasonal variation on different parts of pelagic fishes.

In man V. parahaemolyticus usually causes either diarrhoea, occasionally dysentery like or gastroenteritis of sudden onset varying from mild to severe. The mortality rate is less than 10%. The size of the infecting doze necessary to produce clinical symptoms may vary with the strain but it is probably about 10^6 to 10^9 viable cells according to the data of Takikawa (1958); Aiiso & Fujiwara (1963) and Sakazaki et al. (1968c). The average incubation period is 12 to 24 hours, but ranges from 2-48 hours depending partly on the infecting dose, the nature of the food and the condition of the stomach. The vibrios multiply rapidly in the gut and is excreted in large numbers during illness, but they decrease rapidly with clinical recovery. It is a self limiting infection generally lasting only for a few days with little evidence of spread of the infection from one person to another (Barrow & Miller, 1976). V. parahaemolyticus and closely related organisms have also been isolated occasionally from infected skin or tissue lesions in bathers and fish handlers (Roland, 1970), but possible pathogenicity for fish and shell fish are uncertain.

Outbreaks of this vibrio infection occur only in the summer season in Japan and it is responsible for more than 52% of the total food poisoning cases (Todd, 1978). Between 1972 and 1980 some 200 cases were reported including three out breaks affecting 19, 24 and 40 persons who became ill after eating prawn cocktails made with imported cooked prawns thawed and served without further cooking (Gilbert, 1983). It is one of the most important causative agents of food poisoning in Japan

and was considered a local problem until recently, but it has now been recognised in many countries especially in South East Asia (Chatterjee et al., 1970; Sakazaki et al., 1976). These workers reported the isolation of V. parahaemolyticus from upto 15% patients with diarrhoea in Calcutta. In Thailand and Philippines the isolation rate is greater than that of Salmonella and Shigella. The vibrio have also been isolated from infection of the hands and feets, eyes and ears of the persons who have been in contact with marine shore areas. Battey et al. (1970) reported an outbreak of gastroenteritis in Australia caused by V. parahaemolyticus. Two out breaks of infection with V. parahaemolyticus were reported in the United States (Summer et al., 1971).

Foods implicated in Japanese outbreaks of gastroenteritis have been mainly raw or lightly cooked or processed fish (Okabe, 1974; Barrow & Miller, 1976). Egg, egg products, grain, grain products, vegetable and vegetable products have also been implicated for (Aiso et al., 1965). In other countries such outbreaks originate from the consumption of shrimps and crab meat recontaminated after cooking and held at higher temperature permitting rapid growth (Barker et al., 1974). Thus almost without exception V. parahaemolyticus food poisoning is associated with the consumption of fish (Baross & Liston, 1970; Liston, 1973; Beuchat, 1982).

V. parahaemolyticus is a member of the family Vibrionaceae. Morphologically V. parahaemolyticus is Gram negative rods exhibiting pleomorphisim, slightly curved, straight, coccoid and swollen forms can be observed. All strains of V. parahaemolyticus are motile by means of a single polar flagellum. Vibrio in broth culture has single polar

flagellum, whereas young culture on the surface of nutrient agar this organism may have peritrichous flagella. On agar plates most cultures appear as smooth, moist, circular, opaque colonies with entire edges. Some rough varients have been reported in pure cultures by Twedt et al. (1969). A swarming phenomenon occurs in some instances when low concentration of agar are used. This diminishes with increased concentration of agar.

V. parahaemolyticus is facultatively anaerobic, halophilic bacterium. It can grow in ordinary media containing 1-8% sodium chloride. The optimum concentration of sodium chloride is 2-4%. It fails to grow in the absence of sodium chloride. The optimum concentration of salt for growth may be influenced by the nature of the medium used and the temperature of incubation. In fact V. parahaemolyticus was reported to grow poorly in foods containing 5% or more of sodium chloride (Kodama, 1967).

V. parahaemolyticus prefers an alkaline pH. The recommended pH for the culture media is 7.4 to 8.6 (Anon, 1972). However organisms can grow over a wide range of pH 4.8 to 11.0 (ICMSF, 1980), but the optimal pH for growth is between 7.6 and 8.6. Susceptible foods according to Kodama (1967) are "Proteinatious" with sodium chloride concentration of 1 to 3% and pH above 5.8. A food with a pH value below 5 and sodium chloride content above 5% is not likely to support growth of V. parahaemolyticus. However, some foods, might not attain the inhibitory concentration of salt and pH, shown by the finished products or during processing. Therefore, one must also take the history of each food into

account. V. parahaemolyticus does not cause marked organoleptic changes to seafoods, even at infectious concentration (Lee, 1973).

The effect of pH on V. parahaemolyticus strain 0 in shrimp homogenate was studied by Vanderzant and Nickelson (1972). They showed that viability was not affected by pH between 6 and 10 during 2 hour test period. However rapid inactivation took place at pH 5.0. It is more sensitive to acidity than E. coli and it may be killed in vinegar within 1 hour and in 0.5% acetic acid in a few minutes (Kondo et al., 1960).

The minimum and maxium growth temperature reported are 5-8 and 42-45^{o}C respectively (Liston et al., 1969). The optimum growth temperature is between 35 and 37^{o}C (Horie et al., 1966). Most of the strains do not grow at temperature below 5^{o}C, although the organism may survive for longer periods at lower temperatures (Beuchat, 1975). The generation time under optimum condition is very short, generally in the range of 11 to 13 minutes, though some strains have been reported to have generation time of 8 to 9 minutes (Katoh, 1965) and 5 to 7 minutes (Barrow & Miller, 1974).

The cold sensitivity of this organism was recognised by the low incidence of V. parahaemolyticus gastroenteritis during winter months (Saito, 1967). The extent of inactivation due to low temperature, however, is far less than due to heat. The low temperature inactivation of V. parahaemolyticus is a negative function of temperature. The studies of Matches et al. (1971) in fish homogenate have shown that the log reduction values of 2.2 to 6.2 at -18^{o}C were attained in 12 to 19 days and 3.8 to 7.2 at -34^{o}C in 4 to 12 days.

According to Thomson and Thacker (1973) V. parahaemolyticus survived only for 1 to 3 weeks in oyster at -20°C depending upon the initial concentration of the organism. Isolation of this organism from frozen crab meat and fish fillets stored at -15°C and -30°C after 30 days and 60 days respectively have been reported by Johnson & Liston (1973). These workers also isolated V. parahaemolyticus from inoculated oysters after 130 days of storage at -15°C and -30°C. When lobster tails were heavily contaminated with V. parahaemolyticus at a concentration of 10^4 to 10^6 organisms per ml, it was possible to recover the organisms upto atleast 3 months at -18°C, whereas if the inoculam contained 10^2 to 10^3 organisms per ml, the viability of this organism was for one month only, when the material was stored at the same temperature (Lamprecht, 1980). Further this organism could not be detected after one week at -18°C if the initial inoculam contained less than 100 cells/ml.

V. parahaemolyticus culture 0 from Gulf coast shrimp was inoculated into whole shrimp and shrimp homogenate and kept at 3°C, 7°C, 10°C and -18°C (Vanderzant & Nickelson, 1972). In whole shrimp the initial loss of viability was rapid and resulted in 2 log reduction within 2 days. The loss of viability in shrimp homogenate was not great as in the whole shrimp, and more than 2 log reduction was observed in 8 days. It is also interesting to note that the strain Vanderzant & Nickelson studied was more rapidly inactivated at 3°C than at -18°C. The data therefore suggests that refrigerated temperature may be more determintal to V. parahaemolyticus than freezing. No information is available about

the effect of cold on V. parahaemolyticus, which is present in cooked and shucked clams.

V. parahaemolyticus is very heat sensitive and can be inactivated by mild heat. After heating at 60 or 80°C for 15 minutes, no survivers could be detected in the shrimp homogenate inoculated with 500 cells/ml. Survivers were detected only when the cell concentration was increased to 2 x 10^6/ml., but no survivers were detected after 1 minute at 100°C (Vanderzant et al., 1970). If seafoods are heated to 100°C just before consumption, food poisoning due to V. parahaemolyticus would rarely occur (Sakazaki, 1973b).

Tenmei et al. (1961) reported that V. parahaemolyticus is very sensitive to drying and hot smoking may kill it. Studies of Venugopal et al. (1984) have shown that survival of V. parahaemolyticus smeared on dry fish appeared to be less than 2 hours as it could not withstand the process of sundrying. In our country little information is available about the occurrence of V. parahaemolyticus in dried fishes meant for internal consumption.

V. parahaemolyticus is killed in one minute in distilled water (Takeuchi et al., 1957) probably by the osmotic destruction of the cells. Data regarding the effect of tap water on V. parahaemolyticus and its survival in brackish water and sea water are scanty.

An antigenic scheme for V. parahaemolyticus in which 11,0 groups and 41, K antigens were recognized, were established by Sakazaki et al. (1968b). The H antigen is not included in the scheme since all of the

H antigens of the vibrio reveal the same antigenicity. The K-antigen 2, 14, 16, 27 and 35 were subsequently excluded because they were found to be identical with other K antigens already recognized (Sakazaki, 1983).

Despite its pathogenicity, the public health significance of V. parahaemolyticus when isolated from seafoods during routine surveillence is debatable (Cann et al., 1981). Earlier it was widely accepted that all strains of V. parahaemolyticus, irrespective of its source, were enteropathogenic to man. Later, Kato (1965) found that the strains of this species of vibrio isolated from diarrhoeal stools gave a haemolytic reaction in a specified media, while those isolated from marine sources failed to do so. The medium was later modified by Wagatsuma (1968) to get a more convincing reaction and the test was named as "Kanagawa reaction". Sakazaki et al. (1968) carried out a detailed study of Kanagawa reaction with 3370 cultures and observed that 96.5% of human cultures were Kanagawa-positive, while only 1% of the marine cultures gave a similar reaction. Similar reactions were also reported by other workers (Miyamoto et al., 1969; Batley et al., 1970; Summer et al., 1971; Peffers et al., 1973; Hooper et al., 1974; Barker, 1974). Later, it was confirmed by Sakazaki (1973b) that only those strains which were isolated from human beings gave characteristic illness on feeding tests, while the strains from marine sources failed to produce illness in human volunteers. Sanyal & Sen (1974) also performed tests on volunteers using both Kanagawa-positive and negative strains and they observed that administration of 10^{10} viable cells of Kanagawa-negative strain per ml

did not cause any clinical symptoms, while 10^5 to 10^7 cells of Kanagawa-positive strains per ml produced gastroenteritis in human volunteers.

Even in the outbreaks of food poisoning, the isolation of Kanagawa-positive strains from the causative food is very rare, although all isolates from faecal specimen of the victims are Kanagawa-positive (Sakazaki, 1973a). The inability of marine cultures to give a positive Kanagawa reaction has also been noted by Miyamoto and his colleagues (1969), Barrow & Miller (1976), Cann et al. (1981) and Beuchat (1982). It appears, therefore, that a positive reaction may be closely related to human pathogenicity (Sakazaki, 1973, Beuchat, 1982). A few outbreaks due to Kanagawa-negative strains have been reported by Zen-Yoji et al. (1970).

Several explanations have been advanced to account for the adherence of Kanagawa-positive strain in the stools of patients and the rarity of these pathogenic strains in seafoods. These include the possibility of transformation of negative strain to positive strain during transit in the intestinal tract. High incidence of kanagawa-positive strains among isolates from house flies led Chatterjee (1980) to speculate that flies might be involved in carrying kanagawa-positive strains from excreta.

Sakazaki et al. (1974) suggested that kanagawa-positive V. parahaemolyticus can multiply more rapidly than kanagawa-negative vibrios in the intestine. Carruthers (1977) demonstrated that kanagawa-positive vibrios adhered rapidly to He La cells and to human foetal intestinal cells, whereas kanagawa-negative vibrio did not adhere

to He La cells and adherence to human faetal intestinal cells was at a much slower rate than for kanagawa-positive vibrios.

Twedt et al. (1970) found very high incidence of kanagawa-positive cultures from estuarine sources of the United States (55 to 90%). Quadri & Zuberi (1977) also reported high percentage of kanagawa-positive isolates i.e. 52.5% from fish and shell fish samples from Karachi, Pakistan. 29% of V. parahaemolytiens strains isolated from seafoods of Hong Kong were kanagawa-positive (Chun et al., 1989). Karunasagar & Mohan (1980) observed 25% incidence of kanagawa-positive strains in the environment around Mangalore. Information regarding the kanagawa phenomenon of V. parahaemolyticus isolated from fish and shell fish of marine and brackish water origin harvested at Cochin meant for internal consumption are scanty.

Effects of various antibiotics, detergents, disinfectants and food preservatives against V. parahaemolyticus has been thoroughly investigated in Japan (Yanagizawa, 1967). Among the 12 antibiotics tested, chlorotetracycline was the most effective and Penicillin the least. Pradeep & Lakshmanaperumalsamy (1985) observed that gentamycin is more effective to the strains isolated from brackish water fishes. In our country apparently no information is available about the sensitivity of V. parahaemolyticus isolated from marine sources, especially of kanagawa-positive strains to commonly used antibiotics.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

A. Total bacterial count and incidence of coagulase-positive staphylococci and Vibrio parahaemolyticus in fish and fishery products

A1. Source of samples

Samples of fish and fishery products meant for internal consumption were collected from local landing centres, markets and cold storages. Cured fishes were collected from Mangalore and Tuticorin also. Swab samples were taken from the palms and throats of workers in fish processing factories. Usually the samples were collected on the first working day of each week. The number of samples from different sources were as follows:

Fresh fin fishes	158
Fresh shell fishes	84
Frozen fish and fishery products	93
Cooked and shucked clams	23
Cured fishery products	182
Worker's palms	78
Worker's throats	101
	719

A2. Sampling techniques

a) Fresh fin fishes and shell fishes

Fresh fin fishes of marine and brackish water origin and shell fishes consisting of prawns, cuttle fishes, oysters, mussels, clams and crabs

of marine and brackish water origin were collected from the landing centres and markets situated in and around Cochin. Each sample was immediately transferred into sterile bottles/polythene bags and transported to the laboratory. Analyses were started within 4 hours of collection.

b) Frozen fish and fishery products

Frozen fish and fishery products consisting of cooked, picked and frozen crab meat (body meat and claw meat) prawns, peeled undeveined (PUD), peeled and deveined (PD), mussels and cuttle fishes meant for internal consumption were collected from nine cold storages situated in and around Cochin. The samples were brought to the laboratory in iced condition in an insulated box and analysed immediately on arrival.

c) Cured fishes

Cured fishes consisting of fin fishes and shell fishes were collected from Cochin markets. Few samples were also collected from Mangalore and Tuticorin. The samples collected in sterile polythene bags were transported to the laboratory and examined. Moisture content was determined as per official methods of analysis (AOAC, 1980).

d) Cooked and shucked clams (Villorita sp.)

Cooked and shucked clams were collected from the local markets in sterile polythene bags and transported to the laboratory. The samples were subjected to analysis within 4 hours of collection.

e) Water and mud samples

Water and mud samples mainly from the oyster beds and from the coastal areas were collected throughout the year in sterile bottles/

polythene bags and brought to the laboratory immediately. The samples were examined for Vibrio parahaemolyticus without any further delay.

f) Palms and throats of workers

Swab samples were taken from the palms and throats of fish processing factory workers from 6 factories situated in and around Cochin. Swab samples were then transferred into 10 ml of sterile normal saline (C_1) tubes and transported to the laboratory and kept in the refrigerator till the analysis started.

A3 Preparation of samples

a) Fresh fin fishes and shell fishes

Skin with muscle from both sides of fin fishes, shell and muscle of prawns and muscle alone in case of oysters were cut by using sterile scissors and transferred into sterile sample dishes (approximately 10 gm). Weight of the dish with sample was taken. The contents were then transferred to the sterile cup of a homogenizer, and homogenized, with 100 ml sterile normal saline (C_1). The empty dish was then weighed to get the accurate weight of the material used for the analysis. The homogenate was kept for about 10 minutes and 1 ml of the supernatent was taken out by using 1 ml graduated sterile pipette. It was then serially diluted by using 9 ml sterile normal saline (C_1).

b) Frozen fish and fishery products

10 grams of the muscle were taken from each block by using sterile scissors, transferred to a sterile sample dish and weighed.

Homogenate was prepared and serial dilutions were made as described in A_3. a.

c) Cured fishes

Approximately 10 grams of the samples were taken, homogenate was prepared and serially diluted as described in A_3. a.

d) Cooked and shucked clams

Approximately 10 grams of the samples were taken and treated as described in A_3. a.

e) Palms and throats of factory workers

The swab samples were immersed in 10 ml of sterile normal saline (c_1), then rotated 20 times in the clockwise direction and 20 times in the anticlockwise direction, so that the bacteria present in the swab could be uniformly distributed in the saline. This dilution was considered as 10. From this further dilutions were prepared using sterile 9 ml normal saline and sterile 1 ml pipette.

f) Water samples

The water samples were shaken well before the analysis started. From the original water sample, decimal dilutions were prepared as described earlier by using sterile 3% sodium chloride solution (c_2) for Vibrio parahaemolyticus.

g) Mud sample

10 grams of the mud sample were taken, mixed well with 90 ml sterile 3% sodium chloride solution (c_2) and serial dilutions were made.

A4 Bacteriological analysis of the samples

1. Total bacterial count

a) Fresh fin fishes and shell fishes

One ml each of 10^3, 10^4, 10^5 and 10^6 dilutions of the samples prepared as described under A_3.a. was pipetted into sterile petri dishes in duplicates. Molten sea water agar (A_1) cooled to $40^{\circ}C$, was added to each of the petri dishes. The same medium was added to another two pairs of dishes, each containing 1 ml of normal saline (c_1) but no inoculum of the sample, these dishes served as control to determine the sterility of the media, pipettes, dishes and diluents used. The plates were rotated clockwise and anticlockwise several times for the equal distribution of cells in the medium. The dishes were then kept on the laboratory table for 15 to 20 minutes for solidification of the added agar. After that the plates were incubated in an inverted position for 48 hours at R.T. ($29\pm1^{\circ}C$). At the end of incubation period, all the colonies developed on the agar were counted and the average count of the duplicate plates were recorded. Only those petridishes giving colonies between 30 and 300 were used for the calculation of the bacterial count of the sample.

b) Frozen fish and fishery products

One ml each of 10^3 to 10^6 dilutions of the sample of frozen fish and fishery products were prepared as explained under A_2.b and was then added to the dish as described above (A_4.1.a). Tryptone glucose extract (TGE) agar (A_2) was added to each of the dishes and control dishes as described under A_4.1.a. The plates were incubated at R.T. for 48 hours. The dishes were then examined exactly as in the case of fresh fishes.

c) Cured fishes

Total bacterial counts of cured fishes were taken as described in A_4.1.b.

d) Cooked and shucked clams

As in A_4.1.b.

2) Coagulase-positive staphylococci

a) Fresh fin fishes and shell fishes

Samples were prepared as described under A_3.a. 0.5 ml from 10^2 and 10^3 dilution were pipetted on to the surface of, surface dried Baird Parker medium (A_3). The inoculum was then uniformly spread over the surface of the agar using a sterile 'L' shaped glass rod and the plates were incubated at 37°C for 24 to 36 hours. The black and shiny colonies with narrow white margins surrounded by clear zones extending into the opaque medium after incubation for 36 hours were regarded as presumptive coagulase-positive staphylococci. These colonies were confirmed by the plasma coagulase test (Levine, 1960).

Coagulase test was performed by the tube method using desicated rabbit plasma (coagulase plasma + ethylenediamine tetraacetic acid (EDTA) (Difco). The test was performed by adding 2 drops of an overnight culture grown in Brain heart infusion broth (B_1) to Wasserman tube containing 0.5 ml of plasma. The tubes were incubated in a water bath at 37°C and examined after 2, 4 and 24 hours. Reactions were tabulated according to the scheme of Turner and Schwartz (1958). Only 4+, 3+ and 2+ reactions were considered as positive evidence of coagulase production.

Generally only ten typical colonies from each dish were tested for coagulase production and the total number for coagulase producers in one particular petri dish was calculated on the basis of the results obtained. However, if less than 10 presumptive colonies were present in a particular plate all the colonies were tested.

b) Frozen fish and fishery products

Samples were prepared as described under A_3.b. 0.5 ml from 10^2 and 10^3 dilutions were tested for the presence of coagulase-positive staphylococci. Colonies were counted and coagulase production was tested as described in A_4. 2.a.

c) Cured fishes

Samples were prepared and tested for coagulase-positive staphylococci as described under A_3.b. and $A_4$2.a.

d) Cooked and shucked clams

Samples were prepared and tested for coagulase-positive staphylococci as described under A_3.b and A_4.2.a.

e) Palms and throats of workers

0.5 ml of the 10^1 and 10^2 dilutions of the swab samples were aseptically streaked onto the surface of Baird Parker agar (A_3). Incubation and enumeration of the organisms were done as described in A_4.2.a.

3. Vibrio parahaemolyticus

a) Qualitative studies

The samples consisting of 50 grams each of fresh fin fishes, shell fishes, frozen fishery products, dried fishery products, cooked shucked

clams, cooked picked crab meat and mud samples were blended with 450 ml of 3% sterile sodium chloride solution (C_2) for 1 minute at 8000 r.p.m. Inoculated 10 ml portions of 1:10 dilution into 10 ml of double strength Glucose salt teepol broth (B_2). From the blended samples decimal dilutions were made with 3% sterile sodium chloride solution. One ml of the dilution was inoculated into 10 ml of Glucose salt teepol broth (B_2). It was then incubated at 37°C for 18 hours. A 3mm loopfull of the broth culture was aseptically streaked on surface dried Thiosulfate citrate bilesalts sucrose agar (A_4) plates, and incubated at 37°C for 18 hours in inverted position. The characteristic large (3-4 mm) with light blue or green centre, sucrose non-fermenting dome shaped colonies were regarded as presumptive Vibrio parahaemolyticus and these were further subjected to the biochemical tests, suggested by Sakazaki (1973b).

b) Quantitative studies

Samples were prepared as described under A_4.3.a. The 3-tube Most Probable Number (MPN) technique as described in the Bacteriological Analytical Manual (U.S.D.H.E. and W, 1969) was employed for this studies.

c) Seasonal variation on different parts of pelagic fin fishes, oysters and its environments

In order to study the seasonal variation of Vibrio parahaemolyticus in different parts of pelagic fishes - Glucose salt teepol broth (B_2) was used. Preference was given to the following parts of fin fishes - surface tissue, gills and intestine.

In the case of oysters, muscle, water and mud from its bed. Counts of V. parahaemolyticus in the samples were subsequently derived

based on the combination of the tubes yielding confirmed isolates of the organism from the recommended MPN tables (ICMSF, 1978).

B. Studies on morphological, cultural and biochemical characteristics of the isolated pathogens.

B_1. Coagulase-positive staphylococci

A total of 182 cultures of coagulase-positive staphylococci isolated from fresh fin fishes, shell fishes, frozen fish, cured fishes and fish processing factory workers were used for the study.

a) Cell form and Gram reaction

Cell form and Gram-reaction was studied from growth on nutrient agar (A_{12}) slants. Growth from the edge of an 18 hours old culture was taken and a smear was prepared on a clear microscope slide (Collins & Lyne, 1976). Each smear was stained by using Hucker method (Collins & Lyne, 1976) and observed under the oil immersion objective.

b) Pigment production

The colour of colonies were observed on Brain heart infusion agar (A_5) slants. The slants were streaked with the cultures and incubated at $37^{o}C$ for 5 days, then the colour of the culture was noted.

c) Thermostable nuclease production

Microslides were prepared by spreading the surface of each microscopic slide with 3.0 ml of molten Toluidine blue DNA agar (A_6). Allowed some time for solidifying then cut 2 mm diameter well into the agar (10-12 per slide) using a sterile capillary tube and removed the agar

plugs by aspiration. Approximately 10 µl of the heated samples (15 minutes in a boiling water bath) of broth culture was added to the wells by means of Pasteur pipettes. The slides were incubated in a moist chamber for 4 hours at 37°C and observed. A positive reaction is the appearance of a bright pink halo extending at least 1 mm beyond the periphery of the well.

d) Oxidation and fermentation of mannitol

In order to study the oxidation and fermentation of mannitol heated the tubes containing Hugh and Leifson's medium (B_3) with 1% mannitol in boiling water bath for 10 minutes, cooled and inoculated. One set of tubes were incubated aerobically and the other set anaerobically by sealing the surface of the medium with 2 cm sterile liquid paraffin and incubated at 37°C. Oxid tive metabolism is characterized by acid in aerobic tubes only and fermentative metabolism by the production of acid in both tubes.

e) Acid production from carbohydrates

The ability of strains to produce acid aerobically from lactose and galactose was investigated by using Andrad's carbohydrate broth (B_4) with the said sugars.

f) Phosphatase production

Inoculated a plate of phenolphthalein diphosphate agar (A_7) and incubated overnight at 37°C. Inverted the plates for a few seconds over a shallow vessel containing 5% ammonium hydroxide (C_3). Phosphatase producing colonies turned pink.

g) Gelatinase production

Stabbed the nutrient gelatin tubes (B_{12}) with the culture and incubated the tubes at 37°C for 7 days. The tubes were placed in ice water for 10 minutes, then observed the liquefaction.

h) Catalase production

Emulsified a little portion of the young slant culture in a drop of 10% hydrogen peroxide solution (C_4) on a glass slide. Effervescence of oxygen indicated the presence of catalase.

i) Cytochrome oxidiase test

Placed a 6 cm square piece of filter paper into an empty petri dish and added 3 drops of 1% aqueous solution of tetramethyl paraphenylenediamine dihydrochloride solution (C_5) to its centre. Smeared cells thoroughly on to the reagent impregnated paper in a line of 3-6 mm long with an inoculating loop. Oxidase positive strains turned dark purple in 5-10 seconds.

j) Urease production

Inoculated Christensen's Urea agar (A_8) slant heavily over the entire surface of the slant. Incubated the tubes at 37°C for 24 hours. Urease positive strains produced an alkaline (red colour) reaction in the medium.

k) Nitrate reduction

Inoculated tubes of Nitrate broth (B_{13}) and incubated at 37°C for 12-24 hours. 0.5 ml each of sulfanilic acid (c_6) and α -Naphthylamine

solution (c_7) were added, shaken well and observed the colour. A pink or red colour indicated positive test for nitrite. Zinc dust was added to the negative tubes to reduce the remaining nitrate, if negative the culture reduced nitrate to nitrogen or ammonia and the test is positive.

l) Indole production

The tubes containing 1% Tryptone broth (B_{14}) were inoculated and then incubated at 37°C for 24 hours. 0.3 ml of indole reagent (C_8) was added to the broth culture. Shaken well and allowed the tubes to stand for 10 minutes and observed the results. A dark red colour in the amyl alcohol surface layer constituted a positive test.

m) Methyl red test

Inoculated MRVP broth (B_{15}) and incubated for 5 days at 37°C. A few drops of methyl red solution (C_9) were added to the broth culture. A red colour indicated a positive test.

n) Voges-proskauer test

MRVP broth (B_{15}) was inoculated and the tubes were incubated at 37°C for 48 hours. Pipetted 1 ml of each culture to a separate empty culture tube and added 0.6 ml of naphthol solution (C_{10}) and 0.2 ml of potassium hydroxide solution (C_{11}). Shaken the tubes and allowed to stand for 2-4 hours and observed the changes. A pink or crimson colour in the mixture indicated a positive test.

o) Crystal violet agar test

The appearance of colonies on nutrient agar (A_{12}) containing crystal violet (0.001% W/V) was determined as described by Meyer (1967).

Type A (CV-positive) colonies were yellow to yellow blue in appearance; Type C (CV-negative) colonies were violet to blue in colour after 24 hours incubation at 37°C (Gibbs et al., 1978).

B_2. Vibrio parahaemolyticus

a. Culture maintenance

The following conditions applied throughout this studies unless otherwise stated. Stock cultures of all strains were grown at 37°C on Trypticase soy 3% Nacl agar (A_9) . Stock cultures were maintained on agar slants at room temperature.

A total of 116 strains of V. parahaemolyticus isolated from fin fishes and shell fishes of marine and brackishwater origin were studied. 3% Nacl was added to all media.

b. Morphological characteristics

Cell form (Pleomorphism) and Gram reactions were studied from growth on Trypticase soy agar slants (A_9) as described under B_1.a.

c. Motility

Motility of the strains were observed by stabbing the culture to a depth of 5-10 mm to a tube containing Motility Test 3% Nacl Medium (A_{10}). The tubes were incubated at 37°C for 24 hours. A circular growth from the line of stab represented a positive test.

d. Swarming

Swarming was detected on V. parahaemolyticus maintenance agar (A_{11}) plates containing 1.0 and 2.0% agar.

e. Cultural characteristics

Growth on medium subjected to variable range of temperature and salt concentration was observed after incubating the cultures for 2 days in salt trypticase broth (B_5). The sodium chloride concentration in broth media was 3%, except in the salt range experiments in which concentration of 0,1,6,8 and 10% were tested. The growth was tested at temperatures of 2, 22, 30, 37 and 42°C.

f. Oxidation and fermentation of glucose

Oxidation and fermentation of glucose was observed with Hugh-Leifson's salt medium (B_6) as described under B_1.d.

g. Carbohydrate fermentation

Carbohydrate ermentation was observed in bromcresol purple broth (B_{16}) containing 1% of glucose, lactose, sucrose, mannitol and 0.5% of salicin, inositol, dulcitol, adonitol, maltose, arabinose, xylose, cellobiose, trehalose, rhamnose and sorbitol.

h. Starch hydrolysis

Starch hydrolysis on maintenance agar (A_{11}) plates containing 0.2% soluble starch was tested by the method of Iyer & Karthiayani (1964).

i. Catalase production

Catalase production was tested with 10% hydrogen peroxide solution (C_4) described under B_1.h.

j. Cytochrome oxidase

Cytochrome oxidase production was detected as described in B_1.i.

k. Indole production

Indole production was tested with Kovac's reagent (C_8) as described under B_1.l.

l. Voges-proskauer test

Voges-proskauer test was perfromed as described under B_1.n.

m. Urease production

Urease production was detected by using urea broth (B_7).

n. Nitrate reduction

Reduction of nitrate was observed in nitrate broth (B_{13}) using the reagents as described under B_1.k.

o. Gelatinase production

Gelatin hydrolysis was tested after 5 days of incubation at 37°C in nutrient gelatin tubes (B_{12}) as given in B_1.g.

p. Casein hydrolysis

Casein hydrolysis was observed during a 3 day incubation by using maintenance agar (A_{11}) containing 10% skim milk.

q. H_2S Production

Streaked the triple sugar iron salt agar (A_{14}) slants and stabbed the butt. Incubated at 37°C for 24 hours.

r. Amino acid decarboxylation

Arginine dihydrolase, lysine and ornithine decarboxylase test was performed by inoculating the tubes of arginine dihydrolase (B_8), lysine

and ornithine decarboxylase broth (B_9) and of basal medium (control) with a loopful of the culture. A layer of 10 mm thick of sterile liquid paraffin was added to each tube. Incubated the tubes at 37°C for 24 hours. The medium turned alkaline or purple when decarboxylation occurred.

s. 0/129 pteridine sensitivity

Sensitivity towards 2,4-diamino-6, 7-diisopropylpteridine was tested by using disc diffusion method as described under J_1.c.

C. Survival of coagulase-positive staphylococci at different temperatures

a. Bacteria studied

Coagulase-positive staphylococci isolated from fish processing factory worker (No.301) was used for this study.

b. Substratum used for inoculation

Shrimp homogenate

c. Preparation of shrimp homogenate

A 25% homogenate was prepared by homogenizing the required quantity of cooked shrimp in normal saline (C_1) using a waring blender. 10 ml of the suspension was then transferred into test tubes and sterilized in an autoclave at 15 pounds per square inch pressure for 30 minutes.

d. Preparation of bacterial suspension

On the previous day of inoculation, the coagulase-positive staphylococci strain was inoculated into tubes containing sterile Brain heart infusion broth (B_1) and incubated at 37°C for 24 hours. The broth

culture was then centrifuged for 15 minutes at 5000 r.p.m. and the liquid was aseptically decanted off. 10 ml of sterile normal saline (C_1) was then added to the sediment and the contents of the tube was shaken so as to get a uniform suspension of the cells, and this was centrifuged for 15 minutes. The sediment was once again washed, centrifuged and the cells were resuspended in 10 ml of fresh normal saline (C_1). The suspension was diluted 10 times using sterile normal saline (C_1).

e) Inoculation of the organism into shrimp homogenate

10 drops of the diluted cell suspension was inoculated into each tube containing 10 ml shrimp homogenate. The tubes were gently shaken to have uniform distribution.

f. Enumeration of coagulase-positive staphylococci in the inoculated shrimp homogenate

Immediately after inoculation and shaking 1 ml of the homogenate was plated using Tryptone glucose extract agar (A_2) to find out the initial load of coagulase-positive staphylococci. Since a particular organism alone had been separately inoculated into sterile shrimp homogenate, there was no necessity of using a selective medium and thus TGEA (A_2) was used. Uninoculated sterile homogenate served as control.

g. Storage of the tubes and enumeration

One set of tubes (12 nos.) were stored at -20°C, another set at 2°C, 4°C and the last set at RT (29±1°C). Samples were taken and analysed periodically for coagulase-positive staphylococci by using Tryptone glucose extract agar (A_2) as described earlier.

D. Survival of Vibrio parahaemolyticus at lower temperatures

D_1. In shrimp homogenate

a. Bacteria studied

Vibrio parahaemolyticus -NCMB-1902 obtained from National collection of marine bacteria.

b. Substratum used for inoculation and its prepartion

Shrimp homogenate prepared as described in C.c.With 3% sodium chloride solution (C_2).

c. Preparation of bacterial suspension

V. parahaemolyticus culture was inoculated into Trypticase soy 3% sodium chloride broth (B_{11}) and incubated at 37°C for 24 hours. The broth culture was then centrifuged and washed thrice as described in C.d. with 3% sodium chloride solution.

d. Inoculation of the organism in to shrimp homogenate

10 drops of the washed and diluted cell suspension of V. parahaemolyticus in 3% sodium chloride solution (c_2) was inoculated into each tube containing 10 ml of sterile shrimp homogenate and shaken well.

e. Enumeration of V. parahaemolyticus in the homogenate

Immediately after inoculation and shaking, 1 ml of the sample was withdrawn and plated using Trypticase soy 3% Nacl agar (A_9) and incubated at 37°C for 48 hours, then the count was taken. Since a

particular organism alone had been separately inoculated into sterile, shrimp homogenate, there was no necessity of using a selective medium. Uninoculated sterile homogenate served as control.

f. Storage of the tubes and enumeration

One set of tubes (12 nos.) were kept at $0^{o}C$ and the other set at $-20^{o}C$. Periodically the samples were withdrawn and analysed for V. parahaemolyticus, as described above.

D_2. In crab meat (Scylla serrata) homogenate without sodium chloride and 3 percent sodium chloride

a. Bacteria studied

Vibrio parahaemolyticus -NCMB-1902 obtained from National Collection of Marine bacteria.

b. Substrata used for inoculation and its preparation

Crab meat homogenate was prepared as described in D_1.b. One set was prepared with distilled water and the other set with 3% sodium chloride solution (c_2).

c. Preparation of bacterial suspension

V. parahaemolyticus broth culture was prepared centrifuged, washed and diluted as described in D_1.C.

d. Inoculation of the organism in to crab meat homogenate

As described in D_1.d.

e. Enumeration of V. parahaemolyticus in the homogenate

V. parahaemolyticus count of the inoculated homogenate was taken as described in D_1.e.

f. Storage of the tubes and enumeration

The tubes were kept at -20°C. Periodically the samples were withdrawn and analysed for V. parahaemolyticus as described in D_1.f.

D_3. Survival of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in the shrimp homogenate at lower temperatures

a. Organisms used

1. Kanagawa-positive V. parahaemolyticus strain isolated from clams-No.49.

2. Kanagawa-negative V. parahaemolyticus strain NCMB-1902.

b. Substratum used

Shrimp homogenate with 3% sodium chloride prepared as described D_1.b.

c. Preparation of bacterial suspensions

Cell suspensions of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus were prepared separately as detailed under D_1.c.

d. Inoculation of cell suspension

10 drops of the prepared cell suspensions of both the strains were separately added to separate set of tubes. D_1.d.

e. Enumeration of V. parahaemolyticus in the homogenate

V. parahaemolyticus counts of both strains were taken as described under D_1.e.

f. Storage of the tubes and enumeration

One set of tubes (separately inoculated with kanagawa-positive strain and kanagawa-negative strain) were kept at $2^{o}C$, similarly one set was kept at $-20^{o}C$. samples were withdrawn from both the sets and V. parahaemolyticus load was determined periodically as detailed under D_1.f.

E. Inactivation of Kanagawa-positive and Kanagawa-negative strains of Vibrio parahaemolyticus in tap water

a. Organisms used

a) Kanagawa-positive strain of V. parahaemolyticus isolated from clams-No.49.

b) Kanagawa-negative strain of V. parahaemolyticus NCMB-1902

b. Substratum used

Tap water collected from Cochin area supplied through public distribution system.

c. Chemical analysis of tap water

pH, total dissolved solids, chlorides, total hardness, temporary hardness, permanent hardness, hardness due to calcium and magnesium, alkalinity, sulphate, copper, iron, albuminoid ammonia and free and saline ammonia, were carried out by the Quality Control Laboratory of Central Institute

of Fisheries Technology, Cochin by following the method of APHA (1960) and Taylor (1958).

d. Preparation of substratum

The water sample was sterilized by using seitz filter. 100 ml of the filter sterilized water sample was aseptically transferred into a 500 ml sterile conical flask.

e. Preparation of bacterial suspension

Cell suspension of both strains of V. parahaemolyticus was prepared as detailed in D_1.c.

f. Inoculation of cell suspension into substratum and its enumeration

1 ml of the diluted cell suspension was inoculated into 100 ml of sterile tap water and gently shaken. Immediately 0.2 ml of the suspension was withdrawn and plated on surface dried Brain heart infusion agar (A_5) plates supplemented with 2.5% sodium chloride in duplicate by spread plate method and repeated thereafter with one minute interval for 15 minutes. The plates were incubated at 37°C for 24 hours and the number of colonies were counted.

F. Survival of Kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in different aquatic systems at R.T. ($29 \pm 1^{o}C$)

a. Organisms used

1. Kanagawa-positive strain of V. parahaemolyticus isolated from clams-No.49.

2. Kanagawa-negative strain of V. parahaemolyticus NCMB-1902.

b. Substrata used

1. Normal saline (C_1)
2. 3% sodium chloride solution (C_2)
3. Brackish water
4. Sea water

c. Preparation of substrata and its salinity determination

Each of the above said samples were sterilized by using seitz filter. Salinity of brackish water and sea water was determined before sterilization (APHA, 1960). 100 ml of the filter sterilized water samples were aseptically transferred into sterile 500 ml conical flasks.

d. Preparation of bacterial suspension

Broth culture of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus was prepared, centrifuged, washed and diluted as detailed in D_1.c.

e. Inoculation of cell suspension into substrata and its enumeration

1 ml of the diluted cell suspension of both strains of V. parahaemolyticus was inoculated in to each set of flasks separately. 1 ml of the prepared cell suspension was serially diluted and plated using trypticase soy 3% NaCl agar (A_9) as detailed in D_1.e. to find out the number of cells in the inoculum. Flasks were gently shaken for equal distribution of cells and kept at R.T. ($29\pm1^{o}C$). 1 ml of the sample was withdrawn from the flask and analysed for V. parahaemolyticus as described

in D_1.e periodically. Sterilized and uninoculated water samples served as control.

G. Intercompetition between pathogenic bacteria and the other natural bacterial flora of fishery products at different temperatures for growth and survival.

G_1. Competition between coagulase-positive staphylococci and other bacterial flora present in cooked, picked and frozen crab meat (S. serrata) during defrost

a. Collection of sample

Cooked, picked and frozen crab body meat collected from local cold storage meant for local consumption was brought to the laboratory as described in A_2.b.

b. Determination of bacterial count

Immediately after reaching the laboratory the temperature at the central portion of the block was noted by using a thermometer and approximately 10 g of the sample was aseptically withdrawn. The sample was prepared and analysed for total bacterial count and coagulase-positive staphylococci count as detailed under A_4.1.b and 2.b.

c. Storage of the sample

The sample was kept at room temperature on laboratory table covered with a bell jar for a period of 24 hours.

d. Determination of bacterial count

Periodically approximately 10 g of the sample was withdrawn and analysed for total bacterial count and coagulase-positive staphylococci

count as described under A_4.1.b and 2.b for a period of 24 hours. Simultaneously the temperature at the central portion of the sample was also noted.

G_2. Competition between coagulase-positive staphylococci and the other bacterial flora present in cooked, picked crab meat (S. serrata) at R.T. and iced condition

a. Collection of samples

2 kg of cooked and freshly picked crab meat (body) was collected from a fish processing factory and brought to the laboratory within one hour in sterile polythene bag covered with clean crushed ice.

b. Preparation of sample

The sample was divided into two, 1 kg lots.

c. Bacteriological analysis

Approximately 10 g of the sample from both lots were aseptically transferred into a sterile sample dish. Immediately it was analysed for total bacterial count and coagulase-positive staphylococci count as per the methods described in A_4.1.b and 2.b

d. Storage of the sample

One lot was packed in sterile polythene bag and kept in an ice chest covered with clean crushed ice and the other lot was also packed in sterile polythene bag and kept on laboratory table covered with bell jar at R.T. ($29\pm1^{o}C$)

e. Determination of bacterial count

Analysis of the sample for total bacterial count and coagulase-positive staphylococci count was carried out as described in A_4.1.b and 2.b., periodically for a period of 6 hours.

G_3. Competition between Vibrio parahaemolyticus and other organisms present in cooked, shucked clams (Villorita sp.) at lower temperatures

a. Collection of sample

Cooked, shucked clams meat was collected from the local market and brought to the laboratory within one hour.

b. Analysis of the sample for total bacterial count and V. parahaemolyticus

Homogenate of the sample was prepared immediately as detailed in A_3.d. 3% NaCl was used as diluent. Total plate count was determined as described in $A_4$1.d. For the quantitative estimation of V. parahaemolyticus 3 tube MPN method was used as detailed in $A_4$3.b.

c. Preparation and storage of the samples

50 grams of the sample was weighed and put into sterile small polythene bags (100 nos). First set consisting of 25 bags were kept at $10^{o}C$, second set at $0^{o}C$, third set at $-10^{o}C$ and the last set at $-20^{o}C$. Periodically samples from each set was withdrawn and analysed for total bacterial count and V. parahaemolyticus for a period of 29 days as detailed in A_4.1.d and 3.b.

H. Enterotoxigenicity of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

a. Organisms studied

The number of coagulase-positive staphylococcal strains isolated from different sources and studied for its enterotoxigenicity were as follows:

Frozen fish and fishery products	102
Cured fishes	26
Fish processing factory workers	128
	256

b. Enterotoxin production

Cellophane-over-agar, the method of Hallender (1965) as applied by Jarvis & Lawrence (1970) was used. Circles of cellophane were cut from dialysis tubing using 9 cm filter paper as template. The cellophane circles (15 to 20) were placed alternately with filter paper in a 10 cm glass petri dish, moistened with distilled water to eliminate wrinkling and autoclaved for 20 minutes at 121°C. The sterile cellophane circles were aseptically transferred to petri dishes containing 15 to 20 ml of solidified brain heart infusion agar (A_5). One-tenth milliliter of inoculum (5 ml of Brain heart infusion broth (B_1) in small test tube inoculated with the appropriate staphylococcus culture and incubated at 37°C for 24 hours) was spread over the cellophane with a sterile bent glass rod. The plates were incubated for 24 hours at 37°C after which the growth was harvested from the cellophane with 2.5 ml of 0.01 M Na_2HPO_4(C_{12}). The cell

suspension was centrifuged at 10,000 r.p.m. for 30 minutes and the supernatant fluid was analysed for enterotoxin (Robbins et al., 1974).

c. Detection of enterotoxins

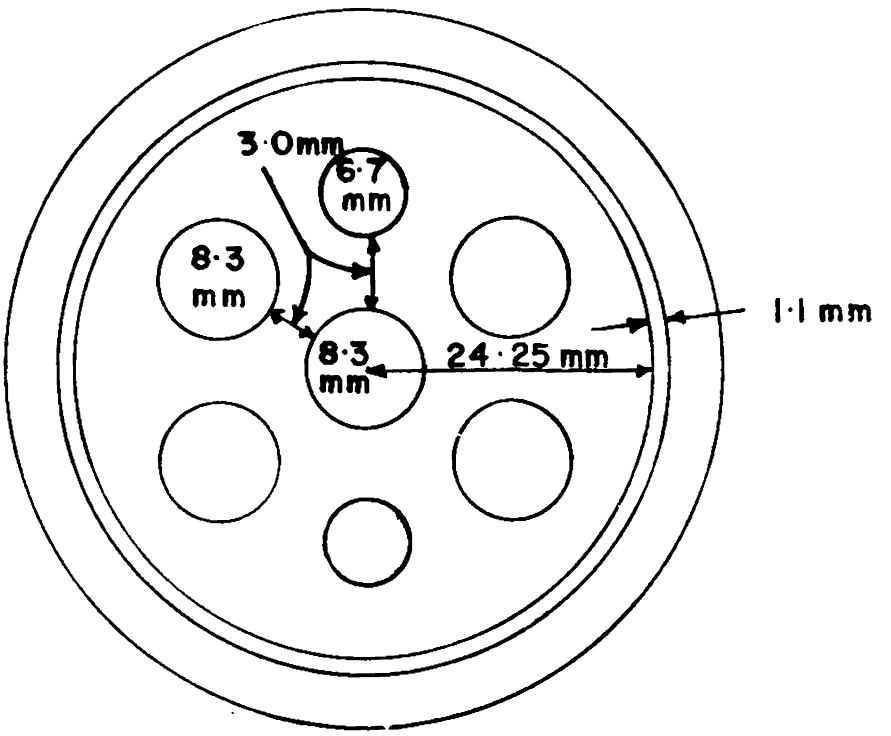
Optimum - sensitivity - plate (OSP) method of Robbins et al. (1974) was used for the detection of enterotoxins in the culture supernatant. Small petri dishes (50 by 12 mm) with tight lids were used. Three milliliters of 1.2% Noble agar (Difco code No.B-142) in phosphate buffered saline (C_{13}) was used for each plate. A 3/16 inch thick plexiglass template (Fig.1) was positioned over each plate, and then cork borers were used to cut seven wells in the agar. The agar plugs were removed with suction. The antiserum was placed in the center well, the control enterotoxin (4.0 µg/ml) in the two small wells and the unknown samples in the four larger outer wells. Filled all the wells level with the top of the agar layer using pasteur pipettes.

For developments the dishes were closed and stored in a humidified box R.T. ($29\pm1^{o}C$) for 48 hours. An airtight plastic rectangular box was used and small petri plates containing water were placed in each corner. The plates were read before and after flooding with 0.1 M Phosphoric acid (C_{14}).

I. Kanagawa phenomenon of Vibrio parahaemolyticus strains isolated from fin fishes and shell fishes of marine and brackish water origin and their environments

a. Cultures used

230 strains of V. parahaemolyticus isolated from different sources as given below were used for this study.



g. 1. Optimum sensitivity plate template for making wells in the agar layer.

Fin fishes	114
Shell fishes	94
Environments	22
	230

b. Medium used

Wagatsuma agar, Modified (A_{13}).

c. Preparation of washed human erythrocytes suspension

Fresh human blood collected from the District Hospital was defibrinated by shaking with sterile glass beeds and centrifuged. Washed the erythrocytes with sterile saline solution (C_1) 3 times, suspended 1 volume of the last sedimented erythrocytes with 4 volumes of saline solution. Washed 20% suspension of the erythrocytes were added to the boiled and cooled to 50°C Wagatsuma agar (A_{13}), mixed and transferred to plates. Surface of the plates were dried by keeping the plates at 56°C for 45 minutes.

d. Preparation of broth culture and spotting

24 hours old V. parahaemolyticus broth culture was prepared in TS-3 broth (B_{11}). Spotted several loopfull of the broth culture on a single surface dried Wagatsuma agar plate in circular pattern. Incubated at 37°C and observed the results after 18-24 hours. Clear transparent zones around the colonies indicated a positive test.

J. Antibiotic sensitivity of coagulase-positive staphylococci and Vibrio parahaemolyticus

J_1. Antibiotic sensitivity of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

a. Organisms studied

The number of coagulase-positive staphylococci strains isolated from different sources and subjected to invitro sensitivity tests to antibiotics were as follows:

From frozen crab meat	71
From frozen prawns	19
From cured fishes	26
From processing factory workers	122
	238

b. Antibiotics used

The antibiotics used in this study, their concentrations per disc and their symbols are given in the chart.

c. Sensitivity testing

Sterile cotton swab was inserted into 18 hours old nutrient broth (B_{10}) culture of the organism and rotated it while pressing against the upper side wall of the tube above the culture fluid level to remove the excess inoculum. The swab was then streaked on to the surface of the pre-set nutrient agar (A_{12}) plates and allowed to dry for 10 minutes at R.T. The antibiotic discs having standard strengths were placed apart on the plates using sterile forceps

in such a way that there is no chance of overlapping of zones of inhibition around the discs. The plates were then incubated at 37°C for 24 hours and the zone of inhibition around each disc was measured and interpreted as per the chart.

Concentrations of different antibiotics tested, their symbols and classification of inhibition zones

Antibiotics	Symbol	Concen. mcg/ disc	Resis-tant mm or less	Inter-mediate mm	Sensi-tive mm or more
Ampicillin	I	10	20	21-28	29
Chloram-phenicol	C	30	12	13-17	18
Erythromycin	E	15	13	14-17	18
Kanamycin	K	30	13	14-17	18
Neomycin	N	30	12	13-16	17
Penicillin	P	10 U	20	21-28	29
Polymyxin-B	X	300 U	8	9-11	12
Streptomycin	S	10	11	12-14	15
Tetracycline	T	30	14	15-18	19

J_2. Antibiotic sensitivity of Kanagawa-positive and Kanagawa-negative strains of Vibrio parahaemolyticus isolated from fin fishes and shell fishes

a. Number of V. parahaemolyticus strains tested

Kanagawa-positive strains	48
Kanagawa-negative strains	36

b. Antibiotic used

In addition to the antibiotics listed in J_1 the following antibiotics were also used in this study.

Antibiotics	Symbol	Concen. mcg/ disc	Resis-tance mm or less	Inter-mediate mm	Sensi-tive mm or more
Gentamycin	J	10	12	-	13
Sulphadiazine	Z	300	12	13-16	17
Tetracycline	T	30	14	15-18	19

c. Sensitivity testing

Broth culture was prepared as described in J_1.c. in nutrient broth (B_{10}) supplemented with 2.5% sodium chloride. Lawn was made on nutrient agar (A_{12}) supplemented with 2.5% sodium chloride and the sensitivity was tested by using disc diffusion method as detailed in J_1.c.

K. Phage pattern of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

a. Number of coagulase-positive staphylococci strains used for phage typing

The number of coagulase-positive staphylococci strains isolated from different sources and used for this study were as follows:

Cured fishes	19
Frozen fishery products	28
Processing factory workers	42
	89

b. Phages used for typing

The basic set consisting 23 phages (for typing human Staph. aureus strains) as follows were used.

Group I	29, 52, 52A, 79, 80
Group II	3A, 3C, 55, 71
Group III	6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85
Group IV	81, 94, 95, 96

c. Preparation of broth culture

A single colony of the purified coagulase-positive staphylococci was inoculated into 50 ml nutrient broth (B_{10}) and incubated at 37°C for 4 to 7 hours.

d. Phage-typing

The above said broth culture was used for inoculating 10 cm petri dish containing nutrient agar (A_{12}). The freshly made nutrient agar plates were dried for 45 minutes with the lid in open position at 37°C. The plates were then flooded with broth culture of the test strain and the excess broth was pipetted off. They were then left for drying with the lids open for about 1 hour at R.T. The phages from the RTD set were applied by a

multipleloop phage applicator (Lidwell Phage Applicator Fig.2). After the phage drops were dried out, the plates were incubated at 30°C overnight.

If the test strain was not typed with the RTD set, the typing was repeated with the RTD x 100 set on another plate.

e. Reading

The plates were first examined with the naked eye, then the reading was taken with the help of a x 5 hand lens, the plates were also examined by indirectly transmitted light, against a dark back ground and the results were recorded as follows:

At RTD

50 or more plaques	++
20 to 49 plaques	+
1 to 19 plaques	±

f. In addition to the above, the following symbols were used

At RTD & 100

Confluent lysis	CL
Confluent lysis with secondary growth	GR/CL
Inhibition of growth no visible plaque	0
Inhibition of growth, with discrete plaque	± or + etc.

L. Media formulae and preparations

A1 Sea Water Agar

Peptone	5.0 g

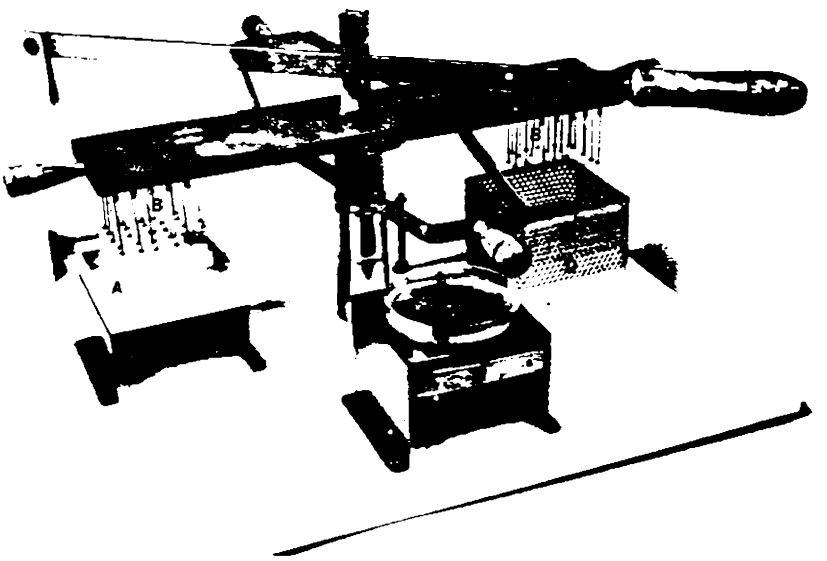


Fig. 2: Lidwell Phage Applicator

Agar	15.0 g
Ferric phosphate	0.1 g

Added the ingredients in 1000 ml of aged filtered sea water and heated to boiling with agitation to obtain complete solution. Autoclaved at 121°C for 30 minutes. Final pH 7.0

A_2 Tryptone Glucose Extract Agar

Beef extract	3.0 g
Tryptone	5.0 g
Dextrose	1.0 g
Agar	15.0 g

Added the ingredients in 1000 ml of distilled water and heated to boiling with agitation. Sterilized at 121°C for 30 minutes. Final pH 7.0

A_3 Baird - Parker Agar

Basal medium

Tryptone	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Sodium Pyruvate	10.0 g
Glycine	12.0 g
Lithium chloride 6 H_2O	5.0 g
Agar	20.0 g

Suspended the ingredients in 950 ml of Distilled water. Heated to boiling to dissolve completely. Dispensed 95 ml portions in screw cap bottles. Autoclaved for 15 minutes at 121°C. Final pH, 7.0±0.2

Bacto Egg Yolk Tellurite Enrichment (Oxoid)

Complete medium

Added 5 ml portions of pre warmed (45-50^{o}C) enrichment to 95 ml of molten basal medium that has been adjusted to 45-50^{o}C. Mixed well and poured 15-18 ml into sterile 15x100 mm petri dishes.

A4 - Thiosulfate-Citrate-Bile salts-Sucrose Agar (TCBS)

Yeast extract	5.0 g
Peptone	10.0 g
Sucrose	20.0 g
Sodium thiosulfate pentahydrate	10.0 g
Sodium citrate dihydrate	10.0 g
Sodium cholate	3.0 g
Ox-gall	5.0 g
Sodium chloride	10.0 g
Ferric citrate	1.0 g
Bromthymol blue (0.2%) solutions	20.0 ml
Thymol blue (1% solution)	4.0 ml
Agar	15.0 g

Added the ingredients to 980 ml of distilled water and heated to boiling with agitation to obtain complete solution. No autoclaving, cooled to 45-50^{o}C, adjusted pH to 8.6 and poured 15-20 ml volume into petridishes.

A5 Brain, Heart Infustion Agar

Difco Code No.B 418

A6 Toluidine Blue-DNA Agar (Lachica et al., 1971)

Deoxyribonucleic acid (DNA)	0.3 g
Calcium chloride	0.0011 g
Sodium chloride	10.0 g
Toluidine blue (1% w/v aqueous solution)	9.2 ml
Tris (hydroxymethyl) aminomethane	6.1 g
Agar	10.0 g

Dissolved the tris (hydroxymethyl) amino methane in 1 litre of distilled water and adjusted pH to 9.0. Added the remaining ingredients except the toluidine blue and heated to boiling to obtain complete solution of the DNA and agar. Added the toluidine blue to this solution and dispensed in adequate volumes in rubber-stoppered flasks. Sterilization is not necessary.

A7. Phenolphthalein Diphosphate Agar

Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Agar	15.0 g

Dissolved ingredients in 1 litre of distilled water and adjusted pH to 7.4. Sterilized at 121^{o}C for 15 minutes. Cooled to 50-55^{o}C and added filter-sterilized 10 ml of 1% solution of phenolphthalein diphosphate

pentasodium, mixed the medium well and dispensed in 15-20 ml amounts into Petri dishes.

A8 Christensen's Urea Agar

Peptone	1.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Potassium dihydrogen phosphate	2.0 g
Phenol red (0.2% solution)	6.0 ml
Urea	20.0 g

Dissolved all ingredients in 94 ml of distilled water, adjusted pH 6.8 - 6.9 and filter sterilized.

Complete medium

Dissolved by boiling 15.0 g agar in 900 ml of distilled water and sterilized at 121°C for 15 minutes. Cooled to 50-55°C and added 100 ml Urea concentrate. Mixed and distributed in sterile tubes and slant with 3 cm butt and a 5 cm slant.

A9 Trypticase Soy 3% NaCl Agar

Trypticase	15.0 g
Phytone	5.0 g
Sodium chloride	30.0 g
Potassium monohydrogen phosphate	2.5 g
Glucose	2.5 g
Agar	15.0 g

-G4235-

Added all the ingredients in 1 litre of distilled water, heated to boiling with agitation. Cooled to 50-55°C, adjusted pH to 7.3 and autoclaved at 121°C for 15 minutes.

A10. Motility test 3% NaCl medium

Beef extract	3.0 g
Peptone	10.0 g
Sodium chloride	30.0 g
Agar	4.0 g

Dissolved all ingredients in 1 litre of distilled water by heating with occasional agitation. Dispensed in approximately 8 ml portions. Autoclaved at 121°C for 15 minutes. Final pH 7.4.

A11. Vibrio parahaemolyticus maintenance agar

Proteose peptone	10.0 g
Yeast extract	3.0 g
$MgSO_4.7\ H_2O$	7.0 g
$MgCl_2$	5.3 g
KCl	0.7 g
NaCl	24.0 g
Agar	15.0 g

Dissolved all ingredients in 1 litre of distilled water by heating. Autoclaved at 121°C for 15 minutes. Final pH 7.2 - 7.4.

A12. Nutrient agar

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g

Added all ingredients to 1 litre of distilled water, heated to boiling, cooled to 50-60°C, adjusted pH to 6.8-7.0. Distributed in tubes for slants or in bulk for plates as required and autoclaved at 121°C for 15 minutes.

A13. Wagatsuma agar, Modified

Yeast extract	5.0 g
Peptone	10.0 g
Sodium chloride	70.0 g
Mannitol	5.0 g
Crystal Violet (0.1% w/v solution in ethyl alcohol)	1.0 ml
Agar	15.0 g

Dissolved all ingredients in 1 litre of distilled water and adjusted pH to 7.5. Heated to boiling for several minutes. Did not autoclave. Cooled to 50°C and added 100 ml of washed 20% suspension of human erythrocytes, mixed and solidified as plates. Dried the plates before use.

A_{14}. Triple sugar iron salt agar

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	15.0 g
Proteose peptone	5.0 g
Glucose	1.0 g
Lactose	10.0 g
Sucrose	10.0 g
Ferrous sulfate	0.2 g

Sodium chloride	30.0 g
Sodium thiosulfate	0.3 g
Phenol red (0.2% solution)	12.0 ml
Agar	12.0 g

Added all ingredients to 988 ml of distilled water, mixed well and heated to boiling. Cooled to 50-60^{o}C and adjusted the pH to 7.3±0.1. Filled the tubes 1/3 and plugged. Autoclaved at 121^{o}C for 12 minutes. Cooled the tubes in slanting position to obtain butts (2.5 cm long) and slants (5 cm long).

B1. Brain heart infusion broth

(Difco. Code No.B 37).

B2. Glucose salt teepol broth (GSTB)

	Single strength	Double strength
Beef extract	3.0 g	6.0 g
Peptone	10.0 g	20.0 g
Sodium chloride	30.0 g	60.0 g
Glucose	5.0 g	10.0 g
Methyl violet	0.002 g	0.004 g
Teepol	4.0 ml	8.0 ml
Distilled water	1000 ml	1000 ml

Dispensed single strength broth in 10 ml portions, double strength broth in 10 ml portions in tubes large enough to accomodate a 10 ml charge of sample. Sterilized at 121^{o}C for 15 min. Final pH, 9.4

B3. Hugh-Leifson medium

Peptone	2.0 g
Sodium chloride	5.0 g
Potassium monohydrogen phosphate	0.3 g
Mannitol	10.0 g
Bromthymol blue (0.2% solution)	15.0 ml
Agar	3.0 g

Added all ingredients to 985 ml of distilled water and heated to boiling. Dispensed in 5 ml quantities into small tubes and autoclaved at 115°C for 15 minutes. Final pH 7.1

B4. Andrade's Carbohydrate broth

Beef extract	3.0 g
Bacto peptone	10.0 g
Sodium chloride	10.0 g
Andrade's indicator	10.0 ml
Distilled water	1000 ml

Dissolved all the ingredients and adjusted the pH to 7.2. Divided the basal broth into two portions. Added 1% Lactose to one portion and 1% galactose to the other portion. Sterilized at 115°C for 15 minutes.

B5. Salt trypticase broth (STB)

Trypticase	10.0 g
Yeast extract	3.0 g
Sodium chloride	30.0 g
Distilled water	1000 ml

Sterilized at 121°C for 15 min. Final pH 7.5

B6. Hugh-Leifson salt medium

Peptone	2.0 g
Glucose	10.0 g
Sodium chloride	30.0 g
Potassium monohydrogen phosphate	0.3 g
Bromthymol blue (0.2% solution)	15 ml
Agar	3.0 g

Added all ingredients to 985 ml of distilled water, heated to boiling. Dispensed in 5 ml quantities in to tubes and autoclaved at 115^{o}C for 15 minutes, pH 7.1.

B7. Urea broth

Urea	20.0 g
Potassium dihydrogen phosphate	9.1 g
Sodium monohydrogen phosphate	9.5 g
Yeast extract	0.1 g
Phenol red (0.2% solution)	5.0 ml

Added all ingredients to 1 litre of distilled water, stirred and sterilized by filtration. Final pH 6.8.

B8. Arginine dihydrolase 3% NaCl broth

Yeast extract	3.0 g
Sodium chloride	30.0 g
Glucose	1.0 g
Bromcresol purple (1% solution)	1.6 ml

Dissolved all ingredients in 1 litre of distilled water and adjusted the pH to 6.8. Added 0.5% of L-arginine. Dispensed in 3 ml quantities into tubes and autoclaved at 121°C for 10 minutes.

B9. Lysine and Ornithine decarb oxylase 3% NaCl broth

Yeast extract	3.0 g
Sodium chloride	30.0 g
Glucose	1.0 g
Bromcresol purple (1% solution)	1.6 ml

Dissolved all ingredients in 1 litre of distilled water and adjusted the pH to 6.8. Divided the basal broth into 3 portions. Added 0.5% L-lysine to the first portion and 0.5% L-ornithine to the second portion. The third portion was the basal broth, control. Dispensed in 3 ml quantities into tubes and autoclaved at 121°C for 10 minutes.

B10. Nutrient broth

Beef extract	3.0 g
Peptone	5.0 g

Dissolved ingredients in 1 litre of distilled water. Autoclaved at 121°C for 15 minutes. Final pH 6.9

B11. Trypticase soy 3% NaCl (TS-3) broth

Trypticase	15.0 g
Phytone	5.0 g
Potassium monohydrogen phosphate	2.5 g
Glucose	2.5 g
Sodium chloride	30.0 g

Dissolved all ingredients in 1 litre of distilled water. Dispensed in 7-10 ml portions in to tubes and autoclaved at 121°C for 15 minutes. Final pH 7.3.

B12. Nutrient gelatin

Beef extract	3.0 g
Peptone	5.0 g
Gelatin	120.0 g

Suspended the ingredients in 1 litre of distilled water, mixed well and heated to boiling. Adjusted the pH to 6.9±0.1. Dispensed into tubes and sterilized at 121°C for 15 minutes.

B13. Nitrate broth

Tryptone	20.0 g
Sodium monohydrogen phosphate	2.0 g
Glucose	1.0 g
Agar	1.0 g
Potassium nitrate	1.0 g

Added all ingredients to 1 litre of distilled water heated to boiling, dispensed in 5 ml volumes into culture tubes and sterilized at 121°C for 15 minutes.

B14. Tryptone broth

Tryptone	10.0 g

Dissolved in 1 litre of distilled water, dispensed in 6 ml portions into culture tubes and sterilized in an autoclave at 121°C for 15 minutes. Final pH 7.3.

B15. MRVP broth

Proteose peptone	5.0 g
Dextrose	5.0 g
K_2HPO_4	5.0 g

Added the ingredients to 800 ml of distilled water, dissolved by heating. Cooled to 20°C diluted to 1 litre. Dispensed in 5 ml portions into culture tubes, sterilized at 121°C for 15 minutes. Final pH 6.9±01.

B16. Bromcresol purple broth

Peptone	10.0 g
Beef extract	3.0 g
Sodium chloride	30.0 g
Bromcresol purple	0.04 g

Dissolved the ingredients in 1 litre of distilled water, divided into fifteen equal parts and added 1% of glucose, lactose, sucrose, mannitol and 0.5% of salicin, inositol, dulcitol, adonitol, maltose, arabinose, xylose, cellobiose, trehalose, rhamnose and sorbitol to each portion.

C1. Normal saline (Physiological saline)

Sodium chloride	8.5 g
Distilled water	1000 ml

Dissolved sodium chloride in distilled water. sterilized at 121°C for 15min.

C2. 3% Sodium chloride solution

Sodium chloride	30.0 g
Distilled water	1000 ml

Dissolved sodium chloride in distilled water. sterilized at 121° for 15min.

C3. 5% Ammonium hydroxide

Ammonium hydroxide (25%)	20 ml
Distilled water	80 ml

C4. 10% Hydrogen peroxide solution

33% Hydrogen peroxide	33 ml
Distilled water	67 ml

C5. 1% Tetramethyl paraphenylene diamine dihydrochloride

Tetramethyl paraphenylene diamine dihydrochloride	1.0 g
Distilled water	100 ml

C6. Sulfanilic acid reagent

Sulfanilic acid	1.0 g
Acetic acid (5 N aqueous solution)	125 ml

C7. Alpha - naphthol reagent

Alpha Naphthol	1.0 g
Acetic acid 5 N	200 ml

C8. Indole reagent (Kovac's, 1928)

Paradimethylamino-benzaldehyde	5.0 g
Amyl alcohol	75 ml
Hydrochloric acid (Conc.)	25 ml

Dissolved benzaldehyde in amyl alcohol and added the hydrochloric acid.

C9. Methyl red solution

Methyl red	0.1 g
Ethyl alcohol	300 ml
Distilled water	500 ml

Dissolved methyl red in alcohol and diluted with water.

Voges - Proskauer test reagents

C10. α Naphthol solution

α Naphthol	5.0 g
Absolute alcohol	100 ml

Dissolved α Naphthol in alcohol.

C11. 40% Potassium hydroxide

Potassium hydroxide	40 g
Creatine	0.3 g
Distilled water	100 ml

Dissolved KOH and creatine in 100 ml distilled water.

C12. 0.01 M Na_2HPO_4

$Na_2HPO_4 \cdot 2 H_2O$	1.78 g
Distilled water	1000 ml

C13. Phosphate buffered saline

Stock solution (0.1 M)

$Na_2HPO_4 \cdot 2H_2O$	12.0 g
$NaH_2PO_4 \cdot 2H_2O$	2.2 g

Distilled water	1000 ml

Dissolved the ingredients in distilled water.

Phosphate buffered saline (0.01M)

Stock solution (0.1 M)	100 ml
0.85% NaCl solution	900 ml

Mixed well, adjusted the pH to 7.5 sterilized at 121^{o}C for 30 minutes.

C14. 0.1 M phosphoric acid

Phosphoric acid	9.8 ml
Distilled water	990.2 ml

CHAPTER - IV

RESULTS

RESULTS

The total bacterial count (TBC) of fresh fin fishes, shell fishes, cooked shucked clams, cured fishery products and frozen fish and fishery products collected from the markets and cold storages situated in and around Cochin meant for internal consumption were determined. The results are presented in Table 1. The results revealed that 15.8 percent of fresh fin fish samples had TBC less than 2.0×10^5 per gram and 12.0 percent of the samples with 10^7 per gram. TBC of none of the fresh shell fish samples were less than 2.0×10^5 per gram and 20.2 percent of the samples revealed 10^7 per gram. In cooked shucked clams, 43.5 percent contained TBC 10^7 per gram and none with less than 2.0×10^5 per gram. TBC of 69.2 percent of the cured fishery products were less than 2.0×10^5 per gram and only 1.1 percent with 10^7 per gram. TBC of 9.7 percent of the frozen fish and fishery products were found to be less than 2.0×10^5 per gram and 15.1 percent with 10^7 per gram. The results were statistically analysed. The mean logarithmic value and Standard Deviation (S.D.) are also given in Table 1.

TBC of fresh fin fishes of marine and brackish water origin are tabulated in Table 2. 50 percent of the fresh lactarius, kilimeen and veloory, 25 percent of jew fish and manangu, 16.7 percent of pearl spot, 12.5 percent of anchovy and 3.8 percent of mackerel had TBC 10^7 per gram. In general the TBC of 15.8 percent of fresh fin fishes were found to be less than 2.0×10^5 and 12 percent of the samples with 10^7 per

Table 1. Total bacterial count of fish and fishery products

Name of the sample	No. of samples examined	No. of samples with total bacterial count/g within the range				Logarithmic scale	
		$\leq 2.0\times10^5$	2.1×10^5 to 9.9×10^5	1.0×10^6 to 9.9×10^6	1.0×10^7 to 9.9×10^7	Mean	S.D.
Fresh fin fishes	158	25	67	47	19	5.958	0.8473
Fresh shell fishes*	84	-	10	48	17	6.613	0.5581
Cooked shucked clams	23	-	3	10	10	6.823	0.6632
Cured fishery products	182	127	47	6	2	4.924	0.7178
Frozen fish and fishery products	93	9	25	45	14	6.237	0.7981

* Bacterial count of live crabs were not taken.

gram. The results were statistically analysed, the mean logarithmic value and S.D. are also given in Table 2.

TBC of fresh shell fishes of marine and brackish water origin are summarised in Table 3. 43.75 percent of oysters (Crassostrea sp.), 33.3 percent of Karikkady (Parapenaeopsis stylifera), 25 percent of thelly (M. affinis/M. dobsoni) and 11.1 percent of naran (P. indicus) had TBC 10^7 per gram. The mean logarithmic value and S.D. are also given in Table 3. TBC of 43.5 percent of cooked, shucked clams (Villorita sp.) was 10^7 per gram (Table 4).

TBC and moisture content of cured fishery products are given in Table 5. The results indicated that TBC of 18.68 percent of the samples were less than 10^4 per gram and 1.6 percent with 10^7 per gram. The moisture content of all the dried prawn samples were found to be less than 20 percent and the same in fin fishes varied from 30 to 70 percent. The results are statistically analysed and the correlation coefficient is also given in Table 5. Correlation between the moisture content and TBC of cured shark, silverbelly, manangu and sardine were found to be highly significant.

Table 6 gives the TBC of frozen fish and fishery products other than cooked, picked and frozen crab meat. 16.7 percent of the samples had less than 2.0×10^5 and 39.6 percent with 10^6 per gram. The mean, logarithmic value and S.D. are given in the table.

TBC of cooked, picked and frozen crab meat (S. serrata) consisting of body meat and claw meat is given in Table 7. 14.3 percent of claw meat samples and 45.8 percent of body meat samples had 10^7 per gram. In general only 2.2 percent with less than 2.0×10^5 per gram and 31.1 percent with 10^7 per gram.

Table 3. Total bacterial count of fresh shell fishes

Name of the sample	No. of samples examined	No. of samples with total bacterial count/g within the range			Logarithmic scale	
		2.1×10^5 to 9.9×10^5	1.0×10^6 to 9.9×10^6	1.0×10^7 to 9.9×10^7	Mean	S.D.
Oyster (*Crassostrea* sp.)	16	-	9	7	6.9355	0.5123
Naran (*Penaeus indicus*)	18	-	16	2	6.609	0.3234
Thelly (*M. affinis*/*M. dobsoni*)	24	6	12	6	6.5383	0.6654
Kazanthan (*M. affinis*)	11	4	7	-	6.1929	0.4233
Karikkady (*Parapenaeopsis stylifera*)	6	N.D	4	2	6.8313	0.5164
Crab (*S. serrata*)	9	N.D.	N.D	N.D.		
Total	84	10	48	17		

N.D = not done

Table 4. Total bacterial count of cooked shucked clams (Villorita sp.)

No. of samples examined	No. of samples with total bacterial count/g within the range			
	$\leq 2.0 \times 10^5$	2.1×10^5 to 9.9×10^5	1.0×10^6 to 9.9×10^6	1.0×10^7 to 9.9×10^7
23	-	3	10	10

Table 6. **Bacterial count of frozen fish and fishery products (other than crab meat)**

Name of the sample	No. of samples examined	No. of samples with total bacterial count/g within the range			Logarithmic scale	
		$\leq 2.0 \times 10^5$	2.1×10^5 to 9.9×10^5	1.0×10^6 to 9.9×10^6	Mean	S.D.
Frozen prawns	26	2	9	15	6.0954	0.5050
Frozen fish	16	4	8	4	5.7139	0.5355
Frozen clams (cooked shucked)	6	2	4	-	5.4525	0.2921
Total	48	8	21	19		

Table 7. Total bacterial count of cooked, picked and frozen crab meat (Scylla serrata)

No. of samples with total bacterial count/g within the range	Body meat A	%	Claw meat B	%	Total A + B	% (A+B) 100 / 45
Less than 2.0×10^5	1	4.2	-	-	1	2.2
2.1×10^5 to 9.9×10^5	3	12.5	1	4.8	4	8.9
1.0×10^6 to 9.9×10^6	9	37.5	17	80.9	26	57.8
1.0×10^7 to 9.9×10^7	11	45.8	3	14.3	14	31.1
Total	24		21		45	

The incidence of coagulase-positive staphylococci and Vibrio parahaemolyticus in fish and fishery products are presented in Table 8. 4.4 percent of fresh fin fishes, none of the fresh shell fishes, 21.7 percent of cooked, shucked clams, 25.3 percent of cured fishery products and 72 percent of frozen fish and fishery products contained coagulase-positive staphylococci and the load varied from 10 to 2.0 x 10^5 per gram. In general 23.1 percent of the samples contained coagulase-positive staphylococci. The results were statistically analysed and the mean logarithmic values and S.D. are also given in the Table. 51.3 percent of fresh fin fishes, 66.7 percent of fresh shell fishes, 39.1 percent of cooked shucked clams, none of the cured fishery products and 2.2 percent of the frozen fish and fishery products contained V. parahaemolyticus. In general V. parahaemolyticus was isolated from 27.4 percent of the samples.

Incidence of coagulase-positive staphylococci and V. parahaemolyticus in fresh fin fishes were studied and the results obtained are given in Table 9. 25 percent of anchovies, 12.5 percent of jew fish, 11.5 percent of mackerel and 7.1 percent of sardine harboured coagulase-positive staphylococci and the load varied from 14-882 per gram. All fresh shell fish samples were found to be free from Staph. aureus (Table 10). 21.7 percent of cooked shucked clams were found to be positive for coagulase-positive staphylococci and the load varied from 156 to 488 per gram (Table 11).

Table 12 presents the results regarding the incidence of coagulase-positive staphylococci and V. parahaemolyticus in cured fishery

Table 8. Incidence of coagulase-positive staphylococci and Vibrio parahaemolyticus in fish and fishery products

Name of the sample	No. of samples examined	No. of samples with coagulase-positive staphylococci/g within the range						Logarithmic scale		No. of samples with V.parahaemolyticus
		1-100	101-999	1.0x 10^3 to 9.9x 10^3	1.0x 10^4 to 9.9x 10^4	1.0x 10^5 to 9.9x 10^5	1.0x 10^6 to 9.9x 10^6	Mean	S.D	
Fresh fin fishes	158	3	4	-	-	-	-	0.098	0.471	81
Fresh shell fishes	84	-	-	-	-	-	-	-	-	56
Cooked shucked clams (Villorita sp.)	23	-	5	-	-	-	-	0.567	1.107	9
Cured fishery products	182	30	12	4	-	-	-	0.497	0.902	-
Frozen fish and fishery products	93	9	12	32	11	3	-	2.332	1.754	2
Total	540	42	33	36	11	3	-			148

Table 10. Incidence of coagulase-positive staphylococci and Vibrio parahaemolyticus in fresh shell fishes

Name of the sample	No. of samples examined	No. of samples with coagulase-positive staphylococci/g with in the range			No. of samples with vibrio parahaemolyticus	Percentage of occurrence of V. parahaemolyticus
		1 - 100	101 - 100	> 1000		
Oyster (Crassostrea sp.)	16	-	-	-	16	100.00
Naran (P. indicus)	18	-	-	-	11	61.11
Thelly (M. affinis/M.dobsoni	24	-	-	-	18	75.00
Kazanthan (M. affinis)	11	-	-	-	8	72.72
Karikkady (P. stylifera)	6	-	-	-	5	83.33
Crab (S. serrata)	9	-	-	-	8	88.88
Total	84	-	-	-	66	78.57

Table 11. Incidence of coagulase-positive staphylococci and vibrio parahaemolyticus in cooked shucked clams (Villorita, sp)

No. of samples examined	No. of samples with coagulase-positive staphylococci/g within the range			No. of samples with V. parahaemolyticus
	1 - 100	101 - 1000	>1000	
23	-	5	-	9

products. Among the samples analysed 62.5 percent of lactarius, (L. lactarius), 50 percent of shark (Scoliodon sp.) 44.4 percent of jew fish, (Pseudosciaena sp.) 33.3 percent of manangu (Thrissocles sp.) veloory (K. kowal), Indian halibut (P. erumei) and kilimeen (N. japonicus) ,27.3 percent of saurida (S. tumbil) 20 percent of ribbon fish, (T. savala) 10 percent of prawns, anchovy, (Anchoviella sp.), sardine (S. longiceps) and 7.7 percent of mackerel (R. kanagurta) were found to be positive for coagulase-positive staphylococci and the load varied from 19 to 5.3×10^3 per gram. Cured lactarius and prawns carried the maximum load. The results were statistically analysed and the results are given in Table 12.

The incidence of coagulase-positive staphylococci and V. parahaemolyticus in frozen fish and fishery products were determined and the results obtained are given in Tables 13 and 14. Among frozen fishery products 100 percent of cooked picked and frozen crab meat, 61.5 percent of frozen prawn (PD) 50 percent of cooked shucked and frozen clams contained coagulase-positive staphylococci and the load was upto 1.2×10^5 per gram. Coagulase-positive staphylococci load of body meat of crab was found to be higher than that of claw meat.

The results on the incidence of V. parahaemolyticus in fresh fin fishes, shell fishes, cooked shucked clams, cured fishery products and frozen fish and fishery products are tabulated in Tables 9 to 14. V. parahaemolyticus was isolated from all species of fin fishes and shell fishes of marine and brackish water origin. 75 percent of thody, 72.7 percent of cat fish, 66.7 percent of pomfret and perch, 64.3 percent of sardine, 62.5 percent of sole, anchovy and jew fish, 53.8 percent of

Table 13. Incidence of coagulase-positive staphylococci and Vibrio parahaemolyticus in frozen fish and fishery products other than crab meat

Name of the sample	No. of samples examined	No. of samples with coagulase-positive staphylococci/g within the range			Percentage of samples with more than 100/g	No. of samples with V. parahaemolyticus
		1 -100	101 -1000	> 1000		
Frozen prawns (PD)	26	5	7	4	42.31	-
Frozen fish	16	3	-	-	-	-
Frozen clams (cooked shucked)	6	1	2	-	33.33	-
Total	48	9	9	4	27.08	-

Table 14. Incidence of coagulase-positive staphylococci and Vibrio parahaemolyticus in cooked, picked and frozen crab meat

Coagulase-positive staphylococci/g (range)	Body meat A	%	Claw meat B	%	Total A + B	% (A+B)100 / 45	No. of samples with V. parahaemolyticus out of 45
0 - 100	-	-	-	-	-	-	
101 - 999	1	4.2	2	9.5	3	6.7	
$1.0x10^3$ to $9.9x10^3$	11	45.8	17	81.0	28	62.2	2
$1.0x10^4$ to $9.9x10^4$	9	37.5	2	9.5	11	24.4	
$1.0x10^5$ to $9.9x10^5$	3	12.5	-	-	3	6.7	
$1.0x10^6$ to $9.9x10^6$	-	-	-	-	-	-	
Total	24		21		45	100.00	

mackerel, 50 percent of manangu, lactarius, seelavu and horse mackerel, 40 percent of silver belly, 38.5 percent of tilapia, 37.5 percent of veloory, 36.4 percent of mullet, 33.3 percent of pearl spot and 25 percent of kilimeen carried V. parahaemolyticus (Table 9).

V. parahaemolyticus was isolated from 100 percent of oysters, 88.9 percent of crabs 83.3 percent of karikkady, 75 percent of thelly, 72.7 percent of kazanthan and 61 percent of naran (Table 10). V. parahaemolyticus was present in 39.1 percent of cooked shucked clams (Table 11) and absent in all cured fishery products (Table 12) and frozen fish and fishery products other than crab meat (Table 13). In cooked, picked and frozen crab meat samples 4.4 percent were found to be positive for V. parahaemolyticus (Table 14).

V. parahaemolyticus was estimated in 98 samples of fresh fin fishes and shell fishes of marine and brackish water origin and the results are tabulated in Table 15. V. parahaemolyticus was present in 67.35 percent of the samples. 45.9 percent of the samples contained 1-100 MPN per gram, 11.2 percent with 101-999 MPN/g, 7.1 percent with 10^3-10^4 MPN/g and 3.1 percent with more than 10^4 MPN/g. In general 10.2 percent of the samples contained more than 1000 MPN/g. 12.5 percent of oyster and 5.9 percent of mackerel harboured more than 10^4 MPN/g. The results were statistically analysed, the logarithmic mean and S.D. are given in Table 15.

Seasonal variation of V. parahaemolyticus in different parts of marine pelagic fishes were studied and the results are presented in Table 16. V. parahaemolyticus was isolated from the surface of the

Table 15. Estimation of Vibrio parahaemolyticus in fresh fin fishes and shell fishes

Name of the sample	No. of samples examined	No. of samples with V. parahaemolyticus within the range (MPN/g)				Total	%	Logarithmic scale	
		1-100	101-999	10^3-10^4	$>10^4$			Mean	S.D.
Mackerel (R. Kanagurta)	17	8	1	2	1	12	70.6	0.579	0.747
Sardine (S. longiceps)	10	5	2	-	-	7	70.0	1.206	0.971
Anchovy (Anchoviella sp.)	6	4	-	-	-	4	66.7	0.983	0.831
Silver belly (Leiognathus sp.)	4	3	-	-	-	3	75.0	1.179	0.841
Tilapia (T. mossambica)	5	3	-	-	-	3	60.0	0.996	0.931
Sole fish (Cynoglossus sp.)	4	3	-	-	-	3	75.0	1.316	0.908
Mullet (Mugil cephalus)	4	2	-	-	-	2	50.0	0.678	1.360
Perch (Epinephelus sp.)	2	1	-	-	-	1	50.0	-	-
Oyster (Crassostrea sp.)	16	9	4	-	2	15	93.75	2.133	0.977
Cooked shucked clams (Villorita sp.)	23	4	3	2	-	9	39.1	0.938	1.326
Naran (P. indicus)	7	3	1	3	-	7	100.0	2.722	1.009
Total	98	45	11	7	3	66	67.35		

Table 16. Seasonal variation of V. parahaemolyticus in different parts of pelagic fishes

Month	Name of the sample	Skin and Muscle		Gills		Intestine	
		TBC/g	V. para: MPN/g	TBC/g	V.para: MPN/g	TBC/g	V.para: MPN/g
January	Mackerel (R. kanagurta)	4.5×10^4	15	5.2×10^6	1.1×10^4	5.3×10^6	0
February	"	7.2×10^3	0	5.8×10^6	62	2.4×10^7	0
March	"	1.5×10^4	1.4×10^3	8.2×10^6	2.7×10^5	4.8×10^6	1.5×10^4
April	"	Spreader	3.6	5.8×10^5	7.3×10^4	8.0×10^5	0
May	"	3.1×10^5	23	1.9×10^7	3×10^3	1.3×10^7	0
June	Sardine (S.longiceps)	6×10^4	0	1.5×10^6	3.6×10^3	3.2×10^7	7.5×10^6
July	Mackerel	1.9×10^4	3.6	3×10^4	0	6×10^6	1.1×10^5
August	"	3.2×10^4	240	1.2×10^7	1.4×10^3	1.3×10^7	1.1×10^4
September	"	Spreader	23	5.8×10^5	0	8.6×10^5	0
October	"	"	23	Spreader	360	9.2×10^6	2×10^4
November	"	7.7×10^3	7.2	3×10^6	360	3.9×10^7	360
December	"	2.4×10^3	3.6	6.1×10^6	0	6.6×10^7	0

samples throughout the year except in the month of February and June. The load varied from 3.6 to 1.4 x 10^3 MPN/g. V. parahaemolyticus was present in the gills of the samples for the whole year except in the month of July, September and December. The load was maximum in the month of March as in the case of skin and muscle. V. parahaemolyticus load in gills varied from 62 to 2.7 x 10^5 MPN/g. V. parahaemolyticus was found to be absent in the intestine of the samples collected in the month of January, February, April, May, September and December. The highest load of V. parahaemolyticus was observed in the intestine of the samples collected in the month of June followed by gills of the sample collected in the month of March.

Table 17 presents the seasonal variation of V. parahaemolyticus in oyster (Crassostrea sp.), its environment and the salinity variation of the water. V. parahaemolyticus was present in oyster throughout the year except in the month of March and the load varied from 3.6 to 290 MPN/g. In mud the load varied from 0 to 1.1 x 10^3 MPN/g. Maximum number of V. parahaemolyticus was present in the mud sample collected in the month of October. In February, March and April the mud samples were found to be free from V. parahaemolyticus. In water the load varied from 0 to 910 MPN/ml. Maximum number of organisms were present in the water sample collected in the month of April. V. parahaemolyticus was absent in the water samples collected in February, March and May. Salinity of the water samples varied from 10.8 to 33.8‰ In mud samples the maximum number of V. parahaemolyticus was present when the salinity of the water came down to 11.6‰.

Table 17. Seasonal variation of Vibrio parahaemolyticus in oyster (Crassostrea sp.) its environment and salinity variations

Month	Vibrio parahaemolyticus MPN/g			Salinity ‰
	Oyster	Mud	Water	
June	290	N.D.	N.D.	N.D.
July	15	"	"	"
August	7.3	"	"	"
September	47	"	"	"
October	93	1.1×10^3	64	11.6
November	9.1	150	9.3	26.4
December	29	91	2	10.8
January	29	3	43	25.7
February	14	-	-	30.0
March	-	-	-	33.8
April	3.6	-	910	33.2
May	3.6	7.3	-	31.8

N.D. Not done

Incidence of coagulase-positive staphylococci in throats and palms of the male and female workers working in six fish processing factories situated in and around Cochin are tabulated in Table 18. Throats of 28.6 percent of male workers and 52.9 percent of female workers showed the presence of coagulase-positive staphylococci. Palms of 22.2 percent of male workers and 55.1 percent of female workers were found to contain coagulase-positive staphylococci. In general coagulase-positive staphylococci was isolated from the throats of 50 out of 101 workers (49.5%) and from the palms of 40 out of 78 workers (51.3%).

Morphological, cultural and biochemical characteristics of 181 strains of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers are given in Table 19. 92.3 percent of the isolates were found to be coagulase 4+ve and 1.7 percent 2+ve. 55.8 percent of the isolates produced white or cream pigments whereas 44.2 percent produced yellow pigments. 100 percent of the staphylococcal isolates fermented glucose (anaerobic) and produced DNase. All the isolates were found to be negative for indole production test and oxidase test. 98.9 percent of the isolates were found to be positive for phosphatase production test, nitrate reduction test and catalase production tests.

The 278 strains of V. parahaemolyticus isolated from different sources (Table 20) were subjected to detailed studies on their morphological, cultural and biochemical characteristics and the results are tabulated in Table 21. All strains were found to be gram-negative and motile. None of the strains swarmed on agar medium with 2.0 percent agar and did not grow at $2^{o}C$. No growth was observed in broth with 0 percent

Table 18. Incidence of coagulase-positive staphylococci in throats and palms of workers of six fish processing factories

Sex	Throat			Palm		
	No. of workers examined	Coagulase-positive staphylococci was isolated from	%	No. of workers examined	Coagulase-positive staphylococci was isolated from	%
Male	14	4	28.6	9	2	22.2
Female	87	46	52.9	69	38	55.1
Total	1 01	50	49.5	78	40	51.3

Table 19. Morphological, cultural and biochemical characteristics of 181 strains of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

Properties		Positive isolates	
		No.	%
Grams reaction (+ve cocci)		181	100.0
Coagulase			
	4+	167	92.3
	3+	9	5.0
	2+	3	1.7
	1+	2	1.1
Pigmentation			
	White / cream	101	55.8
	Golden yellow	80	44.2
Mannitol fermentation (anaerobic)		169	93.4
Glucose fermentation (")		181	100.0
Lactose fermentation		160	88.4
Galactose fermentation		155	85.6
Phosphatase production		179	98.9
Gelatinase "		148	81.8
Oxidase		0	-
Urease production		128	70.7
DNase production		181	100.0
Nitrate reduction		179	98.9
Catalase production		179	98.9
Methyl red		158	87.3
Voges - Proskauer		48	26.5
Indole production		0	-
Crystal violet agar test			
	+ve or Type A (yellow)	56	30.9
	-ve or Type C (violet)	125	69.1

Table 20. Source of Vibrio parahaemolyticus used for detailed studies

Source	No. of isolates
Marine fin fishes	105
Marine shell fishes	79
Brackish water fin fishes	24
Brackish water shell fishes	42
Sea water	9
Brackish water	11
Mud	8
Total	278

Table 21. Morphological, cultural and biochemical characteristics of 278 strains of Vibrio parahaemolyticus isolated from marine and brackish water sources

Characteristics	V. parahaemolyticus NCMB-1902	No. of isolates out of 278	
		Positive	Negative
Grams reaction	-	0	278
Pleomorphism	+	191	87
Motility	+	278	0
Swarming			
1.0% agar	+	225	53
2.0% agar	-	0	278
Temperatures			
$2^{o}C$	-	0	278
$22^{o}C$	+	278	0
$30^{o}C$	+	278	0
$37^{o}C$	+	278	0
$42^{o}C$	+	268	10
Salt conc.			
0%	-	0	278
1%	+	278	0
3%	+	278	0
6%	+	278	0
8%	+	278	0
10%	-	2	276
Oxidation and fermentation of glucose	+	278	0
Catalase production	+	278	0
Cytochrome oxidase production	+	278	0
Starch hydrolysis	+	278	0

Contd...2.

Table 21 contd.

Fermentation of (acid production only)			
Glucose	+	278	0
Sorbitol	-	1	277
Mannitol	+	276	2
Arabinose	-	183	95
Adonitol	-	5	273
Xylose	-	2	276
Maltose	+	270	8
Lactose	-	7	271
Sucrose	-	6	272
Cellobiose	-	65	213
Dulcitol	-	3	275
Salicin	-	1	277
Inositol	-	2	276
Trehalose	+	278	0
Rhamnose	-	0	278
Indole production	+	275	3
Nitrite production	+	278	0
Gelatin liquefaction	+	276	2
Casein hydrolysis	+	230	48
H_2S production	-	0	278
Triple sugar iron agar			
Acid butt, alkaline slant, no gas	+	278	0
Voges-proskauer test	-	3	275
Urease production	+	8	270
L-Lysine decarboxylase	+	278	0
L-Ornithine decarboxylase	+	273	5
L-Arginine dihydrolase	-	0	278
O/129 pteridine sensitivity	+	278	0

NaCl. Only 0.7 percent of the isolates grew in broth with 10 percent NaCl. All of the isolates were found positive for their growth at 22°C, 30°C and 37°C, in broth with 1%, 3%, 6% and 8% NaCl. Oxidation and fermentation of glucose, catalase production, cytochrome oxidase production, starch hydrolysis, fermentation of glucose, trehalose, nitrite production, L-lysine decarboxylase and sensitivity to 0/129 pteridine (2,4 - diamino-6,7 -diisopropyl pteridine).

Survival rate of coagulase-positive staphylococci in sterile shrimp homogenate at different temperatures are plotted in Fig.3. At RT and 8°C there was an increase followed by decrease in count. At 2°C and -20°C the organism did not multiply but survived even after 8 months.

Survival of V. parahaemolyticus in sterile crab meat homogenate with 0 percent and 3 percent NaCl at -20°C is illustrated in Fig.4. No growth was observed at -2°C. The log value came down from 10^6 to 10^2 within 13 days in homogenate with 0 percent NaCl. But in the presence of 3 percent NaCl the same rate of reduction was observed only after 24 days. No. V. parahaemolyticus cells could be detected in the homogenate with 0 percent NaCl after 16 days of storage but in the case with 3 percent NaCl only after 30 days storage.

The survival rate of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus in sterile shrimp homogenate with 3 percent NaCl at 2°C is depicted in Fig. 5. From the graph it is clear that the kanagawa-negative strain of V. parahaemolyticus survived better than the kanagawa-positive strain.

Fig. 6 shows the survival rate of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus in sterile shrimp homogenate with 3% NaCl at -20°C. The rate of destruction for both the strains were found more or less same and they survived upto 21 days of storage.

Inactivation curves for three strains of V. parahaemolyticus, one kanagawa-positive strain and two kanagawa-negative strains in tap water is shown in Fig.7. In tap water 75.6 percent of the cells were inactivated in one minute, 86.6 percent in 2 minutes, 92.7 percent in 3 minutes and 99.9 percent within 15 minutes. The comparison between the time of exposure required to inactivate 90 percent of the cells of the three strains of V. parahaemolyticus by tap water is shown in Table 22. Ninety percent inactivation (D_{10}) was observed between 1.6 and 3.4 minutes. D_{10} (min) was found to be maximum for kanagawa-positive strains. The levels of different salts and organic substances present in tap water is shown in Table 22A.

Survival rate of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus in normal saline and 3 percent NaCl solution at R.T. are illustrated in Fig. 8. The survival rate of these organisms in brackish water and sea water are illustrated in Fig.9. Kanagawa-positive strains survived better than kanagawa-negative strains in all aquatic systems. In normal saline the kanagawa-negative strains did not survive for more than 12 days but the kanagawa-positive strains survived for more than 34 days. In 3 percent NaCl solution both the strains survived more than 34 days. In brackish water (salinity 9.96‰) complete destruction of kanagawa-negative strain was observed within 15 days but the

Table 22. Time of exposure to tap water required to inactivate 90% of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus

Kanagawa phenomenon	D_{10} (Minute)
Kanagawa +ve strain (V.P - 49)	3.4
Kanagawa -ve strain (V.P - 56)	1.6
" (NCMB - 1902)	2.3

Table 22A. The levels of different salts and organic substances present in tap water

pH	..	6.8
Free and saline ammonia, ppm	..	0.04
Albuminoid ammonia ppm	..	0.04
Hardness, Total as $CaCO_3$, ppm	..	34
Hardness permanent as $CaCO_3$, ppm	..	32
Hardness temporary as $CaCO_3$, ppm	..	2
Hardness due to Ca, as $CaCO_3$, ppm	..	22
Hardness due to Mg, as $CaCO_3$, ppm	..	12
Phenolphthalin alkalinity as $CaCO_3$, ppm	..	Nil
Methyl orange alkalinity as $CaCO_3$, ppm	..	22
Copper as Cu, ppm	..	0.003
Iron as Fe, ppm	..	0.02
Chlorides as Cl, ppm	..	7.1
Sulphate as SO_4, ppm	..	19.0
Total dissolved solids, ppm	..	85
Loss on ignition, ppm	..	27
Free chloride, ppm	..	0.02

kanagawa-positive strain survived more than 34 days. In sea water (salinity 28.7‰) complete destruction of kanagawa-negative strain was observed within 21 days but the kanagawa-positive strain survived more than 34 days.

Competition between coagulase-positive staphylococci and the natural bacterial flora of cooked, picked and frozen crab meat during defrost is shown in Fig.10. The TBC increased from 1.3×10^6 to 1.2×10^9 within 24 hours, whereas the count of coagulase-positive staphylococci increased from 6.5×10^3 to 1.0×10^6.

Fig.11 shows the growth pattern of coagulase-positive staphylococci and other natural bacterial flora of cooked picked crab meat under controlled conditions viz. room temperature and under ice for a period of 6 hours. The count of coagulase-positive staphylococci remained almost stationary in the sample kept under iced condition, whereas the count showed a tendency to increase in the sample kept at room temperature.

Competition between V. parahaemolyticus and other natural bacterial flora or cooked, shucked clams for growth at 10^oC and 0^oC is depicted in Fig. 12. At 10^oC the TBC increased from 3.9×10^6 to 1.8×10^8 per gram within 6 days and remained static, whereas V. parahaemolyticus could not be detected after 2 days of storage. At 0^oC the TBC increased by one log within 20 days and V. parahaemolyticus count reduced from 2.8×10^5 MPN/g to 9.1 MPN/g within 20 days of storage.

Fig. 13 shows the competition between V. parahaemolyticus and other natural bacterial flora of cooked shucked clams meat (Villorita sp.) kept at -10^oC and -20^oC. At -10^oC the TBC remained more or less static

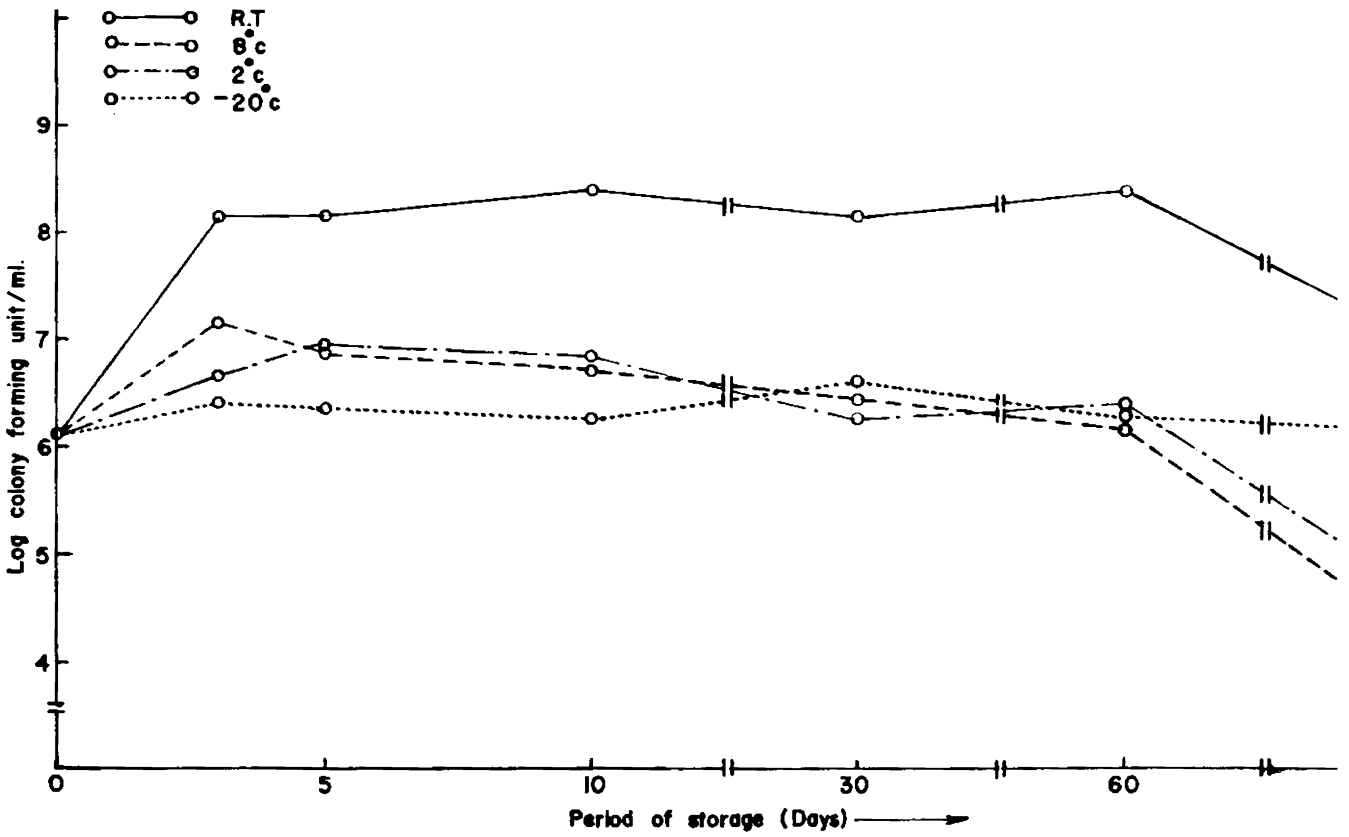


Fig. 3. Survival of coagulase-positive staphylococci at different temperatures in sterile shrimp homogenate

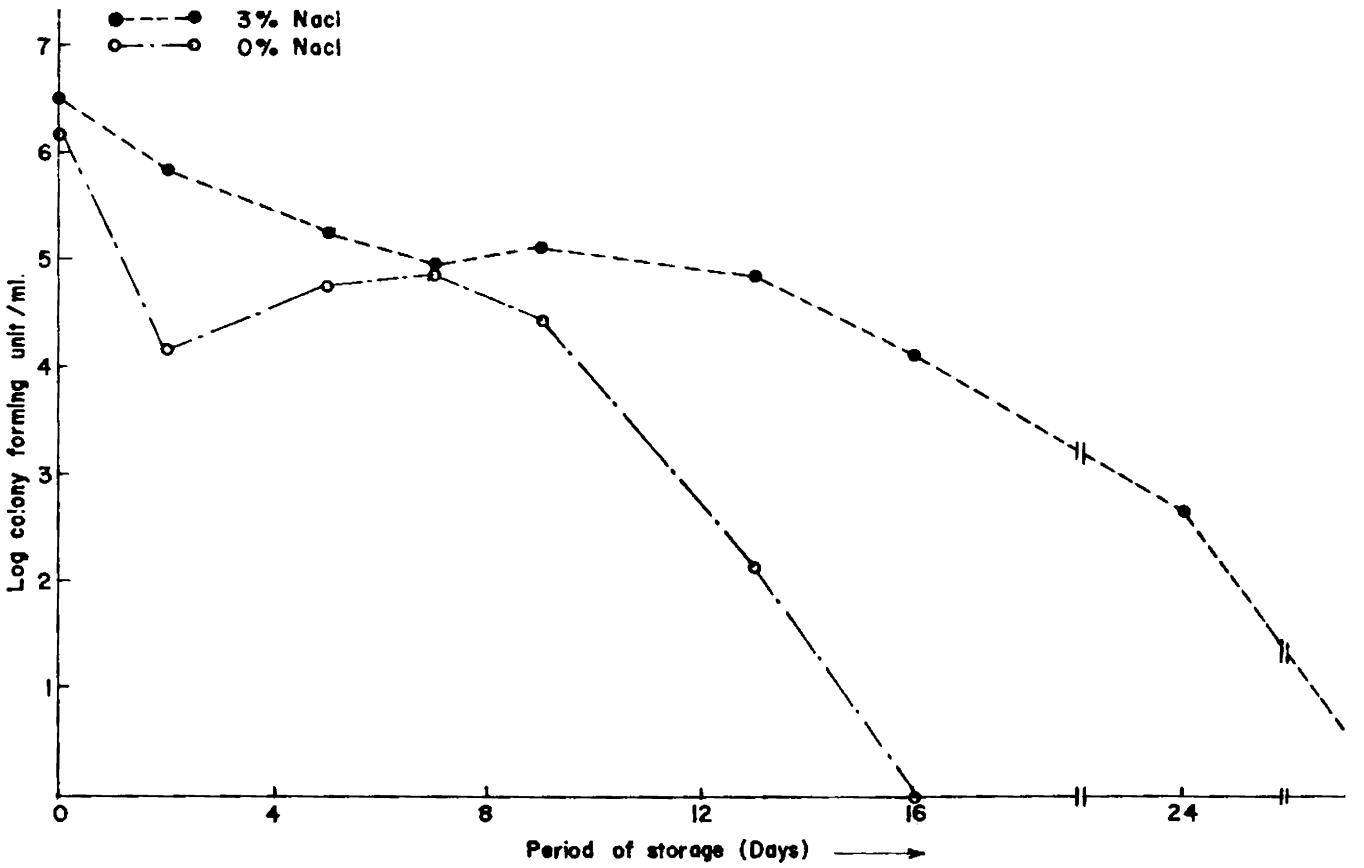


Fig. 4. Survival of Vibrio parahaemolyticus in sterile crab meat homogenate with 0% and 3% NaCl at −20°c.

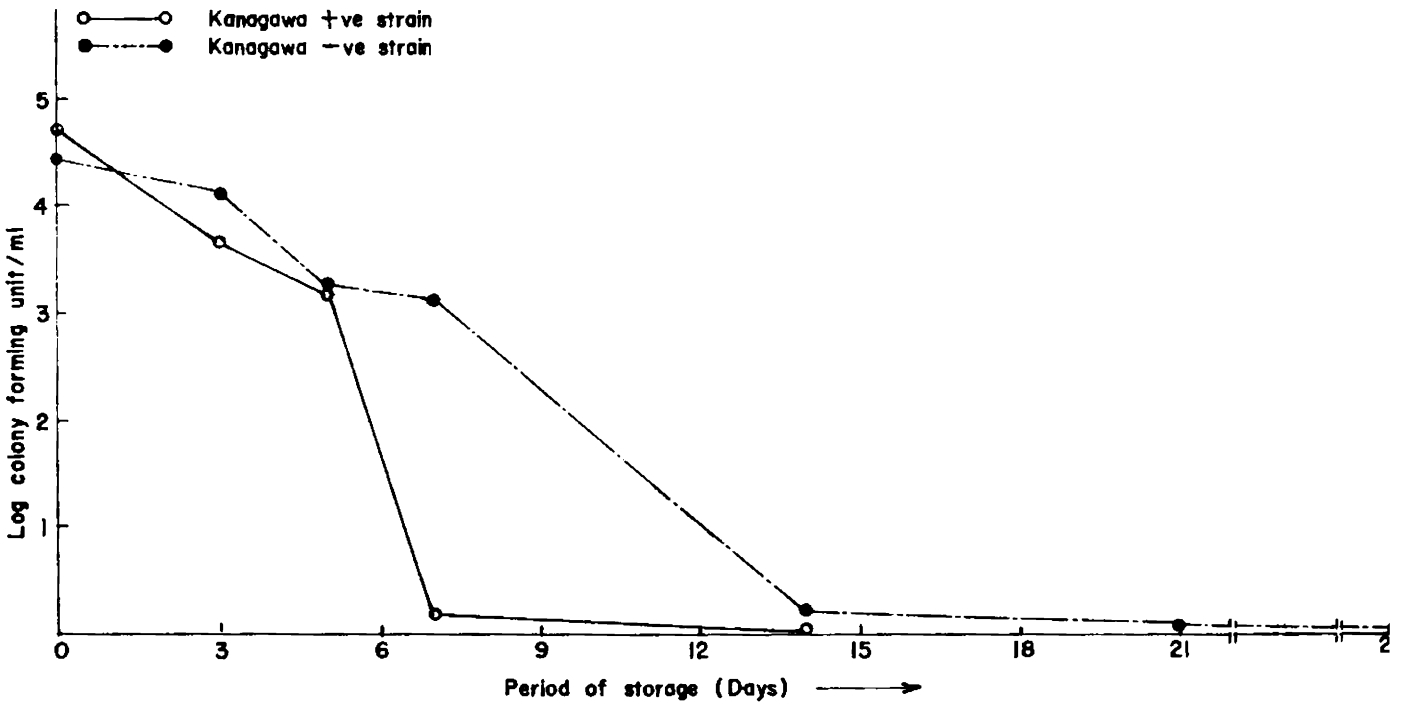


Fig. 5. Survival of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in sterile shrimp homogenate with 3% NaCl at 2°c.

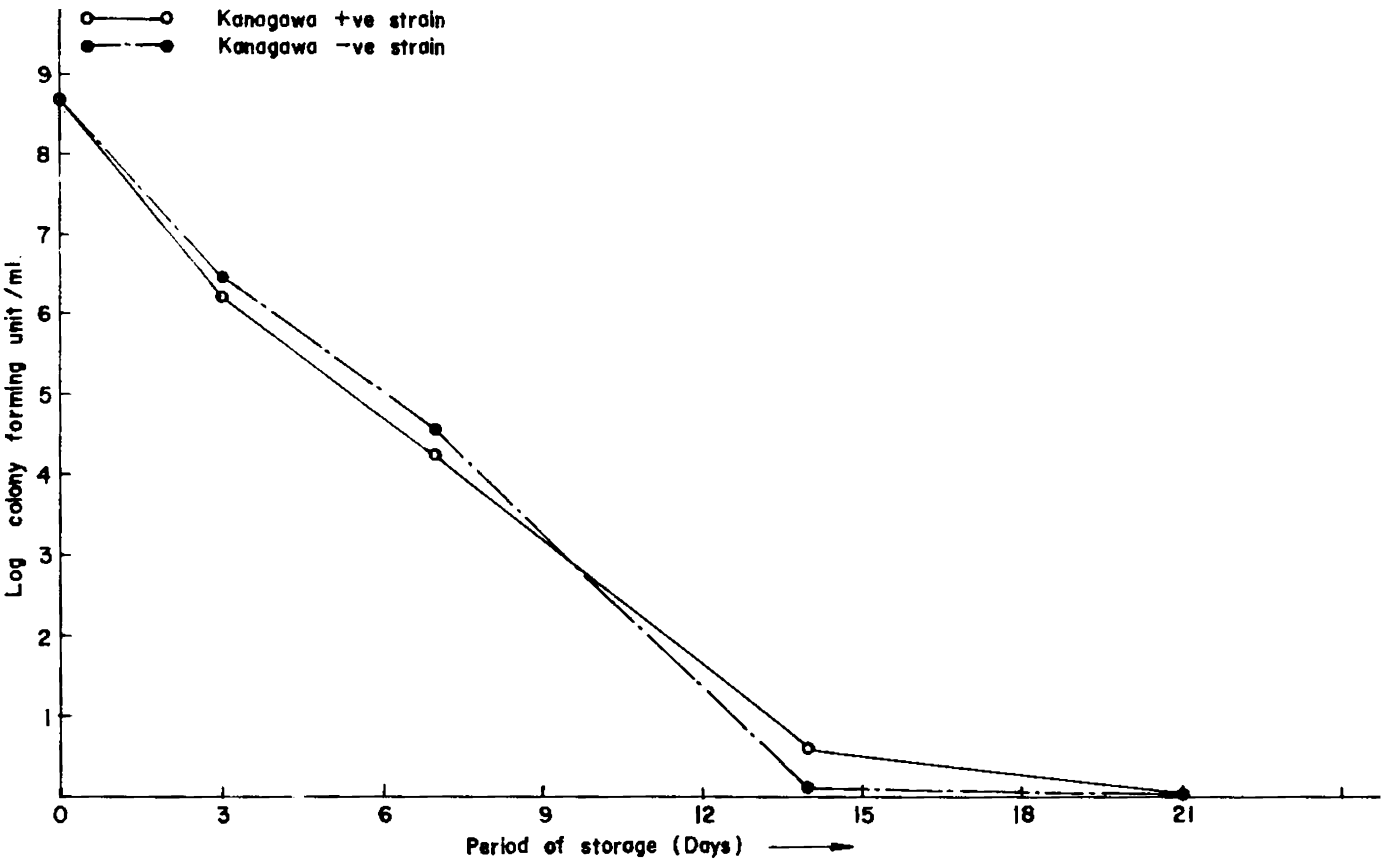


Fig. 6. Survival of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in sterile shrimp homogenate with 3% NaCl at − 20°c

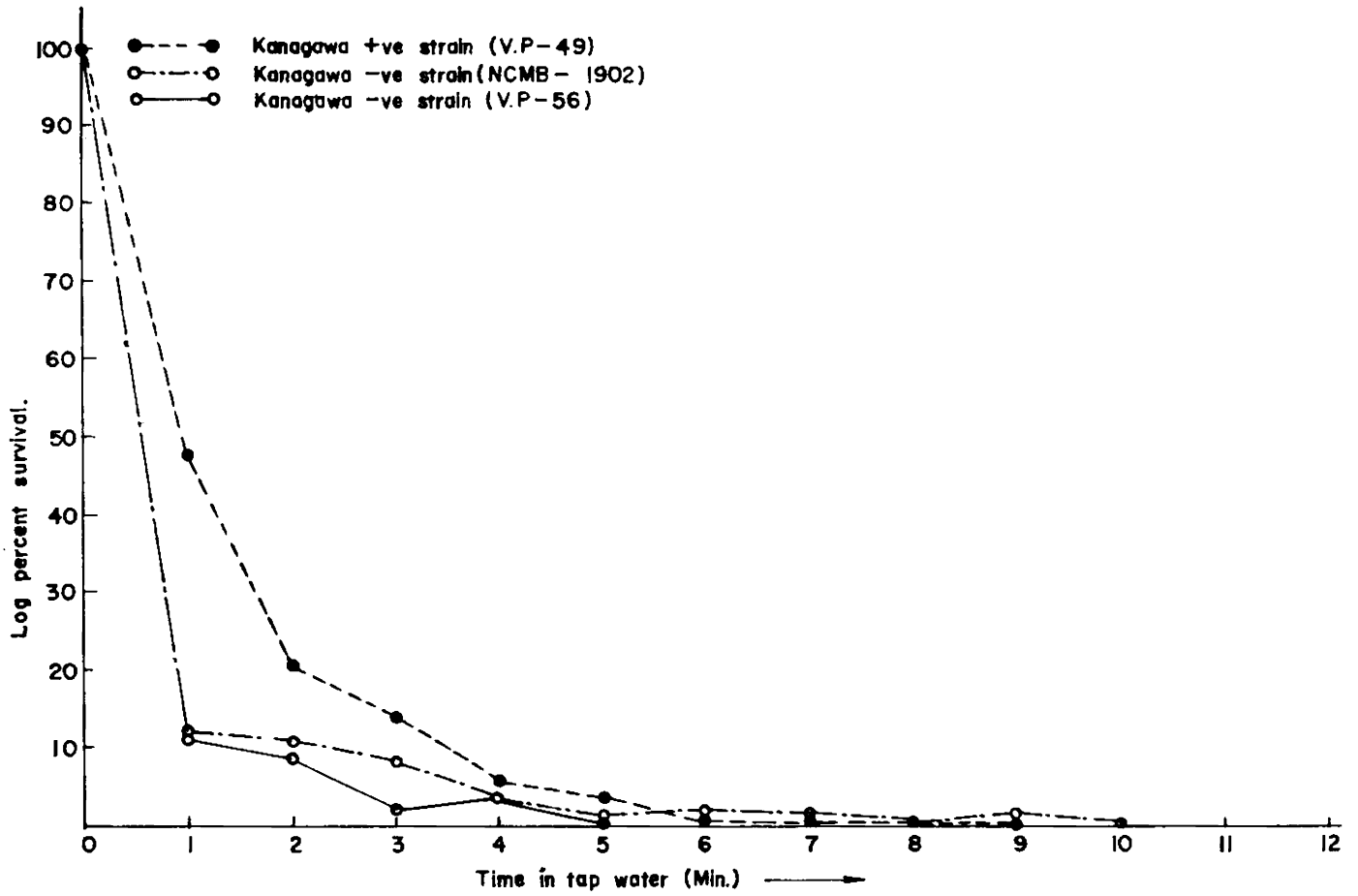


Fig. 7. Inactivation of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in tap water.

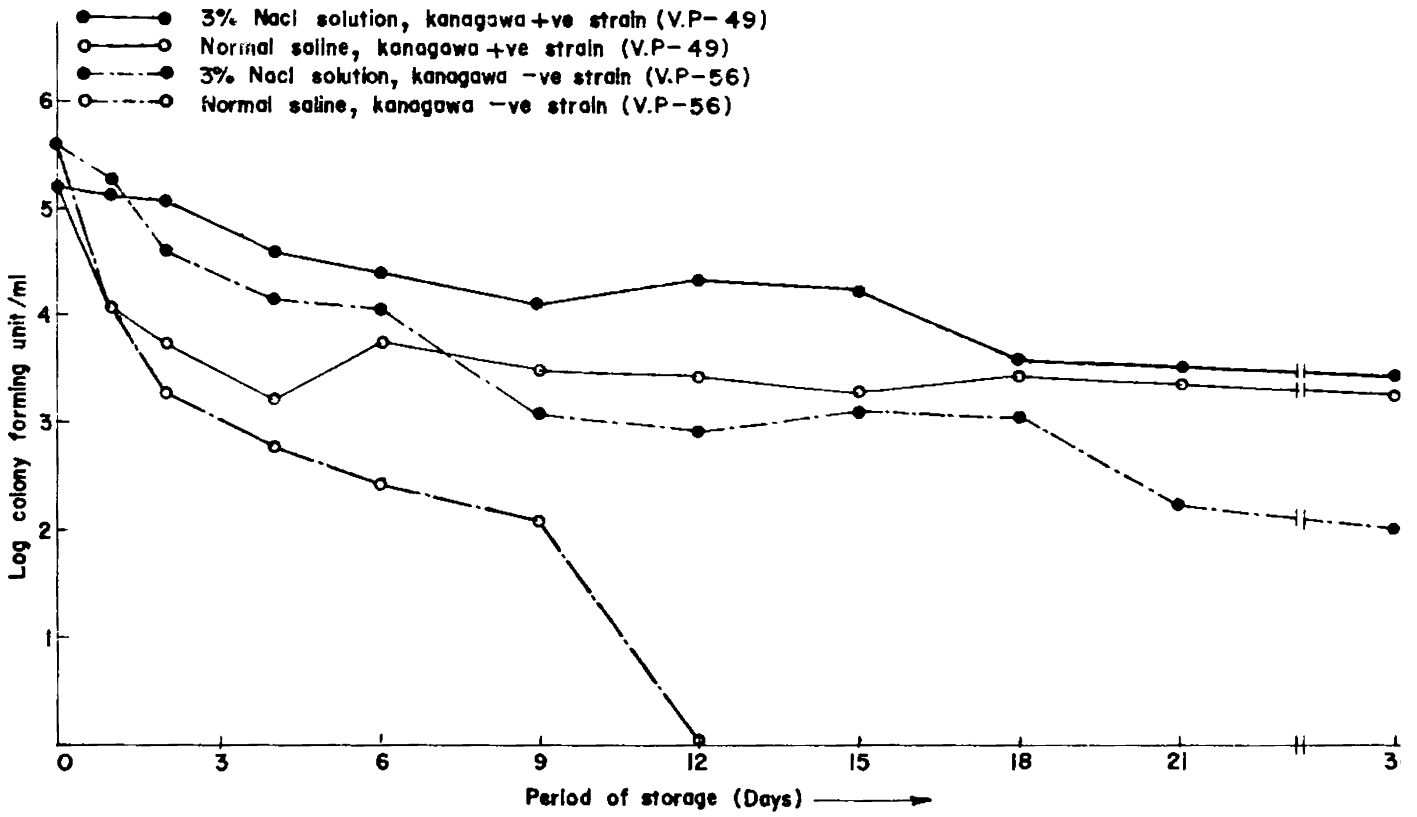


Fig.8. Survival of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in normal saline and 3% Naci solution at R.T.

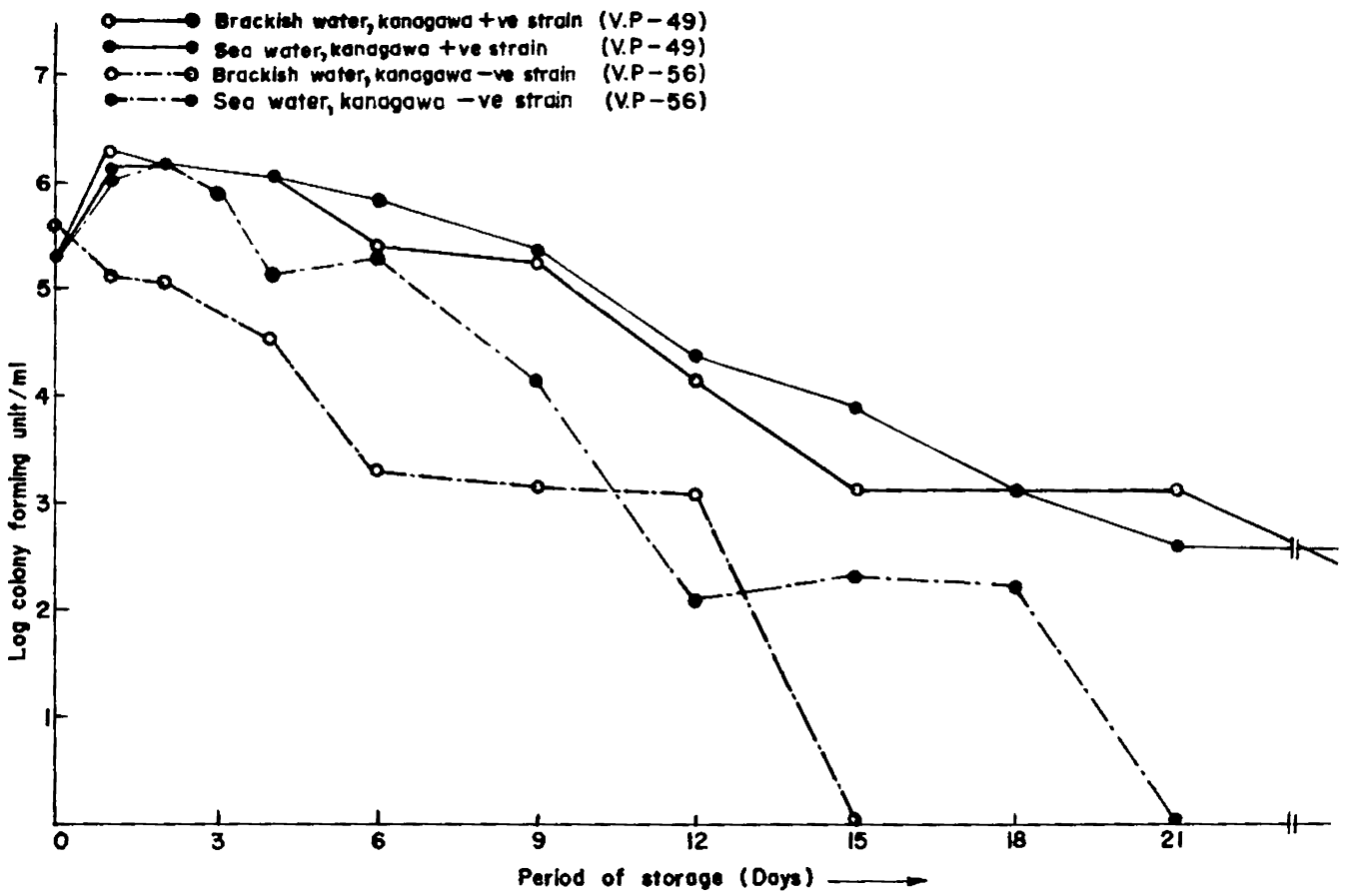


Fig. 9. Survival of kanagawa-positive and kanagawa-negative strains of Vibrio parahaemolyticus in brackish water and sea w at R.T.

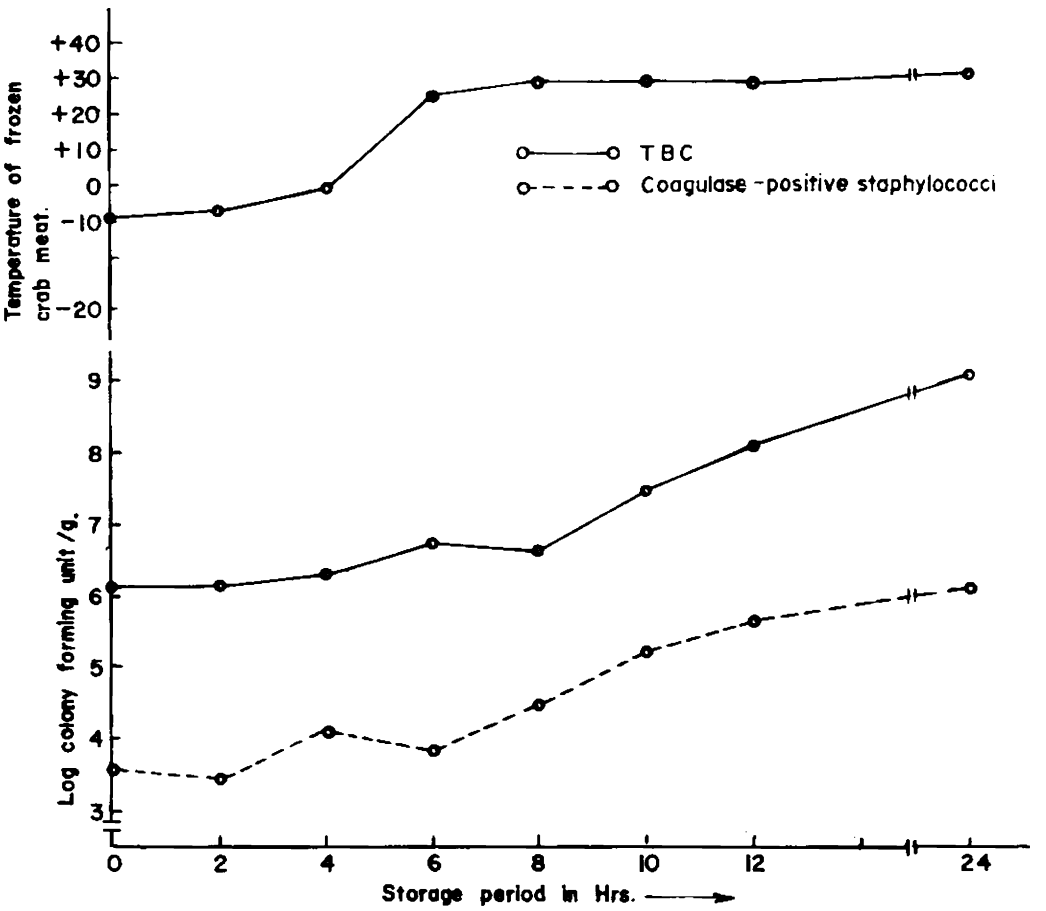


Fig. 10. Competition for growth between coagulase-positive staphylococci and other natural bacterial flora of cooked, picked and frozen crab meat during defrost.

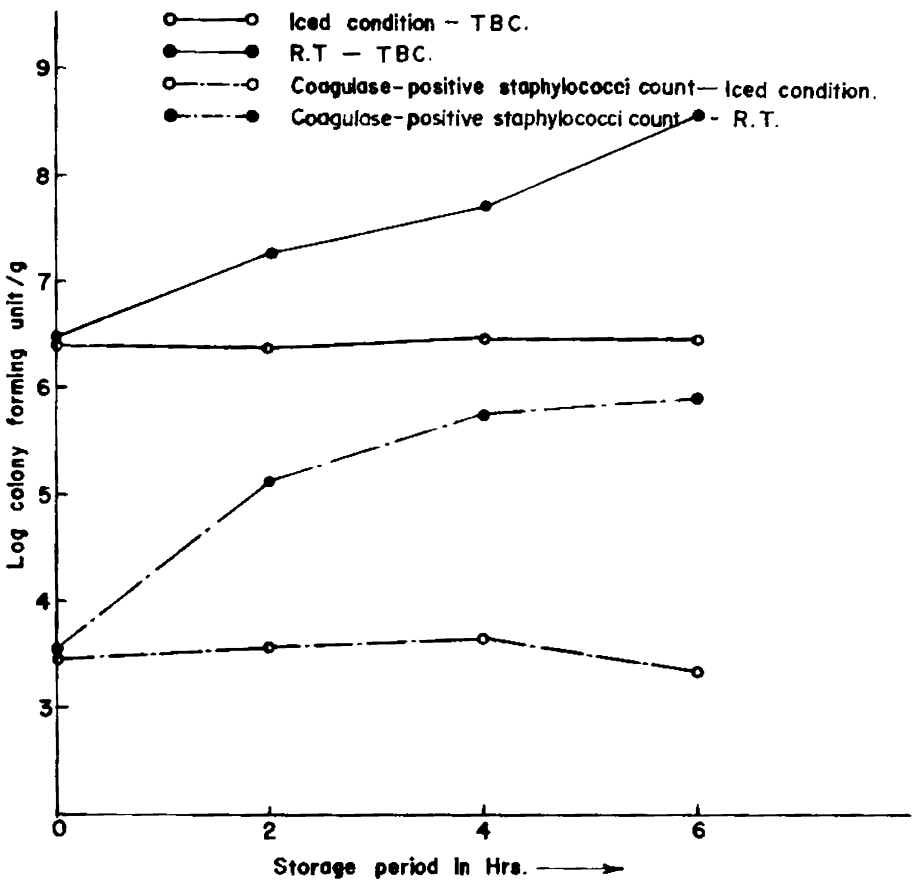


Fig.11. Competition for growth between coagulase-positive staphylococci and other natural bacterial flora of cooked, picked crab meat at R.T ($30^{\circ}\pm1^{\circ}$c) and iced condition

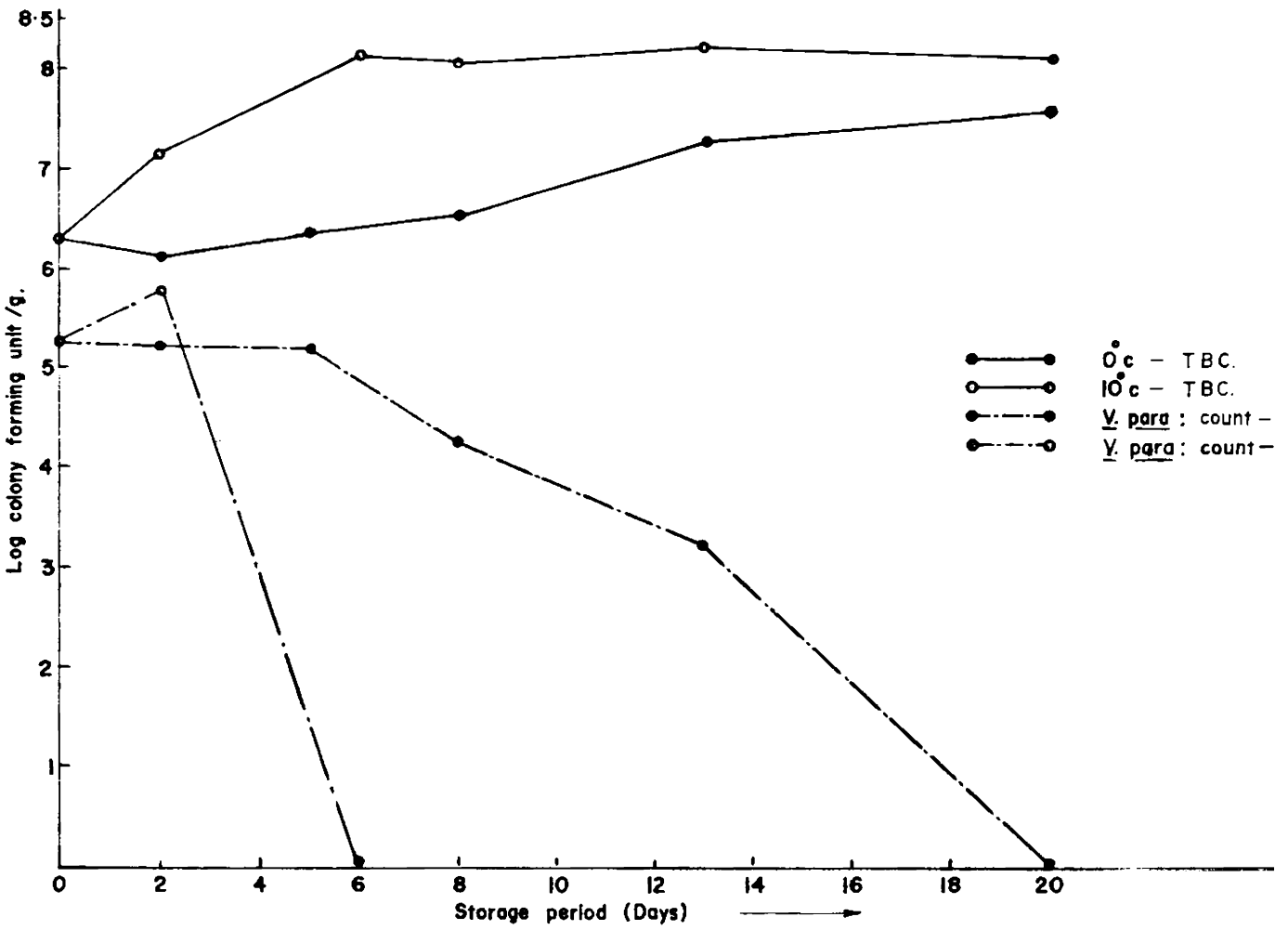


Fig.12. Competition for growth between V. parahaemolyticus and other natural bacterial flora of cooked, shucked clam me at 10°c and 0°c.

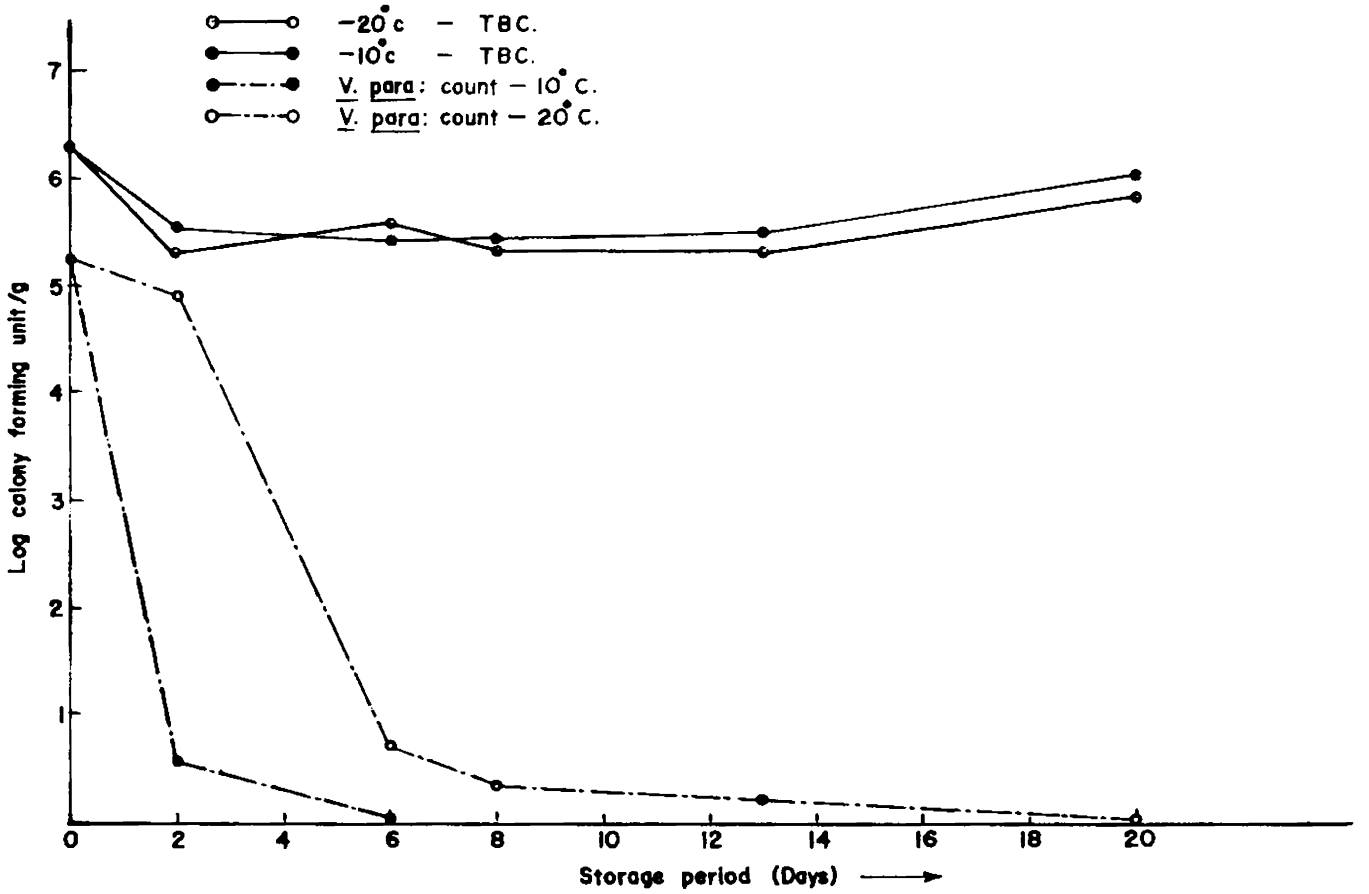


Fig. 13. Competition for growth between V. parahaemolyticus and other natural bacterial flora of cooked shucked clam meat at − 10°c and − 20°c.

and V. parahaemolyticus could not be detected after 2 days of storage. Reduction in TBC was observed in the sample kept at -20°C and complete destruction of V. parahaemolyticus took place within 20 days of storage.

The enterotoxigenicity of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers are tabulated in Table 23. 88.46 percent of the isolates obtained from dried fishery products, 75.49 percent from frozen fishery products and 66.41 percent from fish processing factory workers were found to be enterotoxigenic. In general 72.27 percent of the total of 256 strains were found to be enterotoxigenic.

Distribution of enterotoxins among 185 enterotoxigenic strains of staphylococci isolated from different sources are tabulated in Table-24. On the basis of enterotoxin patterns, type A enterotoxin was produced by the maximum number of isolates followed by Type D, type C, type B and type E.

Incidence of enterotoxigenic staphylococci in fishery products and carriage among fish processing factory workers are tabulated in Table 25. 20.53 percent of the cured fishery products, 68.19 percent of the frozen fishery products were found to contain enterotoxigenic staphylococci. 97.44 percent of the coagulase-positive staphylococci carriers were also found to be the carriers of enterotoxigenic strains.

Kanagawa phenomenon of V. parahaemolyticus isolated from different sources are summarised in Table 26. 20.56 percent of the strains isolated from fin fishes, 22.92 percent from shell fishes, 12.50 percent from water samples and 33.33 percent from mud samples were found to be kanagawa-positive. In general 21.33 percent of the 225 strains

Table 23. Enterotoxigenicity of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers

Source	No. of strains tested	No. of strains produced enterotoxins	%
Cured fishery products	26	23	88.46
Frozen fishery products	102	77	75.49
Fish processing factory workers	128	85	66.41
Total	256	185	72.27

Table 24. Distribution of enterotoxin(s) among 185 enterotoxigenic staphylococci strains isolated from fishery products and fish processing factory workers

Enterotoxin(s) pattern	Isolated from						Total	
	Cured fishery products		Frozen fishery products		Fish processing factory workers		No.	%
	No.	%	No.	%	No.	%		
A	7	30.43	20	25.97	20	23.53	47	25.41
B	1	4.35	4	5.19	6	7.06	11	5.95
C	2	8.70	7	9.10	11	12.94	20	10.82
D	1	4.35	12	15.58	12	14.12	25	13.51
E	1	4.35	1	1.30	3	3.53	5	2.70
AB	2	8.70	-	-	2	2.35	4	2.16
AC	1	4.35	4	5.19	3	3.53	8	4.32
AD	3	13.04	9	11.69	7	8.24	19	10.27
AE	-	-	2	1.60	1	1.18	3	1.62
BC	-	-	-	-	1	1.18	1	0.54
BD	-	-	1	1.30	1	1.18	2	1.08
CD	-	-	6	7.80	6	7.06	12	6.49
DC	1	4.35	-	-	-	-	1	0.54

Table Contd...

Table 24. contd.

DE	1	4.35	-	-	1	1.18	2	1.08
ABC	1	4.35	3	3.90	3	3.53	7	3.78
ABD	1	4.35	1	1.30	2	2.35	4	2.16
ABE	1	4.35	-	-	-	-	1	0.54
ACD	-	-	3	3.90	5	5.88	8	4.32
ACE	-	-	1	1.30	1	1.18	2	1.08
BCD	-	-	1	1.30	-	-	1	0.54
BCE	-	-	1	1.30	-	-	1	0.54
ABCE	-	-	1	1.30	-	-	1	0.54

Table 25. Incidence of Enterotoxigenic staphylococci in fishery products and carriage among workers of fish processing factories

Name of the sample	No. of samples tested	No. of samples with coagulase positive staphylococci	%	No. of samples with enterotoxigenic staphylococci	%
Cured fishery products	112	26	23.2	23	20.53
Frozen fishery products	66	48	72.7	45	68.19
Fish processing factory workers					
Throats	101	50	49.50		97.44
Palms	78	40	51.28		

of V. parahaemolyticus isolated from different sources were found to be kanagawa-positive. 50 percent of the isolates obtained from mussels were found to be kanagawa-positive and all isolates obtained from cooked, shucked clams were found to be kanagawa-negative.

Antibiotic sensitivity of coagulase-positive staphylococci isolated from cured fishery products are given in Table 27. All isolates from cured fishery products were found to be sensitive to kanamycin and streptomycin.

Table 28 shows the antibiotic sensitivity of coagulase-positive staphylococci isolated from frozen fishery products. All the isolates were found sensitive to kanamycin, streptomycin and chloramphenicol.

None of the tested antibiotics were found 100% effective to the coagulase-positive staphylococci strains isolated from fish processing factory workers, but maximum sensitivity was shown towards chloramphenicol (94.2%) followed by kanamycin (91.8%) and streptomycin (87.7%) Table 29.

Antibiotic sensitivity of 238 strains of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers are summarised in Table 30 and plotted in Fig.14. In general maximum sensitivity was shown towards chloramphenicol (96.22%) followed by kanamycin (95.8%) and streptomycin (93.7%). Sensitivity towards other antibiotics like neomycin, erythromycin, polymyxin-B, tetracycline, penicillin and ampicillin were shown by 76.47, 73.53, 67.23, 66.81, 45.38 and 38.66 percent of the isolates respectively.

Table 27. Antibiotic sensitivity of 26 strains of coagulase-positive staphylococci isolated from cured fishery products

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	6	23.08	0	-	20	76.92
Erythromycin	19	73.08	7	26.92	0	-
Kanamycin	26	100.00	0	-	0	-
Neomycin	20	76.92	5	19.23	1	3.85
Penicillin	6	23.08	0	-	20	76.92
Tetracycline	9	34.62	8	30.77	9	34.62
Streptomycin	26	100.00	0	-	0	-
Chloramphenicol	24	92.31	1	3.85	1	3.85
Polymyxin-B	24	92.31	2	7.70	0	-

Table 28. Antibiotic sensitivity of 90 strains of coagulase-positive staphylococci isolated from frozen fishery products

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	58	64.44	6	6.67	26	28.89
Erythromycin	83	92.22	7	7.78	0	-
Kanamycin	90	100.00	0	-	0	-
Neomycin	58	64.44	28	31.11	4	4.44
Penicillin	60	66.67	5	5.56	25	27.78
Tetracycline	70	77.78	13	14.44	7	7.78
Streptomycin	90	100.00	0	-	0	-
Chloramphenicol	90	100.00	0	-	0	-
Polymyxin-B	84	93.33	5	5.56	1	1.11

Table 29. Antibiotic sensitivity of 122 strains of coagulase-positive staphylococci isolated from fish processing factory workers

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	28	22.95	15	12.30	79	64.75
Erythromycin	73	59.84	40	32.79	9	7.38
Kanamycin	112	91.80	3	2.46	7	5.74
Neomycin	104	85.25	11	9.02	7	5.74
Penicillin	42	34.43	7	5.74	73	59.84
Tetracycline	80	65.57	14	11.48	28	22.95
Streptomycin	107	87.70	13	10.66	2	1.64
Chloramphenicol	115	94.20	3	2.46	4	3.28
Polymyxin-B	52	42.62	50	40.98	20	16.39

Table 30. Antibiotic sensitivity of 238 strains of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	92	38.66	21	8.83	125	52.52
Erythromycin	175	73.53	54	22.69	9	3.78
Kanamycin	228	95.80	3	1.26	7	2.94
Neomycin	182	76.47	44	18.49	12	5.04
Penicillin	108	45.38	12	5.04	118	49.58
Tetracycline	159	66.81	35	14.71	44	18.49
Streptomycin	223	93.70	13	5.46	2	0.84
Chloramphenicol	229	96.22	4	1.68	5	2.10
Polymyxin-B	160	67.23	57	23.95	21	8.82

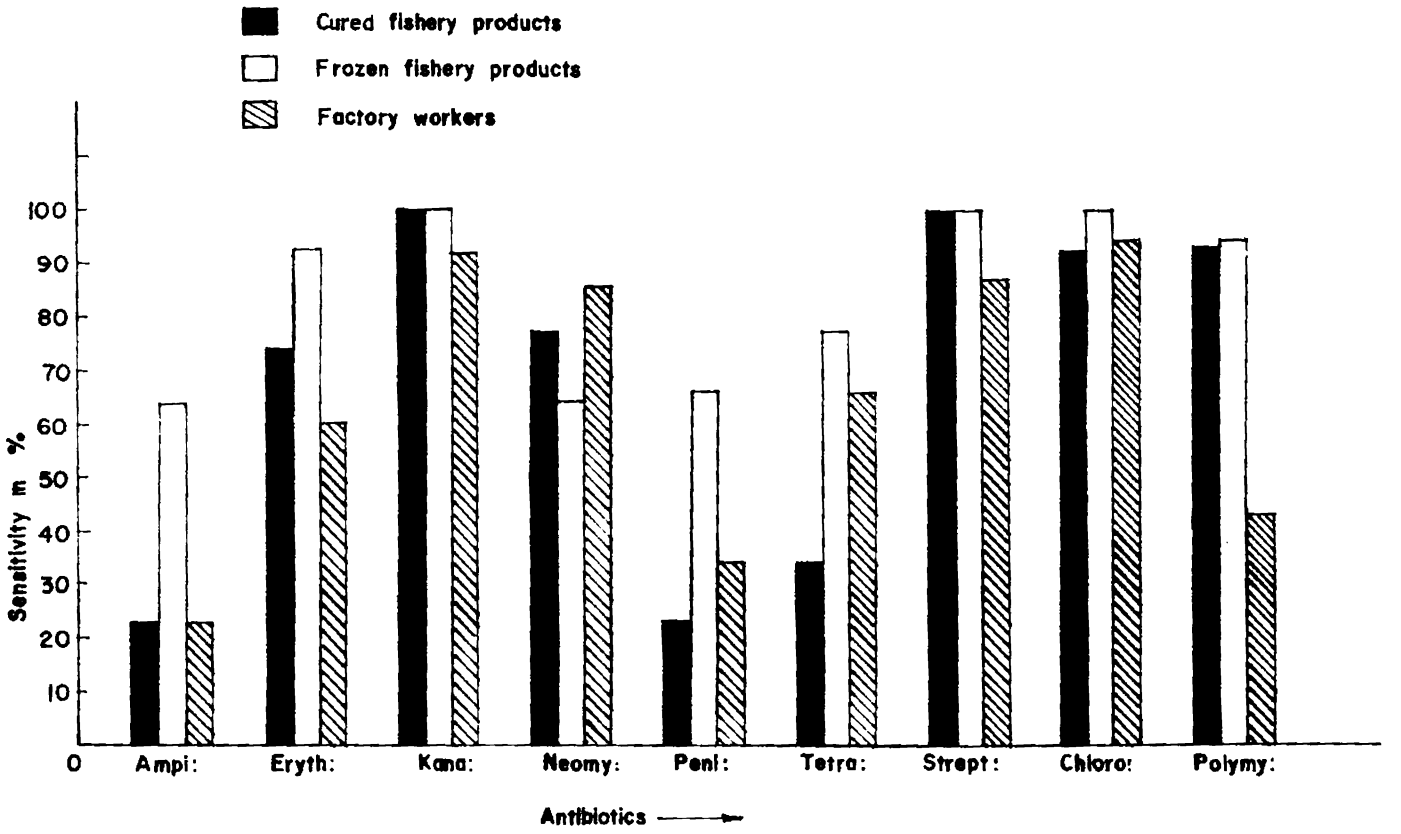


Fig. 14. Antibiotic sensitivity of 238 strains of coagulase-positive staphylococci isolated from cured fishery products, frozen fishery products and fish processing factory workers.

Antibiotic sensitivity of 84 strains of V. parahaemolyticus isolated from fin fishes and shell fishes are tabulated in Table 31. Maximum sensitivity was observed towards chloramphenicol (98.81%) followed by gentamycin (97.62%). Sensitivity towards other antibiotics like polymyxin-B, neomycin, tetracycline, sulphadiazine, ampicillin, kanamycin, streptomycin, erythromycin and penicillin were 52.38, 46.43, 23.81, 21.43, 17.86, 11.9, 11.9, 1.19 and 0 percent of the isolates respectively.

Antibiotic sensitivity of 48 kanagawa-positive strains of V. parahaemolyticus isolated from different sources are listed in Table 32. None of the strains were found sensitive towards penicillin and erythromycin. Maximum sensitivity was shown towards chloramphenicol and gentamycin.

Sensitivity patterns towards the antibiotics by 36 strains of kanagawa-negative strains of V. parahaemolyticus isolated from different sources are given in Table 33. 100 percent of the isolates were found sensitive to chloramphenicol and gentamycin. None of the strains were found sensitive to penicillin.

Antibiotic sensitivity of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus isolated from fin fishes and shell fishes are compared in Fig.15.

Phage pattern of coagulase-positive staphylococci strains isolated from fishery products and fish processing factory workers are summarised in Table 34. 57.9 percent of the isolates from dried fishes, 32.1 percent from frozen fishery products and 47.6 percent from fish processing factory workers were found to be typable. In general 44.9 percent of the isolates

Table 31. Antibiotic sensitivity of 84 strains of Vibrio parahaemolyticus isolated from fin fishes and shell fishes

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	15	17.86	5	5.95	64	76.19
Chloramphenicol	83	98.81	0	-	1	1.19
Erythromycin	1	1.19	5	5.95	78	92.86
Gentamycin	82	97.62	0	-	2	2.38
Kanamycin	10	11.90	30	35.71	44	52.38
Neomycin	39	46.43	42	50.00	3	3.57
Penicillin	0	-	11	13.10	73	86.90
Polymyxin-B	44	52.38	31	36.90	9	10.71
Streptomycin	10	11.90	21	25.00	53	63.10
Sulphadiazine	18	21.43	20	23.81	46	54.76
Tetracycline	20	23.81	54	64.29	10	11.90

Table 32. Antibiotic sensitivity of 48 kanagawa-positive strains of Vibrio parahaemolyticus isolated from fin fishes and shell fishes

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	5	10.42	2	4.17	41	85.42
Chloramphenicol	47	97.92	0	-	1	2.10
Erythromycin	0	-	0	-	48	100.00
Gentamycin	46	95.83	0	-	2	4.17
Kanamycin	5	10.42	16	33.33	27	56.25
Neomycin	17	35.42	28	58.33	3	6.25
Penicillin	0	-	2	4.17	46	95.83
Polymyxin-B	25	52.10	21	43.75	2	4.17
Streptomycin	2	4.17	8	16.67	38	79.17
Sulphadiazine	1	2.10	4	8.33	43	89.58
Tetracycline	3	6.25	37	77.10	8	16.67

Table 33. Antibiotic sensitivity of 36 kanagawa-negative strains of Vibrio parahaemolyticus isolated from fin fishes and shell fishes

Antibiotics	No. of isolates showing sensitivity	%	No. of isolates showing intermediary sensitivity	%	No. of isolates resistant	%
Ampicillin	10	27.78	3	8.33	23	63.89
Chloramphenicol	36	100.00	0	-	0	-
Erythromycin	1	2.78	5	13.89	30	83.33
Gentamycin	36	100.00	0	-	0	-
Kanamycin	5	13.89	14	38.89	17	47.22
Neomycin	22	61.11	14	38.89	0	-
Penicillin	0	-	9	25.00	27	75.00
Polymyxin-B	19	52.78	10	27.78	7	19.44
Streptomycin	8	22.22	13	36.11	15	44.44
Sulphadiazine	17	47.22	16	44.44	3	8.33
Tetracycline	17	47.22	17	47.22	2	5.55

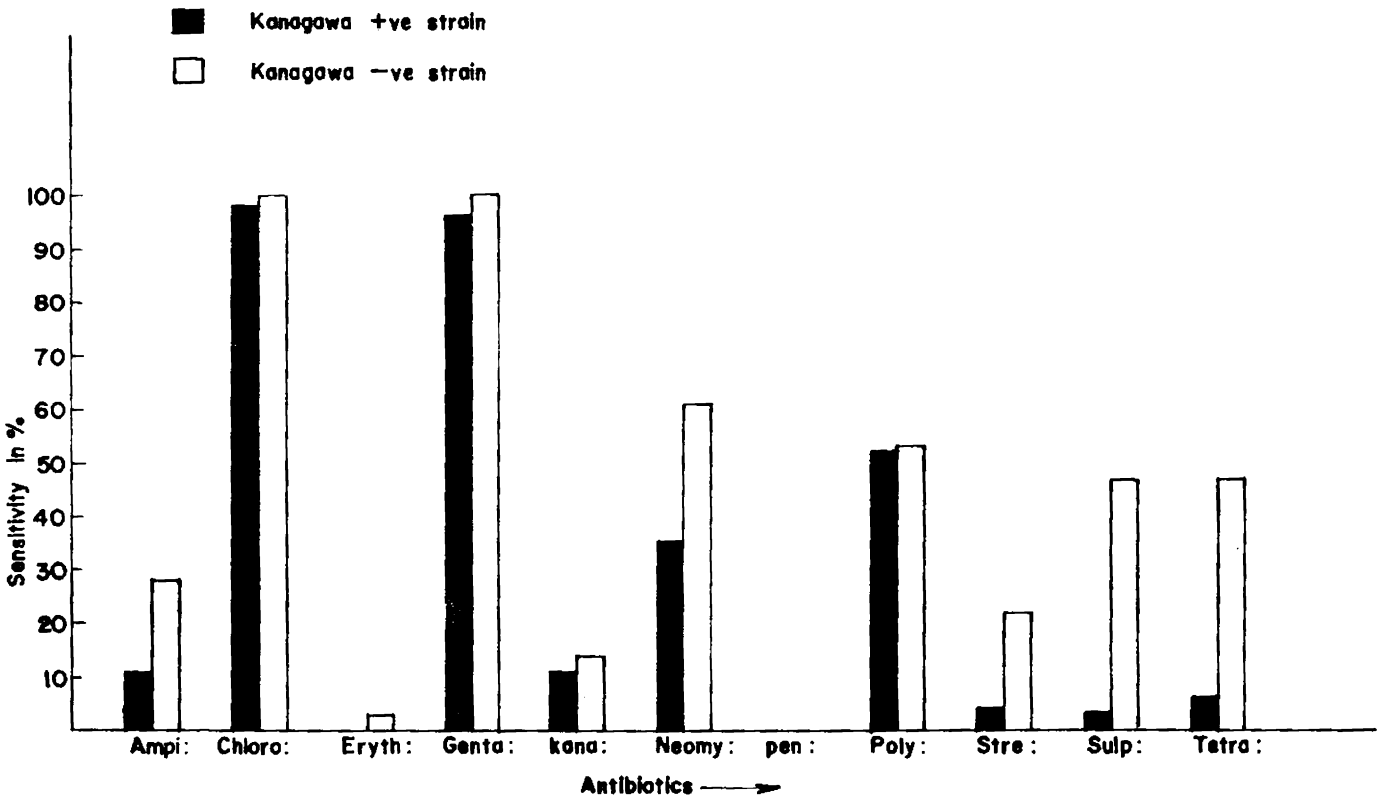


Fig. 15. Antibiotic sensitivity of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus isolated from fin fishes and shell fishes.

Table 34. Pattern of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers to different phages

Source	No. of isolates tested	No. of isolates giving +ve reaction	%	Phage No. and group	
Cured fishes	19	11	57.9	1. 47, 53, 54, 75, 77, 83A, 84	III
				1. 47, 53, 54, 75, 77, 83A, 84, 85	III
				1. 42E, 47, 75	III
				1. 42E, 47, 75 - 81	III & IV
				1. 29, 79, 80 - 53, 77, 85	I & III
				1. 53	III
				1. 54	III
				1. 75	III
				1. 79 - 53, 77, 83A, 85	I & III
				2. 29	I
Frozen prawns	3	1	33.3	1. 52A - 83A, 85	I & II
Cooked picked and frozen crab meat	25	8	32.0	1. 42E, 47	III
				2. 3A, 3C, 55	II
				1. 42E, 47, 54	III
				1. 47	III
				1. 94	IV
				1. 42E, 47	III
				1. 42E	III

Table contd...

Table 34 contd..

Throats of factory workers	30	16	53.3	1.	52A - 3A, 3C	I & II
				2.	3A, 3C	II
				1.	29, 79 - 42E, 47, 95, 96	I, III & IV
				1.	3A	II
				1.	3A, 3C, 55	II
				4.	3C, 55	II
				1.	47, 83A	III
				1.	47E, 54, 75, 84, 85	III
				1.	95	IV
				1.	42E, 47, 53-75, 84, 94	III & IV
				1.	53, 83A	III
				1.	6, 42E	III
Palms of factory workers	12	4	33.3	1.	84, 85	III
				1.	42E, 47, 75, 84 - 94	III & IV
				1.	84	III
				1.	47, 83A, 85	III
Total	89	40	44.9			

were found phage typable. Majority of the typable strains belonged to the phage group III and the rest to group II, IV, I and mixed groups.

Table 35 shows the distribution of typable coagulase-positive staphylococci recovered from different sources within the bacteriophage groups. Among typable strains 54.5 percent from cured fishes, 55.5 percent from frozen fishery products and 35 percent from fish processing factory workers belonged to the phage group III.

Antibiotic sensitivity patterns of phage-typable and non-typable strains of coagulase-positive staphylococci isolated from different sources are compared in Table 36. 52.2 percent of the non-typable strains of staphylococci were found to be sensitive to ampicillin but only 25.0 percent of the typable strains. 60.9 percent of the non-typable strains were found sensitive to penicillin but it was only 30.6 percent in the case of typable strains. In general non-typable strains were found more sensitive to the tested antibiotics than typable strains.

Enterotoxigenicity of phage-typable strains of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers are given in Table 37. 86.84 percent of the typable strains were found to be enterotoxigenic. 42.11 percent of the typable strains produced enterotoxin A either singly or in combinations, followed by enterotoxin C, D, B and E.

Enterotoxin production pattern and phage pattern of typable strains of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers are summarised in Table 38.

Table 35. Distribution of typable coagulase-positive staphylococci within the bacteriophage groups and its source

Source	Phage group	Number	%
Cured fishes	I	2	18.2
	II	0	-
	III	6	54.5
	IV	0	-
	Mixed	3	27.3
Frozen prawns	I	0	-
	II	0	-
	III	0	-
	IV	0	-
	Mixed	1	100.00
Cooked, picked and frozen crab meat	I	0	-
	II	2	25.0
	III	5	62.5
	IV	1	12.5
	Mixed	0	-
Throats of factory workers	I	0	-
	II	8	50.0
	III	4	25.0
	IV	1	6.25
	Mixed	3	18.8
Palms of factory workers	I	0	-
	II	0	-
	III	3	75.0
	IV	0	-
	Mixed	1	25.0
Total	I	2	5.0
	II	10	25.0
	III	18	45.0
	IV	2	5.0
	Mixed	8	20.0
		40	100.00

Table 36. Antibiotic sensitivity pattern of phage-typable and non-typable strains of coagulase-positive staphylococci isolated from different sources

Antibiotics	Typable strains		Non-typable strains	
	No.	%	No.	%
Ampicillin	9	25.0	24	52.2
Erythromycin	24	66.7	34	73.9
Kanamycin	35	97.2	43	93.5
Neomycin	28	77.8	28	60.9
Penicillin	11	30.6	28	60.9
Tetracycline	19	52.8	37	80.4
Streptomycin	33	91.7	45	97.8
Chloramphenicol	36	100.00	44	95.7
Polymyxin-B	28	77.8	32	69.6

Table 37. Enterotoxigenicity of phage typable strains of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

Type of Enterotoxins produced	Typable strains (38 Nos.) No.	%
A	16	42.11
B	5	13.16
C	10	26.32
D	12	31.58
E	3	7.89
Total enterotoxigenic strains	33	86.84
Non enterotoxigenic strains	5	13.16

Table 38. Enterotoxin production pattern and phage pattern of typable strains of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers

Type of enterotoxin(s) produced	Phage groups				
	I	II	III	IV	Mixed
A	0	2	10	0	4
B	0	2	4	0	3
C	0	3	3	1	3
D	0	1	9	0	3
E	0	0	3	0	0
Total	0	8	29	1	13

CHAPTER - V

DISCUSSION

DISCUSSION

A. Total bacterial count (TBC)

In the present study total bacterial count (TBC) of fresh fishes, shell fishes, cooked shucked clams, cured fishery products and frozen fish and fishery products of marine and brackish water origin collected from the markets and cold storages situated in and around Cochin meant for local consumption were enumerated. 15.8 percent, 69.2 percent and 9.7 percent of the fresh fin fishes, cured fishery products and frozen fish and fishery products respectively had total bacterial count less than 2.0×10^5 per gram. In the case of fresh shell fishes and cooked shucked clams all the samples tested indicated bacterial count more than 2.0×10^5 per gram.

Total bacterial count of 41.8 percent of fresh fin fishes, 86.7 percent of shell fishes, 87 percent of cooked shucked clams, 4.4 percent of cured fishery products and 63.4 percent of frozen fish and fishery products were more than 1.0×10^6 per gram. Hence majority of the samples analysed had bacterial load more than what is laid down by ISI.

The results obtained in the present study showed that 12 percent, 20.0 percent, 43.5 percent and 15.1 percent of the fresh fin fishes, shell fishes, cooked shucked clams and frozen fish and fishery products respectively had total bacterial count more than 1.0×10^7 per gram. In the case of cured fishery products all the tested samples except that of two dried prawn samples had total bacterial count less than 10^7 per gram. The mean logarithmic value was found to be maximum in the case of cooked shucked clams and minimum in the case of cured fishery products (Table 1).

The bacterial population levels required to cause organoleptically evident changes vary greatly depending on the kind of food and particularly the type of microorganism (ICMSF, 1978). By the time decomposition can be detected by odour, taste or appearance, most food contain more than 10^6 microorganisms per gram (Elliott and Michener, 1961). Some foods may become unacceptable when they contain 10^7 bacteria per gram. Fish flesh containing 100 million (10^8) bacteria per gram is considered unsuitable for food (Almas, 1981). None of the fish and fishery products had TBC 10^8 or more per gram.

From Table 2 it is clear that 100 percent of kilimeen (Nemipterus japonicus), lactarius (Lactarius lactarius), seelavu (Sphyraena sp.), horse mackerel (Caranx crumenophthalmus), 87.5 percent of anchovy (Anchoviella sp.), 75.0 percent of jew fish (Pseudosciaena sp.), veloory (Kowala kowal) manangu (Thrissocles sp.), thody (Anadontostoma sp.), pomfret (Pampus sp.), perch (Epinephelus sp.), 50 percent of sole (Cynoglossus sp.), 25 percent of pearl spot (Etroplus suratensis), 23.1 percent of tilapia (Tilapia mossambica), 18.2 percent of mullet (Mugil cephalus), cat fish (Tachysurus sp.), 7.1 percent of oil sardine (Sardinella longiceps) and none of silver belly (Leiognathus sp.) samples showed total bacterial load more than 1.0×10^6 per gram. In general 41.8 percent of the fresh fin fishes of marine and brackish water origin collected from the markets situated in and around Cochin had total bacterial count more than 1.0×10^6 per gram. The mean logarithmic value was found to be maximum in the case of lactarius and minimum in the case of silver belly.

In our country Lakshmanan et al. (1984) studied the quality of fish landed at the Cochin Fisheries Harbour and found that only 8.5 percent with unacceptable quality based on TBC$>5.0 \times 10^5$ per gram (IS:4780-1978). In the present study it was revealed that 60.75 percent of the market samples contained more than 5.0×10^5 organisms per gram, so that, it is unacceptable. Studies carried out in the quality of fresh fish in retail markets of Bombay has shown that 38.3 percent of the samples were of unacceptable quality (Iyer et al., 1986). High bacterial counts in the market samples could be either due to surface contamination or because of phenominal growth of bacteria during spoilage.

TBC of fresh shell fishes were found to be higher than that of fin fishes, this may be due to the filter feeding habit of the former (Table 3). 100 percent of oyster (Crassostrea sp.), naran (Penaeus indicus), karikkady (Parapenaeopsis stylifera) and 75 percent of thelly (Metapenaeus dobsoni/M. affinis), 63.6 percent of K zanthan (Metapenaeus affinis) had TBC more than 1.0×10^6 per gram. In general 87 percent of the fresh shell fishes of marine and brackish water origin had total bacterial count more than 1.0×10^6 per gram. The mean logarithmic value of oyster was found to be maximum. TBC of prawns landed at Cochin Fisheries Harbour were in the range of 10^3 - 10^6 per gram (Lakshmanan et al., 1984), but the results of the present study is in full agreement with the results of Cann (1976) i.e. 10^4 to 10^7 per gram. No bacteriological standards has been specified for fresh shell fishes in this country.

TBC of 87 percent of cooked shucked clams collected from the markets had TBC more than 1.0×10^6 per gram (Table 4). Recently

it has been reported that 91.9 percent of the commercially frozen boiled clam meat (Villorita sp.) had TBC more than 1.0 x 10^6 per gram (Varma et al., 1988). High bacterial load is expected in clams because of its filter feeding habit. No bacteriological standard has been specified for this commodity in this country even though it is the cheapest source of animal protein.

Table 5 explains the total bacterial count and moisture content of cured fishery products collected from the markets meant for local consumption. All the cured fishery products except that of prawns had moisture content more than 30 percent. Among fin fishes moisture content was found to be maximum in silver belly followed by shark, jew fish, saurida, mackerel, mullet, pomfret, sardine and kilimeen. ISI had laid down limits for moisture content only for few species of cured fishes. The present studies have shown that none of the cured anchovy, mackerel and only 10 percent of the shark samples collected from the markets had moisture content within the limits laid down by ISI (IS:2883-1964, IS:4302-1967 and IS:5199-1969). In general only 21.4 percent of the market samples had moisture content within the limits. 30 percent and 32.43 percent of the cured fish samples collected from the markets of Andhra Pradesh and Tamil Nadu had moisture content within the limits laid down by ISI (Basu et al., 1989 and Joseph, et al., 1986). Moisture content of all dried prawns were found to be less than 20 percent. Valsan et al. (1985) studied the quality of dry non-penaeid prawns of Bombay markets and found that the moisture content varied from 14.37 percent to 24.6 percent.

TBC of dried prawns collected from Cochin markets were found to be very high i.e. 1.5 x 10^4 to 2.6 x 10^7 per gram. Studies of Valsan et al. (1985) and Basu et al. (1989) have shown that it was 9.8 x 10^3 to 7.5 x 10^5 in the samples collected from Bombay and Andhra Coast. No bacteriological standard has been specified for cured fishery product in our country.

Among cured fin fishes 14.3 percent of sole, 20 percent of anchovy and 7.7 percent of mackerel showed TBC more than 1.0 x 10^6 per gram. In general 18.1 percent of the cured fishery products contained TBC less than 1.0 x 10^4 per gram and 31.9 percent with more than 1.0 x 10^5 per gram. Among cured fin fishes maximum count was observed in sole, mackerel and anchovy i.e. 2.3 x 10^6 to 9.4 x 10^6 per gram. Joseph et al. (1986) observed the highest count of 9.5 x 10^7 per gram in silver belly sample from Nagapattinam whereas the lowest of 1.0 x 10^2 per gram in a sample of sole from Tuticorin.

Significant correlation was observed between the moisture content and TBC of shark, silver belly, manangu and sardine. TBC increased with the increase of moisutre content.

In the present study, 57.7 percent of the frozen PD prawns collected from the cold storages situated in and around Cochin meant for local consumption showed TBC more than 1.0 x 10^6 per gram (Table 6). In India Iyer (1985) observed TBC more than 1.0 x 10^6 per gram only in 22 percent of the frozen PD prawns meant for export. According to Cann (1977) 53 percent and 60 percent of raw frozen shrimps processed in Malaysia and Thailand respectively contained bacterial load more than

1.0×10^6 per gram. The studies of Sumner et al. (1982) in Sri Lanka revealed that 85 percent of frozen prawns had TBC more than 1.0×10^6 per gram. The difference in the samples meant for export and local markets are due to the difference in the level of sanitation. Stringent quality control measures are implemented for products meant for export and no quality control measures are implemented for the products meant for internal consumption.

A part of the bacterial population is indicative of the extent of spoilage of the material, the rest is due to the heavy bacterial load of water and ice used and on the surface to which, the sample comes in contact. The workers handling the material also have been a source of contamination.

Total bacterial count of frozen fin fishes were found to be lower than that of frozen prawns. The high bacterial count in frozen prawns may be due to the high content of free amino acids in prawns (Velankar, 1958).

Compared to other fish and fishery products, cooked, picked and frozen crab meat collected from local cold storages had high bacterial count. 88.9 percent of the samples contained TBC more than 1.0×10^6 per gram (Table 7). The difference in the TBC of body meat and claw meat was statistically analysed and found to be non-significant. The studies of Phillips and Peeler (1972) on the fresh crab meat industry along the Atlantic and Gulf coasts of the U.S.A. have shown that the good fish processing plants had aerobic plate count less than 1.0×10^5 per gram for all types of meats. In plants with poor sanitation the aerobic plate counts were more than 1.1×10^6 per gram.

Phillips and Peeler (1972) studied the reasons for high bacterial count in cooked and picked crab meat and found that cooking time was not sufficient to get sterile product. Since the crabs are not generally sterile, when placed in the coolers which are usually above 4.4°C, there is the possibility of bacterial growth during the overnight cooldown cycle. Puncochar and Pottinger (1954) observed that washing the hands of the women with soap and water followed by sanitizing in chlorine solution greatly reduced the microflora of the hands if done periodically throughout the day. The employees also handled many unsanitised objects during processing and continued picking or packing crab meat without washing or sanitizing their hands.

Picking and packing tables also can be a source of contamination. Puncochar and Pottinger (1954) recommended that the tables be cleaned and sanitized each time the supply of crab is exhausted. The studies of Phillips and Peeler (1972) have shown that crab meat processors can be able to produce products with good microbiological quality by applying simple rules of sanitation. Plants operating under good sanitary conditions produced crab meat with TBC less than 1.0×10^5 per gram (93 percent) and less than 5.0×10^4 per gram (85 percent). Surkiewicz et al. (1972) established that unsanitary conditions during the processing of food products are reflected in the bacteriological results of the finished products.

B. Incidence of coagulase-positive staphylococci

B1. In fish and fishery products

In the present study it is observed that all the fresh shell fish samples were free from coagulase-positive staphylococci. 4.4 percent

of fresh fin fishes, 21.7 percent of cooked shucked clams and 25.3 percent of cured fishes were found to be contaminated with coagulase-positive staphylococci. The occurrence of coagulase-positive staphylococci was maximum in frozen fish and fishery products collected from local cold storages (72 percent). In general 23.1 percent of the fish and fishery products contained coagulase-positive staphylococci (Table 8). Shewan (1962) has reviewed the work carried out by various authors on the incidence of Staph. aureus in fish and fishery products upto 1962. In his review Shewan has stated that 10.3 percent of the fish handled onboard ship, filleted onshore or purchased over counter in the fresh or frozen state contained Staph. aureus.

Out of 19 species of fresh fin fishes of marine and brackish water origin Staph. aureus was present only on anchovy (25 percent), jew fish (12.5 percent) mackerel (11.5 percent) and sardine(7.1 percent). In mackerel the load was less than 100 per gram and on anchovy and jew fish it was less than 1000 per gram (Table 9). 2.5 percent of the samples failed to satisfy the quality specifications for these materials (IS:4780-1978). The studies carried out by Lakshmanan et al. (1984) on fish samples from the Cochin fisheries harbour have shown that 7 percent of the samples failed to satisfy the quality specification. Iyer et al. (1986) studied the incidence of Staph. aureus in fresh fish in different retail markets of Bombay and observed more than 100 organisms per gram on 5 percent of the samples from 3 markets and in other the incidence varied from 0 to 3.3 percent.

All the fresh shell fish samples were found to be free from Staph. aureus (Table 10). 21.7 percent of cooked shucked clams contained

Staph. aureus more than 100 per gram (Table 11). Apparently no other work seems to have been carried out in this regard.

Staph. aureus was isolated from 26.4 percent of the cured fishery products (Table 12). In fin fishes the load was less than 1000 per gram except that of lactarius. In one prawn sample and lactarius sample the load was more than 1000 per gram. The percentage of occurrence was found to be maximum in the case of lactarius (62.5 percent) followed by shark (50 percent). In general 16.5 percent of the samples had Staph. aureus less than 100 per gram, 8.8 percent with 101-1000 per gram and 1.1 percent of the sample with more than 1000 per gram. The mean logarithmic value was found to be maximum in the case of shark followed by lactarius. The results were also statistically analysed to find out the correlation between moisture content and Staph. aureus load. The results showed that correlation was not significant between the above said two factors.

Cured fishes collected from Tamil Nadu coast were found to be free from coagulase-positive staphylococci (Joseph et al., 1986). Valsan et al. (1985) studied the quality of dry non penaeid prawns of Bombay markets and found that coagulase-positive staphylococci was absent in Palaemon tenuipes collected from three markets and was present in Acetes indicus collected from one market and the load varied from 100 to 400 per gram. The present studies have revealed that maximum number of coagulase-positive staphylococci were present in cured lactarius (5.3×10^3 per gram) followed by dried prawns (1.5×10^3 per gram) among cured fishery products. Graikoski (1973) during his studies observed that 12 percent of the dehydrated products examined on selective media contained

Staph. aureus. Apparently no other data is available on the occurrence of coagulase-positive staphylococci in cured fishery products for comparison.

Incidence of coagulase-positive staphylococci was observed in 61.5 percent, 50 percent and 18.8 percent of frozen prawns, cooked shucked and frozen clam and frozen fin fishes respectively collected from local cold storages meant for internal consumption (Table 13). 42.3 percent of frozen prawns, 33.3 percent of cooked shucked and frozen clams and none of the frozen fin fishes had Staph. aureus load more than 100 per gram. In one sample of PUD prawn the load was 1.6×10^3 per gram. Summer et al. (1982) reported that 32 percent, 35 percent and 67 percent of frozen HL, PD and cooked shrimps respectively processed in Sri Lanka showed the presence of more than 100 Staph. aureus per gram. Iyer and Shrivastava (1988) observed that Staph. aureus was present in 38 percent of the cooked frozen shrimps and 16 percent of the samples had Staph. aureus count more than 100 per gram. In the case of headless (HL) peeled and deveined, (PD) peeled and undeveined (PUD) shrimp meant for export, the incidence of the organism was 6, 12 and 16 percent respectively. Compared to frozen fish and fishery products meant for export, products meant for internal consumption had high incidence of Staph. aureus, may be due to the lack of quality inspection system for the later.

Cooked, picked and frozen crab meat had high incidence of coagulase-positive staphylococci, when compared with other fish and fishery products. All the samples contained Staph. aureus and the load varied

from 2.4 x 10^2 to 1.4 x 10^5 per gram (Table 14). 12.5 percent of the body meat samples had Staph. aureus load more than 1.0 x 10^5 per gram. Body meat samples are more subjected to human handling when compared with that of claw meat at the time of picking. International Commission on Microbiological Specification for fish and fishery products prescribes an upper limit of 10^3 cells of Staph. aureus per gram for cooked picked crab meat (Connell, 1980). In the present study it is revealed that 93.3 percent of the cooked picked and frozen crab meat meant for domestic consumption collected from local cold storages had Staph. aureus load more than 1000 per gram. This indicated that they were not of acceptable quality.

Philips and Peeler (1972) had observed absence of Staph. aureus in crabs, which were cooked either by steam or in boiling water and contained only low numbers after picking. They were left at room temperature for several hours and allowed to touch unsanitized or rusty equipments or both. The presence of large number of Staph. aureus in the final product indicated the unhygenic condition prevailing at the processing units.

Staph. aureus count (Geometric mean) was less than 10 per gram in 79 percent of regular, 69 percent of claw and 77 percent of lump meat of good plants. In plants with poor sanitation the counts were above 50 per gram in 21 percent of regular, 24 percent of claw and 15 percent of lump meats (Philips and Peeler, 1972).

Fish caught from the open sea does not contain Staph. aureus (Ridley and Slabyj, 1978). Bryan (1973), Liston (1980), Sumner et al.

(1982), Hobbs (1982) and Iyer (1985) stated that fish handlers are the main source of contamination of the processed product with Staph. aureus. Hence this organism is a useful indicator of hygiene in a process involving human handling (ICMSF, 1978; Liston, 1980 and Hobbs, 1983). The incidence of Staph. aureus has been found to be comparatively higher in cooked fishery products, evidently due to the additional human handling after cooking and the inherent behaviour of the organism to grow competitively in substrata containing very small number of competing micro-organisms (Appleman et al., 1964, Carroll et al., 1968; Neufeld, 1971).

B2. Among fish processing factory workers

Staph. aureus was isolated from the throats of 50 out of 101 workers (49.5 percent) and from the palms of 40 out of 78 workers (51.3 percent) (table 15). The carrier status of the male and female workers were statistically analysed to find out the correlation between the carrier status and sex. The chi-square test showed that there was no correlation between the sex of the workers and carriage status. It had been noted earlier by Rao (unpublished data) that 41.07 percent of the population carried Staph. aureus in their nares, while Shivananda et al. (1978) reported staphylococci from 32 percent of the human nasal swabs. These findings along with the observations made in the present study are in consonance with those of William (1963), that nasal carrier rate of pathogenic staphylococci among normal healthy individuals ranges from 30 to 50 percent.

Iyer (1985) reported the incidence of coagulase-positive staphylococci on the palms of 34 percent of the shrimp handlers while the present

studies have shown that it is 51.3 percent. In a study of process hygiene in the Sri Lanka prawn industry, Sumner et al. (1982) observed that 52 percent of the workers carried Staph. aureus on their fingers. The percentage of incidence of coagulase-positive staphylococci on the palm of the workers in the present study is in agreement with that reported in Sri Lanka prawn industry.

In the case of processed products particularly cooked, picked and frozen crab meat such handlers are likely to pose serious public health problems. It is well known that the main reservoir for Staph. aureus is man and site of multiplication is nose. Staph. aureus is present in the nasal passage and throats, on the hair and on the skin of 50 percent or more of healthy individuals (Bergdoll, 1979). These organisms multiply freely in the salt rich secretion of the human nose and exists as symbionts. Showers of droplets from the nose charge the skin and clothing as well as the air. Use of handkerchiefs and touching the nose can transmit large numbers of organisms to the hands (Stephen, 1959). According to Hobbs (1973), Gilbert and Wieneke (1973) Lee and Pfeifer (1975) Jay (1978) and Hobbs (1982) food handlers are the main source of contamination of the processed product with Staph. aureus. Therefore this organism is a useful indicator of human hygiene.

C. Incidence of Vibrio parahaemolyticus

C1. Qualitative studies in fish and fishery products

In the present study Vibrio parahaemolyticus was isolated from 66.7 percent, 51.3 percent, 39.1 percent and 2.2 percent of the fresh shell fishes, fresh fin fishes, cooked shucked clams and frozen fish and fishery

products respectively of marine and brackish water origin. All the cured fishery products were found to be free from Vibrio parahaemolyticus (Table 8).

Among fresh fin fishes of marine and brackish water origin V. parahaemolyticus was present in all the 19 species of fishes studied but it was found to be maximum in thody (75 percent) followed by cat fish (72.7 percent) and minimum in kilimeen (Table 9). In our country De et al. (1977) showed the incidence of V. parahaemolyticus in marine fishes in Calcutta to be 35.2 percent. Natarajan et al. (1979b) reported 36.8 percent occurrence in fishes from brackish water environments, Karunasagar and Mohan Kumar (1980) observed the incidence to vary from 8.3 to 33.3 percent. Nair et al. (1980) isolated V. parahaemolyticus from 35.6 and 40.6 percent of the freshly harvested fishes from estuary and mangroves respectively and from 44 percent of the market samples. It was only 6 percent and 13 percent of the fish collected from the markets in Korea and Japan respectively (Liston, 1973) the low incidence may be attributed to difference in climatic conditions.

V. parahaemolyticus was isolated from all kinds of shell fishes collected from the markets (Table 10). 100 percent of oyster, (Crassostrea sp.) 88.9 percent of crab (Scylla serrata), 83.3 percent of karikkady (P. stylifera), 75 percent of thelly (M. dobsoni/M. affinis) 72.7 percent of kazanthan (M. affinis) and 61 percent of naran (P. indicus) were found to be positive for V. parahaemolyticus. In general 78.6 percent of the fresh shell fishes of marine and brackish water origin contained

V. parahaemolyticus. Cann and his colleagues (1981) isolated V. parahaemolyticus from 70 percent and 67 percent of Malaysian shrimps collected from fishing vessels and landing places respectively. 39.1 percent of the cooked shucked clams were also found to be positive for V. parahaemolyticus (Table 11), which may be attributed to cross contamination. The Puget sound studies (Liston, 1973) indicated that the incidence of V. parahaemolyticus is highest in shell fishes, particularly oysters and clams. The results of the present study agrees with the findings of Liston (1973). Incidence of this species of Vibrio has been reported in 15 percent, 19 percent and 30 percent of raw clams, mussels and oysters respectively of Canadian Maritime Provinces (Thomson and Thacker, 1972). 2.4 percent and 8 percent of the mussels and oysters respectively in The Netherland (Kampelmacher et al., 1972). The low incidence of V. parahaemolyticus in the shell fishes of temperate region may be due to the low temperature.

The present studies have revealed that all the cured fishery products collected from the local markets meant for domestic consumption were found to be free from V. parahaemolyticus (Table 12). Venugopal et al. (1984) could isolate V. parahaemolyticus from only one sample of salted white bait. Apparently no other information is available on this aspect.

All the frozen fish and fishery products except that of cooked, picked and frozen crab meat were found to be free from V. parahaemolyticus. The same species of Vibrio was present in 4.4 percent of the cooked picked and frozen crab meat samples collected from local cold storage meant for internal consumption (Tables 13 and

14). In view of the reported heat sensitivity of V. parahaemolyticus, the recovery of the organism from cooked, picked and frozen crab meat suggests contamination of the product after processing. Studies of Iyer (1985) have revealed that V. parahaemolyticus was present in 5 percent and 3 percent of the frozen HL and PUD shrimps respectively. Vanderzant et al. (1973) could not isolate V. parahaemolyticus from frozen breaded shrimps. Cann and his colleagues (1981) found that all the frozen Malaysian shrimps meant for export were free from V. parahaemolyticus eventhough 70 percent and 67 percent of the raw material collected from fishing vessels and landing places contained this pathogen.

The incidence of V. parahaemolyticus in market samples of sea foods reflects the sensitivity of the organism to handling and processing techniques, as well as the actual occurrence in nature. Incidence of V. parahaemolyticus in our market fish has been observed as 51.3 percent. This was only about 13 percent in summer in Japan (Zen-Yoji et al., 1965) in Korea about 6 percent (Chun et al., 1967) and very much lower in Europe (Kampelmacher et al., 1970).

C2. Quantitative studies in fish and fishery products

Quantitative studies of this organism in fish and fishery products have shown that shell fishes are the major reservoir of V. parahaemolyticus. 12.5 percent of oyster and 5.9 percent of mackerel contained V. parahaemolyticus more than 10,000 MPN\gram. In general 17.4 percent of shell fishes and 5.8 percent of fin fishes had V. parahaemolyticus load 101-999 per gram and 10.9 percent of shell fishes and only 3.8 percent of fin fishes had V. parahaemolyticus load 10^3 - 10^4 MPN per gram

(Table 15). Studies carried out by Nair (1985) have shown that, among fishes, market samples examined yielded a higher frequency of isolation as compared to freshly caught estuarine and marine samples. Secondary contamination and exposure of fishes to favourable tropical ambient temperature could be the major factors contributing to the higher incidence level of V. parahaemolyticus in market samples. The present studies have revealed that the densities of V. parahaemolyticus in estuarine shell fishes was much higher as compared to shell fishes from Arabian sea. This is in agreement with the findings of Nair (1985) with respect to fishes from Bay of Bengal. Earlier investigations (Kaneko et al., 1974; Varya and Hirtle, 1975) have shown similar results and that the open sea is an inhospitable environment for V. parahaemolyticus with the high salinity.

It appears that V. parahaemolyticus occurs in counts of 100 organisms or less per gram of sea food in the majority of instances. However these results must be interpreted with serious concern since sea foods sold at the markets may take several hours before they are actually brought to the eating establishment. Sea foods in these locales might undergo some time temperature abuse resulting in proportional increase in the levels of V. parahaemolyticus. A hygienic standard of 10^4 organisms per 100 gram of fish meat has been proposed (Sakazaki et al., 1979). ICMSF (1974) has recommended an acceptability limit for V. parahaemolyticus of 10^2 organisms per gram of lobster and shrimp. The present studies show that the concentration of V. parahaemolyticus in 11.54 percent of fresh fin fishes and 32.6 percent of fresh shell fishes were above this limit.

C3. Studies on seasonal variation of V. parahaemolyticus in different parts of pelagic fishes

The studies on seasonal variation of V. parahaemolyticus on skin and muscle, gills and intestine of marine pelagic fin fishes have shown that V. parahaemolyticus was present in skin and muscle of mackerel throughout the year except the sample collected in February and June (Table 16). The load varied from 3.6 to 1.4 x 10^3 MPN per gram. Maximum load was observed in the sample collected in the month of March.

Gills of mackerel samples collected in the months of July, September and December were found to be free from V. parahaemolyticus. The load was maximum in the month of March as in the case of skin and muscle and the load varied from 62 to 2.7 x 10^5 MPN per gram.

V. parahaemolyticus was found to be absent in the intestine of the samples collected in the months of January, February, April, May, September and December. Maximum load of V. parahaemolyticus was observed in the intestine of the samples collected in the month of June followed by gills of the samples collected in the month of March.

C4. Studies on seasonal variation of V. parahaemolyticus in oyster and its environments

The present studies on seasonal variation of V. parahaemolyticus oyster (Crassostrea sp.) and its environment have shown that V. parahaemolyticus was present in oyster throughout the year except in the month of March and the load varied from 3.6 to 290 MPN per gram. None of the mud samples collected from the oyster bed in the months of

February, March and April revealed the presence of V. parahaemolyticus and maximum load was observed in the month of October i.e. 1.1 x 10^3 MPN per gram and during which time the salinity of the surrounding water was less.

V. parahaemolyticus was found to be absent in the water samples collected from the oyster beds in the months of February, March and May and maximum in the month of April i.e. 910 MPN per ml (Table 17). The results were statistically analysed to find out the correlation between the salinity and V. parahaemolyticus load on different substrata and correlation was found significant between the salinity and V. parahaemolyticus load in oyster. V. parahaemolyticus load of oyster increased with the decrease in salinity of the surrounding water.

The studies by Nair (1985) on the seasonal variation of V. parahaemolyticus in fishes have revealed lowest detection rate and counts during monsoon months (October-November) and increase in summer season (March-July).

The increased incidence or level of V. parahaemolyticus in fin fishes and shell fishes is perhaps a direct response to higher temperature as reported earlier (Thomson and Thacker, 1972 and Ayres and Burrow, 1978). Liston (1974) states that V. parahaemolyticus not only increase absolutely in numbers as ambient temperature rises, but multiplies at higher rate than other mesophilic vibrios, so that it increases disproportionately with the mesophilic population.

D. Studies on morphological, cultural and biochemical characteristics of coagulase-positive staphylococci and V. parahaemolyticus isolated from different sources

D1. Coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

All cultures (181) were found to be gram positive cocci, appearing singly, in pairs and in clusters. 92.3 percent of the 181 strains of Staph. aureus isolated from fish, fishery products and fish processing factory workers were found to be coagulase 4+ve and the rest 3+ve to 1+ve. 55.8 percent of the isolates produced white/cream pigments whereas 44.2 percent of the isolates produced golden yellow pigments. 100 percent of the isolates fermented glucose and 93.4 percent of the isolates fermented mannitol (anaerobic). 88.4 percent and 85.6 percent of the isolates fermented lactose and galactose respectively. Phosphatase production was observed in 98.9 percent of the isolates and gelatinase production in 81.8 percent of the isolates. 98.9 percent of the isolates reduced nitrate and catalase production was observed in 98.9 percent of the isolates, 87.3 and 26.5 percent of the strains were found to be positive for methyl red and Voges-proskauer test. None of the strains produced indole and oxidase. 30.9 percent of the strains were found to be positive or Type A (yellow) and 69.1 percent of the isolates were negative or Type C (violet) in crystal violet agar test. Urease was produced by 70.7 percent of the isolates and DNase by 100 percent of the strains (Table 19).

As is evident from the table the characteristic of coagulase-positive staphylococci isolated from fish, fishery products and fish processing

factory workers are in agreement with the characteristics of coagulase-positive staphylococci isolated from mastitic milk (Maria et al., 1980). 87 percent of the isolates from the above source were coagulase 4+ve, 100 percent fermented glucose, 93.4 percent of the strains fermented mannitol (anaerobic). 100 percent of the isolates produced phosphatase, gelatinase was produced by 91.3 percent of the isolates. Nitrate was reduced by 100 percent of the isolates, all strains were also found positive for thermonuclease production.

D2. V. parahaemolyticus isolated from fishes and its environments

Close morphological, cultural and physiological similarity existed between the 278 strains of V. parahaemolyticus isolated from marine and brackish water sources and the type strain (NCMB-1902/ATCC 17802). All strains proved to be gram negative, motile rods. 225 strains swarmed on 1 percent agar. Ability of some strains of V. parahaemolyticus to swarm on 1 percent agar has already been reported (Sakazaki et al., 1963). Cultural characteristics of all strains in graded temperature and salt concentration showed typical of V. parahaemolyticus except 10 strains which did not grow at 42^{o}C and 2 strains which grew at 10 percent NaCl.

Biochemically all strains isolated in the present study showed very good similarity to the characteristics of the type strain (Table 21) except the utilization of sorbitol by one strain, arabinose by 183 strains, adonitol by 5 strains, xylose by 2 strains, lactose by 7 strains, sucrose by 6 strains, cellobiose by 65 strains, dulcitol by 3 strains, salicin by 1 strain and inositol by 2 strains. Indole was not produced by 3 strains, gelatin was not liquefied by 2 strains, casein hydrolysis was not observed in 48 strains,

3 strains were found positive for Voges-Proskauer test and five strains were found negative for ornithine decarboxylase test.

All the isolates of Bartley and Slanetz (1971) from the coastal waters of new Hemisphere produced acid from sorbitol. In the present study it is found that majority of the isolated strains utilized arabinose but it was only 15 percent in the study of Sakazaki et al. (1963), 79.7 percent in the study of Twedt et al. (1969) and 100 percent in the study of Earle and Crisley (1975). 87.9 percent, 100 percent of the isolates of V. parahaemolyticus from clams were found positive in lactose, cellobiose tests respectively (Earle and Crisley, 1975). The literature contains many reports of strains that utilize sucrose (Baross and Liston, 1970, Twedt et al., 1969 and Vanderzant et al. 1973). Intraspecies variation among strains of V. parahaemolyticus were also reported by earlier investigators (Twedt et al., 1969; Zen Yoji et al., 1973). In general V. parahaemolyticus is urease negative, 8 isolated strains and type strain were found urease positive. As stated by Skerman (1967) 100 percent similarity between any 2 strains subjected to larger number of tests appears to be a rariety.

E. Studies on survival of coagulase-positive staphylococci in sterile fish and fishery products at different temperatures

At room temperature ($30\pm1^{o}C$) the coagulase-positive staphylococci multiplied rapidly in sterile shrimp homogenate within the first 3 days of storage and the count remained more or less steady for upto 60 days then started declining (Fig.3). The same trend was observed for the storage at $8^{o}C$ and $2^{o}C$, but with less magnitude. At $-20^{o}C$ the count

remained more or less steady. After 60 days of storage there was decrease in the number of viable cells in all the samples kept at different temperatures except at -20°C.

Apparently no information is available on the survival of Staph. aureus in sterile fish and fishery products at lower temperatures for comparison. Iyer (1985) studied the survival of Staph. aureus in non-sterile shrimps during freezing at -40°C and subsequent storage at -20°C and found that in raw shrimps the cells survived upto 4 months and in cooked shrimps upto 6 months. The peculiar behaviour of Staph. aureus may be due to some inherent factor influencing the viability of the organism. The rapid destruction rate of the organism during frozen storage while suspended in raw shrimps may be due to the slow enzymic action of the natural bacterial flora, active even at low temperature and the low destruction rate in sterile shrimp homogenate may be due to the absence of above said factors

F. Studies on survival of V. parahaemolyticus in sterile fishery products without sodium chloride and with 3 percent sodium chloride

The log value of V. parahaemolyticus came down from 10^6 to 10^4 within 2 days of storage in sterile crab meat (S. serrata) homogenate with 0 percent NaCl kept at -20°C but the rate was found to be very slow in the same condition with 3 percent NaCl (Fig.4). The destruction was more or less at the same rate at the 7th day of storage. V. parahaemolyticus survived in crab meat homogenate with 0 percent NaCl at -20°C for upto 16 days but with 3 percent NaCl they survived upto 30 days.

Iyer (1985) reported that 99.9 percent of V. parahaemolyticus were destroyed during freezing at -40°C and there was gradual reduction in numbers during storage at -20°C and all the cells lost viability in 7 to 9 days. Thomson and Trenholm (1971) observed that specimen of shell fishes held in deep freeze at -20°C for more than 2 weeks seldom contained V. parahaemolyticus. Vanderzant and Nickelson (1972) observed substantial reduction in the number of V. parahaemolyticus when inoculated into whole and homogenized shrimps held at -18°C, but they could isolate the organism even on the 8th day of storage. According to Thomson and Thacker (1973), V. parahaemolyticus survived only for about 2 weeks in oyster if the inoculum was less than 1.0×10^6 per ml. These results are in agreement to the results obtained in the present study. Johnson and Liston (1973) could isolate this organisms from frozen crab meat and fish fillets stored at -15°C and -30°C, after 30 days and 60 days respectively. These investigators could also isolate the organism from inoculated oysters stored at -15°C and -30°C after 130 days. Lamprecht (1980) could isolate the organism from inoculated (10^4 to 10^6 per ml) lobster tails upto atleast 3 months at -18°C. However the organism survived only upto one month if the inoculum was between 10^2 and 10^3 organisms per ml. The results of the present study obviously do not agree with those of the above two studies. The difference may be attributed to the difference in the individual strains of V. parahaemolyticus used in the experiments.

In sterile shrimp homogenate with 3 percent NaCl stored at 2°C, kanagawa-negative strains of V. parahaemolyticus survived better than

kanagawa-positive strains. For kanagawa-positive strains the rate of destruction was very high from 5th to 7th days of storage but for kanagawa-negative strains that was from 7th to 14th day of storage. Within 14 days of storage all kanagawa-positive strains of V. parahaemolyticus lost their viability but the viability of kanagawa-negative strains remained upto 28 days of storage (Fig.5).

The rate of destruction of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus were found to be more or less same in strile shrimp homogenate with 3 percent NaCl kept at -20°C. Both the strains remained viable upto 21 days of storage (Fig. 6). At -20°C the rate of destruction was found to be gradual. Apparently, no similar work seems to have been done in sterile fish substrata.

G. Studies on survival of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus in different aquatic systems

The rate of inactivation of kanagawa-negative strains of V. parahaemolyticus were found to be higher than that of kanagawa-positive strains in sterile tap water (Fig.7). The time of exposure to drinking water required to inactivate 90 percent of the organism (D_{10}) of kanagawa-positive strain was 3.4 minutes but the average value of that of kanagawa-negative strain was 1.95 minutes (Table 22). On an average within one minute 75.6 percent, in 2 minutes 86.6 percent, in 3 minutes 92.7 percent, in 4 minutes 95.4 percent, in 5 minutes 98 percent and within 15 minutes 99.9 percent of the organisms were found to be inactivated in tap water.

Studies of Lee (1972) have shown that, the time of exposure to distilled water required to inactivate 90 percent of the organism was between 0.9 and 4.4 minutes, but Takeuchi et al., (1957) reported that V. parahaemolyticus cells are killed in one minute in distilled water probably by the osmotic destruction of the cells. For this reason washing of the fish and of equipments such as containers, chopping board, kitchen knives, dishes etc., with tap water may effect some decrease in the number of viable V. parahaemolyticus cells, although it is also known that traces of salts and organic substances present in the fresh water allow their survival (Yanagisawa and Takeuchi, 1957). The longer survival of V. parahaemolyticus in tap water may be due to this effect.

In sterile normal saline kanagawa-negative strains could survive only for 12 days whereas kanagawa-positive strains survived more than 34 days at R.T. ($30\pm1^{o}C$). In 3 percent sterile NaCl solution both kanagawa-negative and kanagawa-positive st ains could survive more than 34 days but the rate of destruction was found to be more for kanagawa-negative strains (Fig.8). Karunasagar (1987) stated that survival of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus in sterile phosphate buffered saline and fresh water was very poor, most of the cells destroyed within 24 ho

In sterile brackishwater (salinity 9.96‰ kanagawa-negative strains of V. parahaemolyticus could survive only for 15 days, whereas kanagawa-positve strains survived more than 34 days (Fig. 9). Similarly in sterile sea water (salinity 28.7‰) kanagawa-negative strains of V. parahaemolyticus survived for 21 days whereas kanagawa-positive

strains survived more than 34 days. Therefore it is clear that kanagawa-positive strains of V. parahaemolyticus can survive longer period in different aquatic systems compared to kanagawa-negative strains. Karunasagar (1987) also have observed that kanagawa-positive strains of V. parahaemolyticus could survive better than kanagawa-negative strains of V. parahaemolyticus in sterile sea water at ambient temperatures. Barrow and Miller (1974) noted that in autoclaved sea water both kanagawa-positive and kanagawa-negative strains gradually diminished in number but kanagawa-negative strains survived longer.

Some workers have sought to explain the presence of kanagawa-positive strains in the patients stools and kanagawa-negative strains in the environment on a process of selection of kanagawa-negative strains in the natural environment and vice versa in the patients gut (Joseph et al., 1982). But the studies of Karunasagar (1987) have revealed that kanagawa positivity of V. parahaemolyticus is a highly stable genetic character.

H. Intercompetition

H1. Studies on competition between coagulase-positive staphylococci and other natural bacterial flora of cooked, picked crab meat

From Fig.10 it is clear that TBC of cooked, picked and frozen crab meat during defrost increased from 1.3×10^6 to 1.2×10^9 per gram within 24 hours. The Staph. aureus load increased from 6.5×10^3 to 1.0×10^6 per gram within the same condition. At 0 hours the temperature of the central point of the product was $-10^{\circ}C$ but,

it became equal to R.T. at 6 hours and later it was more than that of R.T. Apparently no similar work seems to have been done on fish substrata for comparison but Peterson et al. (1962) studied in chicken pies and observed the same trend.

Fig. 11 shows the competition for growth between coagulase-positive staphylococci and other natural bacterial flora of cooked picked crab meat at R.T. and iced condition. TBC of the product at 0 hours was 4.8×10^6 per gram. At iced condition TBC of the product remained the same, whereas in the sample kept at R.T. has gone upto 5.2×10^8 per gram within 6 hours of storage. The same trend was observed in the case of coagulase-positive staphylococci. The initial load (at 0 hours) was 5.0×10^3 per gram. At iced condition a slight reduction in Staph. aureus load was observed within 6 hours, but the load increased to 9.0×10^5 in the same sample kept at R.T.

The studies of Iyer (1985) have shown that Staph. aureus were unable to grow along with the natural bacterial flora present in shrimp homogenate. At 28°C the growth of staphylococci was inhibited in 15 to 18 hours followed by an out growth of the natural bacterial flora. It is evident from this study that proper icing could atleast check the further growth of Staph. aureus in cooked meat already contaminated with this organism.

H2. Studies on competition between V. parahaemolyticus and other natural bacterial flora of cooked, shucked clams at different temperatures

At 10°C the TBC of cooked shucked clams (Villorita sp.) increased from 3.9×10^6 to 1.8×10^8 per gram within 6 days of storage and

remained static upto 20 days of storage. V. parahaemolyticus could not be detected after 6 days of storage even /the initial load was 2.8 x 10^5 /t MPN per gram. At 0^oC the TBC increased by one log within 20 days of storage and the V. parahaemolyticus was reduced from 2.0 x 10^5 to 9.1 MPN per gram (Fig.12). This may be due to the cumulative effect of lower temperature and antagonism between V. parahaemolyticus and other bacteria. Goatcher and Westhoff (1975) reported the repression of V. parahaemolyticus by Pseudomonas sp.

At -10^oC and -20^oC reduction in TBC was observed upto 13 days of storage and a slight increase was noticed in the subsequent days of storage. V. parahaemolyticus load decreased significantly within 2 days when the sample was stored at -10^oC, the same rate of reduction was also observed in the sample kept at -20^oC between 2nd to 6th day of storage (Fig.13). No V. parahaemolyticus cells could be detected in the sample kept at -10^oC on 6th day and subsequent days of storage, but in the sample kept at -20^oC they survived upto 20 days in the presence of other bacterial flora. These studies have shown that storage at -10^oC is more lethal to V. parahaemolyticus than at -20^oC.

The sharp reduction in counts of V. parahaemolyticus in the first one day of chilling and freezing experiments in this study is in agreement with the findings of Johnson and Liston (1979) in oyster, Asakawa (1967) for the vibrios in tuna meat frozen /at -10^oC and -15^oC. Vanderzant and Nickelson (1972) reported a sharp decrease in viable cells during the first 2 days of storage at 10 and -18^oC in whole shrimp and in shrimp homogenate and Covert and Woodburn (1972) noted similar results in fish homogenate frozen /at -5 ± 1^oC and -18 ± 1^oC.

/stored

I. Studies on enterotoxigenicity of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers

Table 23 shows the enterotoxigenicity of coagulase-positive staphylococci isolated from different sources. 88.46 percent of the isolates from cured fishery products, 75.49 percent from frozen fishery products and 66.41 percent from fish processing factory workers were found to be enterotoxigenic. In general 72.27 percent of the total of 256 isolates of Staph. aureus were found to be enterotoxigenic and they produced enterotoxins A, B, C, D and E either singly or in combinations.

Table 24 narrates the distribution of enterotoxins among the 185 enterotoxigenic isolates obtained from different sources. Among the isolates from cured fishes maximum number (30.43 percent) of the enterotoxigenic strains produced enterotoxin A alone followed by AD (13.04 percent). When production of each toxin alone and in combination with others is considered as many as 16 produced 'A', 6'B', 5'C', 7'D' and 3'E'. Thus it is seen that majority (16 out of 23) of the enterotoxigenic strains of Staph. aureus isolates from cured fishery products produced enterotoxin A followed by D.

As the isolates from cured fishery products, majority of the isolates from frozen fishery products produced enterotoxin A either alone or in combination with other toxins followed by enterotoxin D. This is interesting when compared to the reported predominance of enterotoxin C producing strain in milk, meat and human carriers (Rao, 1976, 1977a, 1977b) and the reported predominance of enterotoxin 'B' producing isolates in milk

also

product Khoa (Varadaraj and Nambudripad, 1982). In this connection, it is also interesting to recall the predominance of enterotoxin D producing strain in frozen foods in USA (Casman et al., 1967) and predominance of enterotoxin A producing strains in meat products in U.K. (Simkovicova and Gilbert, 1971; Payne and Wood, 1974). It was also reported that isolates from poultry produced only enterotoxin D (Harvey et al., 1982). Enterotoxin C was produced by majority of the isolates recovered from food poisoning cases in India (Rajalakshmi and Rajyalakshmi, 1982).

As in the case of cured and frozen fishery products, the isolates from the throats and palms of fish processing factory workers produced enterotoxin A, more frequently followed by enterotoxin D and C. In all, 44 isolates were found to elaborate enterotoxin A, 34 enterotoxin D, 30 enterotoxin C, 6 enterotoxin E and 5 enterotoxin B either singly or in combinations with one or more of the other toxins. Most enterotoxigenic isolates from the apparently health population produced enterotoxin C, (Rao, unpublished data). Rajalakshmi and Rajyalakshmi (1982) reported that highest number of isolates from human patients involved in a food poisoning episode in Hyderabad produced enterotoxin C (48.4 percent) followed by A and B (12.9 percent each).

Enterotoxin A was reported to be most potent in causing food poisoning followed by D, C and B (Casman and Bennet, 1965). Food implicated in staphylococcal food poisoning may contain 0.01 mcg or less of enterotoxin per gram of food (Casman and Bennett, 1965; Bergdoll, 1972).

In general 20.5 percent of the cured fishery products, 68.19 percent of frozen fish and fishery products collected from markets and cold storages

situated in and around Cochin meant for internal consumption contained enterotoxigenic staphylococci. 97.44 percent of the Staph. aureus carriers were also found to be the carriers of enterotoxigenic strains.

Coagulase-positive staphylococci do not constitute the normal flora of fresh fish, but only get contaminated either from handlers or from the surfaces with which they come in contact.

In order to understand the role of fish handlers in the contamination of fishery products with coagulase-positive staphylococci, the available information on enterotoxin production pattern of coagulase-positive staphylococci isolated from fish handlers and fishery products needs to be considered. It is observed that maximum number of enterotoxigenic isolates from frozen fishery products, cured fishery products and fish processing factory workers elaborated enterotoxin A_1 followed by enterotoxin D. This shows the possibility that workers might have served as source of contamination for the products.

J. Studies on kanagawa-phenomenon of V. parahaemolyticus

From Table 26 it is clear that 20.56 percent of V. parahaemolyticus strains isolated from fin fishes of marine and brackish water origin were found to be kanagawa-positive. The percentage of occurrence of kanagawa-positive strains of V. parahaemolyticus were more in the intestine of fin fishes i.e. 36.84, percent whereas it was 19.05 percent in the case of gills and 16.41 percent in the case of skin and muscle.

22.9 percent of the isolates from shell fishes were found to be kanagawa-positive. The percentage occurrence of kanagawa-positive

strains were more in oyster samples i.e. 28.57 percent, whereas it was 21.57 percent from prawns, 18.18 percent from crabs. All the isolates from cooked, shucked clams were found kanagawa-negative.

The occurrence of kanagawa-positive strains of V. parahaemolyticus was maximum in mussel i.e. 50 percent and minimum in water samples (12.5 percent). In general 21.33 percent of the 225 strains of V. parahaemolyticus isolated from fin fishes, shell fishes and their environments were found to be kanagawa-positive.

Thomson and Vanderzant (1976) observed only 4 kanagawa-positive strains out of 2218 isolated from water and sediment from Galveston Bay. Ayres and Barrow (1978) found no kanagawa-positive strain out of 1484 isolates obtained from British coastal waters. Sutton (1974) recorded 2 percent kanagawa-positive strain from oysters grown in Sydney area.

Twedt et al. (1970) found higher incidence of kanagawa-positive cultures from estuarine sources of the United States (55-90 percent). In Asia Qua dri and Zuberi (1977) were the first to report a very high percentage of kanagawa-positive isolates (52.5 percent) from fish and shell fish samples from Karachi. The present findings are in agreement with that of Karunasagar and Mohankumar (1980). They observed 25 percent incidence of kanagawa-positive strains in the environment around Mangalore. Bandekar et al. (1982) observed 12 percent kanagawa-positive strains among isolates from shrimps in Bombay.

Studies of Karunasagar (1987) have shown that kanagawa-positive V. parahaemolyticus strains in the environment are not derived from fecal

to be

contamination. Contradicting reports have appeared in the literature regarding the relation between indices of pollution and levels of V. parahaemolyticus in the natural environment (Horie et al., 1967; Baross and Liston, 1970; Kaneko and Colwell, 1973; Sutton, 1974; Thomson et al., 1976). The studies of Karunasagar (1987) confirmed that even kanagawa-positive strain of V. parahaemolyticus found in the environment is not derived from fecal contamination and is probably autochthonous flora of the estuaries just as their kanagawa-negative counterparts.

K. Studies on antibiotic sensitivity of coagulase-positive staphylococci and V. parahaemolyticus isolated from different sources

K1. Coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers

All the coagulase-positive staphylococci strains isolated from cured fishery products were found to be sensitive to kanamycin and streptomycin (Table 27) and all the isolates from frozen fishery products were found to be sensitive to kanamycin, streptomycin and chloramphenicol (Table 28). None of the tested antibiotics were found to be effective on all the isolates of coagulase-positive staphylococci obtained from fish processing factory workers.

Antibiotic sensitivity patterns of coagulase-positive staphylococci isolated from different sources are plotted in Fig.13. It is evident from the figure that the isolates obtained from fish processing factory workers were less sensitive to all tested antibiotics compared to the strains obtained from dried and frozen fishery products except for neomycin. In general the isolates obtained from frozen fishery products were found

to be more sensitive than from other sources. Apparently no such studies in this aspect is reported.

Antibiotic sensitivity pattern of all the 238 strains of coagulase-positive staphylococci isolated from different sources are summerised in Table 30. 100 percent sensitivity was not observed against any of the tested antibiotics but maximum sensitivity was observed against chloramphenicol (96.22 percent) followed by kanamycin (95.80 percent) and streptomycin (93.70 percent). Sensitivity towards other antibiotics like neomycin was 76.47 percent, erythromycin was 73.53 percent, polymyxin-B was 67.23 percent, tetracycline was 66.81 percent, penicillin was 45.38 percent and ampicillin was 38.66 percent of the isolates respectively. In other words maximum resistance was observed against ampicillin (52.52 percent) followed by penicillin (49.58 percent).

The kanamycin sensitivity of staphylococci in this study is in complete agreement with the reports of James (1962) and Kapur et al., (1978). But the staphylococcal isolates from market beef showed only 46.94 percent sensitivity towards kanamycin (Nanu and Soman, 1980). Streptomycin sensitivity of Staph. aureus in this study is almost in agreement with the reports of Murty and Makholia (1963) and Rao (1966) who found it to be 89.80 percent and 86.86 percent. Chloramphenicol, ampicillin, erythromycin and penicillin sensitivity of the isolates were in agreement with the reports of Vijayalakshmi and Bhaskeran (1981), where they observed it to be 92.3 percent, 63.5 percent, 87.2 percent and 59.6 percent respectively.

The results of antibiotics sensitivity tests presented in Table 30 shows that chloramphenicol, kanamycin, streptomycin, neomycin and erytromycin are the effective antibiotics, since 70 percent or more of the strains of Staph. aureus were sensitive to these antibiotics.

Coagulase-positive staphylococci do not constitute the normal flora of fresh marine fishes. In view of the antibiotic sensitivity pattern of coagulase-positive staphylococci isolated from fishery products and fish handlers it can be assumed that fish and fishery products get contaminated by Staph. aureus from fish handlers. Enterotoxin (s) production pattern of the Staph. aureus strains isolated from fishery products and fish processing factory workers also supports the above statement.

In Poland, Kurylowiez and Slopek (1946) studied 200 strains of Staph. aureus and found only 1 percent penicillin resistant strains, but all the isolates from market beef were found resistant to penicillin (Nanu and Soman, 1980). The high incidence of antibiotic resistant strains among Staph. aureus has been attributed to the unrestricted and often unnecessary use of antibiotics. The antibiotic resistant strains of Staph. aureus in fishery products and fish processing factory workers pose a threat to public health activities and clinical practices. Hence the unrestricted and often unnecessary use of antibiotics has to be checked.

K2. Vibrio parahaemolyticus isolated from fin fishes and shell fishes of marine and brackish water origin

Sensitivity of 84 strains of V. parahaemolyticus isolated from fin fishes and shell fishes of marine and brackish water environments against

11 antibiotics are tabulated in Table 31. Maximum sensitivity was observed against chloramphenicol (98.81 percent) followed by gentamycin (97.62 percent). Sensitivity against other antibiotics like polymyxin-B was 52.38 percent, neomycin was 46.43 percent, tetracycline was 23.81 percent, sulphadiazine was 21.43 percent, ampicillin was 17.86 percent, kanamycin and streptomycin were 11.90 percent, erythromycin was 1.19 percent of the isolates. None of the V. parahaemolyticus strains were found sensitive to penicillin.

Sensitivities to antibiotics of strains isolated from human gastroenteritis were reported by Sanyal et al., (1973) and Sen et al., (1977). Pradeep and Lakshmanaperumalasamy (1985) studied the antibiotic sensitivity of V. parahaemolyticus isolated from water, sediment, plankton, fish and prawn of Cochin backwater and found that gentamycin and chloramphenicol were more effective and no strain was sensitive to penicillin. Sen et al., (1977) studied the antibiotic sensitivity of strains isolated from human gastroenteritis cases and observed the same trend. Sakazaki et al., (1963) found that strains of V. parahaemolyticus isolated in Japan were sensitive to tetracycline and chloramphenicol, but the concentrations of the antibiotic tested was much higher. Kaneko and Colwell (1978) reported sensitivity of V. parahaemolyticus, isolated from water sediment and plankton of Rhode river, to chloramphenicol and neomycin were 100 percent, streptomycin was 88 percent, polymyxin-B was 58 percent and penicillin was 8 percent of the isolates. Sensitivity of isolates of the present study was found to be less than those reported from Rhode river. Frequencies in antibiotic resistance among bacteria were reported to be dependent on the amounts and kinds of antibiotics used in that area (Colwell and Sizemore, 1974). This is evidenced

by the occurrence of more antibiotic resistant bacteria in hospital sewage (Grabow and Prozesky, 1973). Antibiotics are also used extensively as chemotherapeutic agents in fish and prawn culture system. As a result antibiotic resistant bacteria are detected in increasing numbers in fish (Aoki et al., 1974).

Antibiotic sensitivity pattern of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus against the tested antibiotics are plotted in Fig.15 . kanagawa-negative strains were found to be more sensitive towards the antibiotics than kanagawa-positive strains. Resistance of kanagawa-positive strain was much higher towards, streptomycin, sulphadiazine and tetracycline when compared with that of kanagawa-negative strain. Apparently no similar work seems to have been done on kanagawa-positive strains of V. parahaemolyticus. However Sen et al. (1977) reported that gentamycin and chloramphenicol were found to be highly sensitive towards the V. parahaemolyticus strains isolated from human gastroenteritis cases.

L. Studies on phage pattern of coagulase-positive staphylococci isolated from fishery products and fish processing factory workers

11 coagulase-positive staphylococci isolates out of 19 (57.9 percent) from cured fishery products, 9 out of 28 (32.1 percent) from frozen fishery products and 20 out of 42 (47.6 percent) from fish processing factory workers were found to be phage typable (Table 34). Majority of the typable strains belonged to the phage group III (45 percent) followed by phage group II (25 percent) IV (5 percent), I (5 percent) and rest in the mixed group (20 percent).

Among typable strains of coagulase-positive staphylococci, 54.5 percent from cured fishes, 55.5 percent from frozen fishery products and 36 percent from fish processing factory workers belonged to the phage group III. 50 percent of the typable strains isolated from the throats of fish processing factory workers belonged to the phage group II, whereas 75 percent from the palms of the workers belonged to the phage group III (Table 35). Jay (1961) reported that 71 percent of the coagulase-positive staphylococci isolated from market meats were found to be typable. The largest number of typable strains belonged to group III (55 percent) followed by mixed group. Five reports taken on random from the literature on hospital staphylococci showed an average of 53 percent for group III (Jay, 1961). Papavassiliou and Obliger (1959) found that 68 percent of 84 phage typable staphylococci recovered from milk belonged to group III, 29 percent belonged to mixed group composed of III and IV.

Phage typing has been used for strain characterization and discrimination in Staph. aureus for more than 35 years. The method has proved extremely useful for tracing the sources of outbreaks of food poisoning as it is most successfully applied to incidents of simultaneous infection in which relatively small numbers of strains were isolated and where there is less possibility of variation in the phage sensitivity of the strains.

M. Phage pattern and antibiotic sensitivity of coagulase-positive staphylococci

The results in Table 36 indicates that non - typable strains of Staph. aureus were more sensitive to ampicillin, erythromycin, penicillin,

tetracycline and streptomycin when compared to typable strains. Typable strains of Staph. aureus were more sensitive to kanamycin, neomycin, chloramphenicol and polymyxin-B when compared to non-typable strains. Wallmark and Finland (1961) found that majority of non-typable strains isolated from hospitalized patients were resistant to penicillin, streptomycin, tetracycline and erythromycin. Agarwal et al. (1963) reported that the resistant strains were more in group III than in group I.

N. Phage pattern and enterotoxigenicity of coagulase-positive staphylococci

It was observed that 86.84 percent of the typable strains of coagulase-positive staphylococci isolated from fish, fishery products and fish processing factory workers produced enterotoxin A, B, C, D and E either singly or in combinations (Table 37). 42.11 percent of the typable strains produced enterotoxin A, 13.16 percent enterotoxin B, 26.32 percent enterotoxin C, 31.58 percent enterotoxin D and 7.89 percent enterotoxin E either singly or in combinations.

Enterotoxin production pattern and phage group of typable strains of coagulase-positive staphylococci is summarised in Table 38. None of the strains belonged to phage group I produced toxin and maximum toxin was produced by strains belonged to phage group III followed by the mixed group mainly I&III. Majority of the strains produced A or both A and D. This is in agreement with the findings of Gilbert (1974). These were also the most commonly detected enterotoxins in samples of food borne out breaks (Gilbert, 1974). These results suggests a correlation between the production of enterotoxin A or A and D and sensitivity to phages of lytic

group III, which would explain the high proportion of the strains from cases of food poisoning that is sensitive to these phages.

CHAPTER - VI

SUMMARY

SUMMARY

Fish, being a highly perishable commodity, has to be handled and processed under good hygienic conditions. Microbial activity is the most important cause for quality deterioration and spoilage of fish and fishery products. In order to maintain the quality of raw material and the products, it is highly essential to keep microbial growth at the minimum. It is generally accepted that the flesh of newly caught healthy fish is sterile. Bacteria are found in variable numbers in three sites on the fish: the slime coat, the gills and the intestine. The bacteria of public health significance like Escherichia coli, faecal streptococci, coagulase-positive staphylococci, salmonella and Vibrio cholerae may contaminate fish and fishery products at different stages of handling and processing depending upon the level of hygiene and sanitation. Vibrio parahaemolyticus is present in fishes of marine and brackish water origin. The available scientific information on total bacterial count and incidence of organisms of public health significance in fish and fishery products meant for internal consumption appears to be insufficient to enable for the formulation and recommendation of quality standards for fish and fishery products in domestic trade. Therefore the present investigation was undertaken to study the total bacterial count and the incidence of organisms of public health significance mainly coagulase-positive staphylococci and V. parahaemolyticus in fish and fishery products collected from the markets and cold storages situated in and around Cochin meant for internal consumption. The isolates of Staphylococcus aureus and V. parahaemolyticus were subjected to detailed studies on their morphological, cultural and

biochemical characteristics, seasonal variations, survival, antibiotic sensitivity and their enteropathogenicity. Studies were also carried out to find out the source of contamination of fish and fishery products with Staph. aureus. The strains of Staph. aureus isolated from fishery products and fish processing factory workers were phage typed.

The results indicated that 41.8 percent of fresh fin fishes, 86.7 percent of shellfishes, 87 percent of cooked-shucked clams, 4.4 percent of cured fishery products and 63.4 percent of frozen fish and fishery products contained total bacterial count more than 1.0×10^6 per gram. Indian Standard Institution has allowed a maximum bacterial count of 1.0×10^6 per gram for peeled and deveined prawns and for other fish and fishery products it is less than 5.0×10^5 per gram; when the results obtained in these studies are compared with the ISI Standards cited above the samples fall into unacceptable quality.

TBC of fresh shell fishes were found to be higher than that of fresh fin fishes. Among the shell fishes analysed 100 percent of oyster, naran, karikkady, 75 percent of thelly and 63.3 percent of kazanthan had TBC more than 1.0×10^6 per gram.

In peeled and deveined prawns, 57.7 percent contained TBC more than 1.0×10^6 per gram. The total bacterial count in frozen fin fishes were lower than frozen prawns.

All the cured fishery products except that of prawns had moisture content more than 30 percent. Among cured fin fishes moisture content was found to be maximum in silver belly (67 percent). 54.9 percent of the samples had moisture content within the limits prescribed by ISI.

18.1 percent of the cured fishery products had TBC less than 1.0×10^4 and 31.9 percent with more than 1.0×10^5 per gram. Maximum count was observed in cured sole, mackerel and anchovy i.e. 2.3×10^6 to 9.4×10^6 per gram. Significant correlation was found between the moisture content and TBC of dried shark, silver belly, manangu and sardine.

Cooked, picked and frozen crab meat had higher TBC compared to all other fish and fishery products. 88.9 percent of the samples had TBC more than 1.0×10^6 per gram. The difference in the TBC of body meat and claw meat was found to be non-significant.

Coagulase-positive staphylococci was present in 4.4 percent of fresh fin fishes, 21.7 percent of cooked, shucked clams and 25.3 percent of cured fishery products, 72 percent of the frozen fish and fishery products and 100 percent of the cooked, picked and frozen crab meat. In general, 23 percent of 540 samples examined contained Staph. aureus. All the fresh shell fish samples were found to be free from Staph. aureus.

Among fresh fin fishes, Staph. aureus was present in anchovy, jew fish, mackerel and sardine. In mackerel the load was less than 100 per gram and in anchovy and jew fish it was less than 1000 per gram. 2.5 percent of the fresh fish samples failed to satisfy the quality specifications for these materials.

In cured fishery products the Staph. aureus load was less than 1000 per gram in all samples except that of prawns and lactarius. In one prawn sample and lactarius samples the load was more than 1000 per gram. 62.5 percent of lactarius and 50 percent of shark harboured Staph. aureus. In cured fishery products, the correlation between moisture content and Staph. aureus load was found to be non-significant.

The incidence of Staph. aureus was observed in 61.5 of frozen prawns, 50 percent of cooked shucked and frozen clams, and 18.8 percent of frozen fin fishes. Out of that 42.3 percent of frozen prawns, 33.3 percent of cooked shucked and frozen clams had Staph. aureus load more than 100 per gram.

All the samples of cooked, picked and frozen crab meat contained Staph. aureus and the count varied from 2.4×10^2 to 1.4×10^5 per gram. International Commission on Microbiological Specification for fish and fishery products prescribes an upper limit of 10^3 cells of Staph. aureus per gram of cooked, picked crab meat (Connell, 1980). The present study revealed that 93.3% of the cooked, picked and frozen crab meat analysed had Staph. aureus count more than 1000 per gram.

Staph. aureus was isolated from the throats of 49.5 percent and from the palms of 51.3 percent of the workers in the different fish processing factories. The chi-square test revealed that the correlation between the sex of the workers and Staph. aureus carrier status was non-significant.

V. parahaemolyticus was isolated from 51.3 percent of fresh fin fishes, 66.7 percent of fresh shell fishes, 39.1 percent of cooked shucked clams and 2.2 percent of frozen fish and fishery products studied. All the cured fishes were found to be free from V. parahaemolyticus.

The quantitative studies on V. parahaemolyticus have shown that shell fishes are the major reservoir of V. parahaemolyticus. 12.5% of oyster and 5.9% mackerel contained V. parahaemolyticus more than

10,000 MPN (Most probable number) per gram. The present studies have revealed that the densities of V. parahaemolyticus in estuarine shell fishes were much higher when compared to those from Arabian sea.

The studies on seasonal variation of V. parahaemolyticus on skin and muscle, gills and intestine of marine pelagic fin fishes have shown that V. parahaemolyticus was present on skin and muscle of the fish throughout the year except in the month of February and June and load varied from 3.6 to 1.4 x 10^3 MPN per gram. V. parahaemolyticus was absent in the gills of the samples collected in the months of July, September and December and the load varied from 62 to 2.7 x 10^5 MPN per gram. Maximum load of V. parahaemolyticus was observed in the intestine of the samples collected in the month of June, and absent in January, February, April, May, September and December.

V. parahaemolyticus was present in Oyster throughout the year except in the month of March and the load varied from 3.6 to 290 MPN per gram. In mud the load varied from 3 to 1.1 x 10^3 and in water from 2 to 910 MPN per gram.

Morphological, cultural and biochemical characteristics of 181 strains of coagulase-positive staphylococci isolated from fishery products and fish handlers were studied. Similarly the characteristics of 278 strains of V. parahaemolyticus isolated from fishes and their environments were studied and compared with that of type strain (NCMB-1902/ATCC 17802).

At room temperature the coagulase-positive staphylococci multiplied significantly in sterile shrimp homogenate within the first 3 days of storage and the count remained more or less steady for upto 60 days

and thereafter started declining. The same trend was observed on storage at $8^{o}C$ and $2^{o}C$ but with low magnitude. At $-20^{o}C$ the count remained more or less steady. The organism remained viable even after 8 months of storage.

The log value of V. parahaemolyticus came down from 10^{6} to 10^{4} within 2 days storage in sterile crab meat homogenate with 0 percent NaCl kept at $-20^{o}C$. The rate of destruction was found to be very slow in the same condition with 3 percent NaCl. V. parahaemolyticus survived in crab meat homogenate with 0 percent NaCl at $-20^{o}C$ for upto 16 days but with 3 percent NaCl they survived upto 30 days.

In sterile shrimp homogenate with 3 percent NaCl stored at $2^{o}C$ kanagawa-negative strains of V. parahaemolyticus survived better than its kanagawa-positive counter parts. Within 14 days of storage all kanagawa-positive strains of V. parahaemolyticus lost their viability but the viability of kanagawa-negative strains remained upto 28 days of storage. The rate of destruction of kanagawa-positive and kanagawa-negative strains of V. parahaemolyticus was found to be more or less same in sterile shrimp homogenate with 3 percent NaCl kept at $-20^{o}C$ and both the strains remained viable upto 21 days of storage.

The time of exposure to tap water required to inactivate 90 percent of the organism (D_{10}) of kanagawa-positive strains of V. parahaemolyticus was 3.4 minutes, but for kanagawa-negative strains it was 1.95 minutes. In sterile normal saline, 3 percent NaCl solution, brackish water and sea water the kanagawa-positive strains of V. parahaemolyticus could survive better than its kanagawa-negative counter parts.

Total bacterial count of cooked, picked and frozen crab meat during defrost increased from 1.3 x 10^6 to 1.2 x 10^9 per gram and Staph. aureus load increased from 6.5 x 10^3 to 1.0 x 10^6 per gram. TBC and Staph. aureus load did not increase when the sample was kept at iced condition, but at RT the TBC increased from 4.8 x 10^6 to 5.2 x 10^8 per gram and Staph. aureus load from 5.0 x 10^3 to 9.0 x 10^5 per gram.

At 10^oC the TBC of cooked, shucked clams increased from 3.9 x 10^6 to 1.8 x 10^8 per gram within 6 days of storage and remained static upto 20 days of storage. V. parahaemolyticus could not be detected after 6 days of storage even when the initial load was 2.8 x 10^5 MPN per gram. At 0^oC the TBC increased by one log within 20 days of storage and V. parahaemolyticus load reduced from 2.8 x 10^5 to 9.1 MPN per gram. At -10^oC and -20^oC reduction in TBC was observed upto 13 days of storage and a slight increase was noticed in the subsequent days of storage. V. parahaemolyticus load decreased drastically within 2 days when the sample was kept at -10^oC, the same rate of reduction was also observed in the sample kept at -20^oC between 2nd to 6th day of storage. V. parahaemolyticus cells were not detected in the sample kept at -10^oC on 6th day and subsequent days of storage, but in the sample kept at -20^oC they survived upto 20 days in the presence of other natural bacterial flora.

88.46 percent of the Staph. aureus strains isolated from cured fishery products, 75.49 percent from frozen fishery products and 66.41 percent from fish processing factory workers were found to be enterotoxigenic and they produced enterotoxins A, B, C, D and E either

singly or in combinations. Majority of the enterotoxigenic strains produced enterotoxin A followed by enterotoxin D. It is found that 20.53 percent of the cured fishery products, 68.19 percent of frozen fish and fishery products collected from the markets and cold storage situated in and around Cochin meant for internal consumption contained enterotoxigenic staphylococci. 94.44 percent of the Staph. aureus carriers were also found to be the carriers of enterotoxigenic strains.

21.33 percent of the V. parahaemolyticus strains isolated from fin fishes and shell fishes of marine and brackish water origin and their environments were found to be kanagawa-positive - thus enteropathogenic. Occurrence of kanagawa-positive strains of V. parahaemolyticus was more in the intestine of fin fishes (36.84 percent) whereas it was only 19.05 percent in the case of gills and 16.41 percent in the case of skin and muscle.

All the Staph. aureus strains isolated from cured fishery products were found to be sensitive to kanamycin and streptomycin, but all the isolates from frozen fishery products were found to be sensitive to kanamycin, streptomycin and chloramphenicol. None of the tested antibiotics were found to be sensitive to all the strains of Staph. aureus obtained from fish processing factory workers.

Kanagawa-negative strains of V. parahaemolyticus were found to be more sensitive to the tested 11 antibiotics than its kanagawa-positive counter parts. Resistance of kanagawa-positive strains of V. parahaemolyticus was much higher towards streptomycin, sulphadiazine and tetracycline when compared to kanagawa-negative strains.

57.9 percent of the Staph. aureus strains isolated from cured fishery products, 32.1 percent from frozen fishery products and 47.6 percent from the fish processing factory workers were found to be phage typable. Majority of the typable strains belonged to the phage group III (45 percent) followed by phage group II (25 percent), IV (5 percent), I (5 percent) and the mixed group (20 percent). Among typable strains of Staph. aureus 54.5 percent from cured fishes, 55.4 percent from frozen fishery products and 36 percent from fish processing factory workers belonged to the phage group III.

86.84 percent of the typable strains of Staph. aureus isolated from fish, fishery products and fish processing factory workers produced enterotoxin A, B, C, D and E either singly or in combinations. None of the strains belonging to the phage group I produced any of the enterotoxins. Maximum toxins were produced by strains belonging to the Phage group III, foliowed by mixed group, mainly I/III.

The results obtained in these investigations reveal the need for formulating quality standards with reference to Staph. aureus and V. parahaemolyticus for fish and fishery products meant for internal consumption.

REFERENCES

REFERENCES

Agarwal, D.S., Bhujwala, R.A. & Bhatia, S.L. (1963) Indian J. Med. Res. 51:851

Aiiso, K. & Fujiwara, K. (1963) Annual report of Institute of Food Microbiology, Chile University, 15: 34

Aiiso, K., Fujiwara, K., Simidu, U., Katoh, H., Hasuo, K., Otsuka, T., Araki, T. & Uchida, A. (1965) 18th Ann. Rept. Inst. Food Microbiol. Chiba Univ. 000

Allison, V.D. (1949) Proceedings of the Royal Society of the Medicine, 42:216

Almas, A. Karl (1981) Chemistry and microbiology of fish and fish processing. Dept. of Biochemistry, Norwegian Institute of Technology. p.46. University of Trondheim.

Anand, C.P. & Shetty Rudra, T.M. (1977) Fish. Technol. XIV:98

Angelotti, R., Foter, M.J. & Lowis, K.M. (1961) Appl. Microbiol. 9:308

Anon (1963) Mon. Bull. Minst. Hlth. 22:220

Anon (1964) Mon. Bull. Minst. Hlth. 23:189

Anon (1971) Morbidity and Mortality Weekly Report, Octo. 2, 20(39), 356.

Anon (1972) Isolation and identification of Vibrio parahaemolyticus. Suppl. to FDA - Bacteriological Analytical Manual, 2nd Ed. U.S. Dept. of H.E.W., Washington, D.C.

Anon (1974) International Symposium on Vibrio parahaemolyticus (Fujino, T., Sakaguchi, G., Sakazaki, R. & Takeda, Y. Eds.) Saikon Publishing Co., Tokyo.

Anon (1980) Monthly Import Detention List. Report No.80-10 Food and Drug Admn, Washington, D.C.

AOAC (1980) Official Methods of Analysis (Horwitz, W. Ed.) 13th Edn., Association of Official Analytical Chemists, Washington.

Aoki, T., Egusa, S. & Arai, T. (1974) Antimicrob. Agents Chemother, 6:534.

APHA (1960) Standard Methods for the Examination of Water and Waste Water Including Bottom Sediments and Sludges. APHA, AWWA and WPCF, 11th edn.

Appleman, M.D., Bain, N. & Shewan, J.M. (1964) J. Appl. Bact. 27:69.

Asakawa, S. (1967) J. Fac. Fish. Anim. Husb. Hiroshima, Univ. 7:113.

Ayres, P.A. & Barrow, G.I. (1978) J. Hyg. (Camb) 80:281.

Baer, E.F., Duran, A.D., Leininger, H.V., Read, R.B., Schwab, A.H. & Swartzentruber, A. (1976) Appl. Environ. Microbiol. 31:337.

Baird Parker, A.C. (1974) Ann. N.Y. Acad. Sci. 236:7.

Bandekar, J.R., Chandu, R., Nerkar, D.P. & Lewis, N.F. (1982) Occurrence of kanagawa-positive Vibrio parahaemolyticus strains in shrimps (Penaeus indicus).

Banik, A.K., Chaudhuri, D.R. and Bose, A.N. (1976) J. Fd. Sci. Technol; 13:72.

Barber, M., Hayhoe, F.G. & Whitehead, J.E.M. (1949) Lancet, 2, 1120.

Barber, L.E. & Deibel, R.H. (1972) Appl. Microbiol. 24:891.

Barker, W.H. (1974) In "International Symposium on Vibrio parahaemolyticus (Fujino, T., Sakaguchi, G., Sakazaki, R. & Takeda, Y. Eds.) p.47 Saikon publishing Co., Tokyo.

Baross, J. & Liston, J. (1970) Appl. Microbiol. 20:179.

Barrow, G.I. (1974) Postgraduate Medical Journal. 50, 612.

Barrow, G.I. & Miller, D.C. (1974) "International Symposium on Vibrio parahaemolyticus (Fujino, T., Sakaguchi, G., Sakazaki, R. & Takeda, Y. Eds.) p. 205, Saikon Publishing Co., Tokyo.

Barrow, G.I. & Miller, D.C. (1976) Microbiology in Agriculture Fisheries and Food (Skinner, F.A. & Carr, J.G. Eds.) p. 181 Academic Press, London.

Bartley, C.H. & Slanetz, L.W. (1971) Appl. Microbiol. 21:965.

Barua, D., Mithal, S. & Aikat, B.W. (1959) Bull. Inst. Post Grad. Med. Ed. and Res. Calcutta, 1, 77.

Basu Subrata, Imam Khasim, D., Gupta, S.S. & Rao, C.C.P. (1989) Fish. Technol. 26:114.

Batley, Y.M., Wallace, R.B., Allan, B.C. & Keeffe, B.M. (1970) Med. J. Aust. 1:430.

Baumgart, J. (1970) Arch. Lebensm. Hyg. 21:35.

Beaven, D.W. and Burry, A.F. (1956) Lancet, 2, 211.

Beckers, H.J., Schothorst Van, M., Spreekens, K.J.A. & Costerhuis, J.J. (1981) Food Sci. and Technol, 13:203.

Bergdoll, M.S. (1970) Fd. Technol. 23:132.

Bergdoll, M.S. (1972) The Staphylococci by Cohen, J.O. (ed.) Wiley Inter Science, New York.

Bergdoll, M.S., Pace, P.J., Krumbiegel, E.R. & Skinner, H.G. (1972) Morbid. Mortal. Weekly Rep. 21:422.

Bergdoll, M.S. (1973) The microbiological safety of food. Hobbs, B.C. & Christian, J.H.B. (Eds.) p.287 Academic Press, London.

Bergdoll, M.S. (1979) Food-Borne Infections and Intoxications. Riemann, H. and Bryan, F.L. (Eds) p. 452, 53, 57 Academic Press, New York.

Beuchat, L.R. (1975) J. Milk Food. Technol. 23:630.

Beuchat, L.R. (1977) Can. J. Microbiol. 23:630.

Beuchat, L.R. (1982) Fd. Technol. 36:80.

Bhaskaran, C.S. & Jayakar, P.A. (1969) Pathol. Microbiol. 34:98.

Bhujwala, R.A. & Mohapatra, L.N. (1972) Indian J. Pathol. Bact. 2, 66.

Breckinridge, J.C. & Bergdoll, M.S. (1971) New Engl. J. Med. 284:541.

Bryan, F.L. (1968) J. Milk. Fd. Technol. 31:110.

Bryan, F.L. (1973) Microbial safety of fishery products. Chichester, C.O. and Graham, H.D. (Eds) p. 273 Academic Press, New York.

Cann, D.C. (1974) Fishery products, Kreuzer, R. (Ed) p. 338 FAO, Fishing News (Books) Ltd., England.

Cann, D.C. (1976) Proc. Conf. Handl. Process. Mark. Trop. Fish. p. 377 Tropical products Institute, London.

Cann, D.C. (1977) Proc. Conf. Handl. Proc. Mark. Trop. Fish. Tropical Products Institute, 5-9 July.

Cann, D.C., Taylor, L.V. & Merican (1981) J. Hyg. 87:845.

Carroll, B.J., Love, T.D., Ward, B.Q. & Waters, M.E. (1968) Fishery Industrial Research, 3:5.

Carruthers, M.M. (1977) J. of Infectious Disease, 132:555

Casman, E.P. & Bennett, R.W. (1963) J. Bacteriol. 86:18.

Casman, E.P. & Bennett, R.W. (1965) Appl. Microbiol. 13:181.

Casman, E.P., Bennett, R.W., Dorsey, A.E. & Issa, J.A. (1967) J Bact. 94:1875.

Chatterjee, B.D. & Aikat, B.K. (1964) Indian. J. Med. Res. 52, 15.

Chatterjee, B.D., Gorbach, S.L. & Negoy, K.N. (1970) Bull. Wld. Hlth. Org. 42, 460.

Chatterjee, B.D. & Neogy, K.N. (1972) Bull. Calcutta. Sch. Trop. Med. 20:29.

Chatterjee, B.D. (1980) Indian J. Microbiol. 20, 179.

Chesbro, W.R. & Auborn, K. (1967) Appl. Microbiol. 15:1150.

Chun, D., Chung, J.K., Lee, J.K., Shin, D.K. & Moon, S.K. (1967) Mod. Med. (Korea) 6:105.

Chun, K.Y., Woo, M.L., Lum, L.Y. & French, G.L. (1989) J. Appl. Bact. 66, 57.

Collins, C.H. & Lyne, M.P. (1976) Microbiological Methods p. 106 Butterworths, London.

Colwell, R.R. & Sizemore, R.K. (1974) Marine Technology Society, Tenth Annual Conference Proceedings, p. 427.

Connell, J.J. & Shewan, J.M. (1980) Advance in Fish Science and Technology. Connell, J.J. (Ed) p.56 Fishing News Books Ltd., England.

Covert, D. & Woodburn, M. (1972) Appl. Microbiol. 23:321.

Dack, G.M. & Lipputz, G. (1962) Appl. Microbiol. 10:472.

De, S.P., Banerjee, M., Deb, B.C., Sengupta, P.G., Sil, J., Sircar, B.K., Sen, D., Ghosh, A. & Pal, S.C. (1977) Indian J. Med. Res. 65:21.

Digiacinto, J.V. & Frazier, W.C. (1966) Appl. Microbiol. 14:124.

Donnelly, C.B., Leslie, J.E., Black, L.A. & Lewis, K.H. (1967) Appl. Microbiol. 15:1382.

D'Souza, J.J., Collee, J.C. & Shah, J.M. (1965) Indian J. Pathol. Bact. 8:222.

Earle, M. Paul & Crisley, F.D. (1975) Appl. Microbiol. 29:635.

Elliott, R.P. & Michener, H.D. (1961) Appl. Microbiol. 9:452.

Engvall, E. & Perlmann, P. (1971) Immunochemistry, 8, 871.

Evans, J.B., Beuttner, L.G. & Niven, C.F. (1950) J. Bact. 60:481.

Frazier, W.C. (1976) Food Microbiology, p. 448 Tata Mc. Graw Hill Publishing Co. Ltd., New Delhi.

Fung, D.Y.C. (1972) J. Milk Food Technol. 35:577.

Fujino, T., Okuno, Y., Nakada, D., Aoyama, A., Fukai, K., Murai, K. & Ucho, T. (1951) J. Japan. Ass. Infect. Dis. 25, 11 (in Japanese).

Genigeorgis, C. & Sadler, W.W. (1966) J. Bacteriol. 92:1383.

Genigeorgis, C., Riemann, H. & Sadler, W.W. (1969) J. Food Sci. 34:62.

Genigeorgis, C., Foda, M.S., Mantis, A. & Sadler, W.W. (1971) Appl. Microbiol. 21:862.

Genigeorgis, C. (1974) Proc. Annu. Food Hyg. Symp. 16th Teachers Food Hyg. Coll. Vet. Med. p.34.

Ghosh Ray, B. & Paul, S.C. (1961) Ind. Jour. Microbiol., 1, 100.

Ghosh, D.N. & Chattoraj, S.B. (1963) Indian J. Publ. Hlth. 7:1

Ghosh, S.S. (1970) Ph.D. Thesis, NDRI, Karnal.

Gibbs, P.A., Patterson, J.T. & Harvey, J. (1978) J. of Appl. Bact. 44, 57.

Gilbert, R.J., Wieneke, A.A., Lanser, J. & Simkovicova, M. (1972) J. Hyg. 70:755.

Gilbert, R.J. & Wieneke, A.A. (1973) The microbiological safety of foods, Hobb, B.C. and Christian, J.H.B. (Eds), p. 273 Academic Press, New York.

Gilbert, R.J. (1974) Post Grad. Med. J. 50:603.

Gilbert, R.J. (1983) Food Microbiology Advances and Prospects, p.52 Robert, T.A. and Skinner (Eds.) Academic Press, London.

Goatcher, J. Lydia and Westhoff C. Dennis (1975) J. Food Sci. 40:533.

Goyal, R.K. and Madhavan, H.N. (1961) Antiseptic 58, 487.

Grabow, W.O.K. & Prozesky, O.W. (1973) Antimicrob. Agents. Chemother, 3:175.

Graikoski, T. John (1973) Microbial Safety of fishery products. p. 102 Academic Press, London.

Gupta, S.P. and Chakravarthi, R.N. (1954) Indian. J. Med. Res. 42, 159.

Haines, W.C. & Harmon, L.G. (1973) Appl. Microbiol. 25:436.

Hajck, V. & Marsalek, E. (1973) Zentralbl. Bakteriol. (Orig) A, 223:63.

Hallender, H.O. (1965) Acta. Pathol. Microbiol. Scand. 63:299.

Hallender, H.O. (1966) Acta. Pathol. Microbiol. Scand. 67:117.

Harvey, J., Patterson, J.T. and Gibbs, P.A. (1982) J. Appl. Bact. 52:251.

Hobbs, B.C. (1960) Roy. Soc. Hlth. J. 80:267.

Hobbs, G., Cann, D.C., Wilson, B.B. and Horsley, R.W. (1971) J. Food. Technol. 6:233.

Hobbs, B.C. (1973) The microbiological safety of foods. Hobbs, B.C. and Christian, J.H.B. (Eds) p. 129 Academic Press, London.

Hobbs, B.C. (1974) Food Manufacture, 49:29.

Hobbs, B.C. (1976) Microbiology in Agriculture Fisheries and Food. Skinner, F.A. and Carr, J.G. (Eds). p. 161 Academic Press, London.

Hobbs, G. & Hodgkiss, W. (1982) Development in Food Microbiology. Davies, R. (Ed). p.71 Applied Science Publishers Ltd., U.K.

Hobbs, G. (1982) Antonie Van Leeuwenhock. 48:619.

Hobbs, G. (1982) TD 1755, Torry Research Station, Aberdeen, Scotland.

Hodge, B.E. (1960) Public. Hlth, Rept. (U.S) 75:355.

Hooper, W.L., Barrow, G.I. & Mc. Nab, D.J.N. (1974) Lancet, 1:1100.

Horie, S., Okuzumi, M., Kato, N. & Saito, N. (1966) Bull. Jap. Soc. Sci. Fish. 32:424 (in Japanese).

Horie, S., Shaeki, K. & Okuzumi, M. (1967) Bull. Jap. Soc. Fish. 33:697.

ICMSF (International Commission on Microbiological Specification for Foods) (1974) Microorganisms in Foods 2. p. 101-102, University of Toronto Press, Toronto, London.

ICMSF (International Commission on Microbiological Specifications for Foods) (1978) Microorganisms in Foods 1. Their significance and methods of enumeration. Second Edition, University of Toronto, Press, Toronto, London.

ICMSF (1980) Microbial Ecology of Foods (Vol. I) Factors Affecting Life and Death of Microorganisms. Academic Press, New York.

IS: 3851 (1966) Specification for Dry-salted Cat fish. Indian Standards Institution, New Delhi.

IS: 5199 (1969) Specification for Dry-salted Shark. Indian Standards Institution, New Delhi.

IS: 2237 (1971) Specification for frozen prawns. Indian Standards Institution, New Delhi.

IS:4302 (1977) Specification for Dry-salted Mackerel. Indian Standards Institution, New Delhi.

IS: 2883 (1973) Specification for Dry-salted white Baits (Stolephorus sp.) (First revision). Indian Standards Institutions, New Delhi.

IS: 4780 (1978) Specification for pomfret, Fresh (First Revision) Indian Standards Institutions, New Delhi.

Iyer, K.M. and Karthiayani, T.C. (1964) Curr. Science 33 (1) 18.

Iyer, T.S.G., Chaudhuri, D.R. and Pillai, V.K. (1966a) Fish. Technol. 3, 44.

Iyer, T.S.G. and Chaudhuri, D.R. (1966b) Fish. Technol. 3:113.

Iyer, T.S.G. (1985) Ph.D. Thesis, submitted to Kerala University.

Iyer, T.S.G., Damle, S.P., Garg, D.K., Narayanan Nambiar, V. and Vasu, N.M. (1986) Fish. Technol. 23:78.

Iyer, T.S.G. & Shrivastava (1988) Fish. Technol. 25:132.

Jadhav, M.G. and Magur, N.G. (1970) Fish. Tech. VII, 86.

Jarvis, A.W. and Lawrence, R.C. (1970) Appl. Microbiol. 19, 698.

Jay, J.M. (1961) Appl. Microbiol. 9:228.

Jay, J.M. (1962) Appl. Microbiol. 10, 252.

Jay, J.M. (1970) Modern Food Microbiology. D. Van Nostrand Company, New York.

Jay, J.M. (1978) Modern Food Microbiology, 2nd Ed. D. Van Nostrand Company, New York.

Johnson, H.C. & Liston, J. (1973) J. Food Sci. 38:437.

Joseph, S.W., Colwell, R.R. & Kasper, J.B. (1982) C.R.C. Crit. Rev. Microbiol. 10:77.

Joseph George, K., Muraleedharan, V., Kalaimani, N. & Unnikrishnan Nair, T.S. (1986) Fish. Tech. 23:63.

Kallander, A. (1953) Nord. Hyg. T. 34:1.

Kampelmacher, E.V., Mossel, D.A.A., Van NoorleJansen, L.M. & Vincentile, H. (1970) J. Hyg. 168:189.

Kampelmacher, E.V., Noorle Jansen, Van, L.M., Mossel, D.A.A. & Groen, F.J. (1972) J. Appl. Bacteriol., 35:431.

Kaneko, T. & Colwell, R.R. (1973) J. Bacteriol. 113:24.

Kaneko, T. & Colwell, R.R. (1974) Appl. Microbiol. 28:1009.

Kaneko, T. & Colwell, R.R. (1978) Microbial. Ecol. 4:135.

Kapur, M.P., Singh, R.P. and Khanna, E.M. (1978) Indian Vet J. 55, 848.

Karthiayani, T.C. and Iyer, K.M. (1967) Fish. Technol. IV, 89.

Karunasagar, I. & Mohan Kumar, K.C. (1980) Indian. J. Med. Res. 72:619.

Karunasagar, I. (1987) Occurrence, Activities and Survival of Vibrio parahaemolyticus. Final Report of Research Scheme funded from U.S. held rupees.

Kato, T., Obara, Y., Ichinoe, H., Nagashima, K., Akiyama, S., Takizawa, K., Matsushima, A., Yamai, S. & Miyamoto, Y. (1965) Shokuh in Eisei Kenkya, 13:83 (in Japanese)

Katoh, H. (1965) Jap. J. Bact. 20:94.

Khot, J.B., Sherikar, A.T., Nadgauda, A.M. and Ajinkya, S.M. (1982) Proc. Symp. Harvest and Post-harvest Technology of Fish. SOFT (I), Cochin.

Koch, R. (1878) Untersuchungen Uberdie Actiologie der Wundinfection Skrankhciten, F.C.W. Vigel (Leipzig).

Kodama, T. (1967) Vibrio parahaemolyticus, 11 Fujino and Fukumi, H. (Eds) p. 351, Nayashoten, Tokyo, Japan.

Kondo, R., Yoshimura, Y., Yamaguchi, M., Tanaka, K. & Yanagisawa, F. (1960) Jap. J. Publ. Hlth. 7:752 (in Japanese).

Kraft, A.A., Oblinger, J.L., Walker, H.W., Kawal, M.C., Moon, N.J. & Reinbold, M.C. (1976) Microbiology in Agriculture, Fisheries and Food. Skinner, F.A. and Carr. J.H. (Eds). p. 141. Academic Press, London.

Kurylowiez, W. & Slopek, S. (1946) Pol. Tygodn. Lek. 1:27.

La Chapelle, N.C., Joj, E.H. & Halverson, C.W. (1966) Am. J. Public Health, 56:94.

Lakshmanan, P.T., Cyriac Mathen, Varma, P.R.G. & Iyer, T.S.G. (1984) Fish. Tech. 21:98.

Lall, R., Sen, D., Saha, M.R., Bose, A.K., De, S.P., Palchoudhury, N.C. & Pal S.C. (1979) Indian J. Med. Res. 69:17.

Lamprecht, E.C. (1980) J. Sci. Fd. Agric. 31:1309.

Lee, J.S. (1972) Appl. Microbiol. 23:166.

Lee, J.S. (1973) J. Milk Food Technol. 36:405.

Lee, J.S. & Pfeifer, D.K. (1975) Appl. Microbiol. 30:72.

Lee, J.S. & Pfeifer, D.K. (1977) Appl. Environ. Microbiol., 33, 853.

Leviene, M. (1960) An introduction to the laboratory technique in Bacteriology. The Mac millan Co., New York.

Licciardello, J.J. & Hill, W.S. (1978) J. Food Protect. 41:948.

Liston, J. and Colwell, R.R. (1963) Proc. Sym. Marine Microbiol. p.611, Charles, C. Thomas Publisher, Springfield, Illinois, U.S.A.

Liston, J., Matches, J.R., & Baross, J. (1969) Proc. of Tech. Conf. on Fish Insp. and Qual. Control. Halifax Canada 15-25 July, FAO.

Liston, J. (1973) Microbial Safety of fishery products. Chichester, C.O. and Graham, H.D. (Eds.) Academic Press, New York.

Liston, J. (1974) International Symposium on Vibrio parahaemolyticus (Fujino, T., Sakaguchi, G., Sakazaki, R. & Takeda, Y. Eds.) p. 123 Saikon Publishing Co. Ltd., Tokyo.

Liston, J. (1980) Advances in Fish Science and Technology (Ed. Connell, J.) p.151 Fishing News Books Ltd., England.

Lotter, L.P. & Leistner, L. (1978) Appl. Environ. Microbiol. 36:377.

Lovell, R.T. & Barkate, J.A. (1969) J. Fd. Sci., 34:268.

Maria Lusia Garcia, Benito Moreno & Bergdoll, M.S. (1980) Appl. and Environ. Microbiol. 39:548.

Matches, J.R. & Liston, J. (1968) J. Food Sci. 33:641.

Matches, J.R., Liston, J. & Dancault, L.P. (1971) Appl. Microbiol. 21:951.

Mathen, C., Francis Thomas, Mathew, A. & Joseph, A.C. (1975) Fish. Technol. 12, 16.

Matthias, J.Q., Shooter, R.A. & Williams, R.E.O. (1957) Lancet, 1: 1172.

Mc Lean, R.A., Lilly, D.H. & Alford, J.A. (1968) J. Bacteriol. 95:1207.

Meyer, W. (1967) Intern. J. System. Bacteriol. 17, 387.

Michener, H. & Elliott, R. (1964) Advances in Fd. Res. 13:349.

Miller, W.A. (1955) Effect of freezing ground Pork and Subsequent Storing above 32^{O}F upon bacterial flora.

Milling, M. (1971) Can. J. Public. Health. 62:382.

Minor, T.E. & Marth, E.H. (1972) Appl. Microbiol. 23:833

Miyamoto, Y., Nakamuma, K. & Takizawa, K. (1961) Jap. J. Microbiol. 5:477.

Miyamoto, Y., Kato, T., Obara, Y., Akiyama, S., Takizawa, K. & Yamai, S. (1969) J. of Bacteriol. 100:1147.

Mochmann, H., Richter, U., Karsch, W., Witte, W. & Meyer, W. (1976) Unter-Suchungen Über die Enterotoxin Bildung Von Staphylococcus aureus - Stammen Unterschiedlicher Herkumft, Zentralbl. Bakteriol. Parasitenk. Abt I, Orig. A. 234, 434.

Morris, C.A., Conway, H.D. & Everall, P.H. (1972) Lancet 11:1375.

Mossel, D.A.A. (1982) Microbiology of Foods - The ecological essentials of assurance and assessment of safety and quality. Third edition. The University of Utrecht. The Netherlands.

Muller, H., Pappel, H., Mannheim, W., Dajuni, H. & Ann, U. Van (1973) Fakale Tragerrate Und Enterotoxin buildungsvermogen Von Staphylococcus aureus beim Menschen, Zentralbl Bakteriol (Orig). A. 233:180.

Munch Peterson, E. (1960) Aust. J. Dairy Tech. 15:25.

Murty, D.K. and Makholia, B.D. (1963) Indian. Vet. J. 40, 393.

Myers, R.M. and Acharya, K. (1956) Indian. J. Med. Sci. 10, 614.

Nair, N.V., Sengupta, D.N. & Ghosh, S. (1975) Indian J. Med. Res. 63:558.

Nair, B.G., Abraham, M. & Natarajan, R. (1980) Can. J. Microbiol. 26:1264.

Nair, B.G. (1985) Indian J. Med. Res. 82:281.

Nakanishi, H. & Murase, M. (1974) International Symposium on Vibrio parahaemolyticus (Fujino, T., Sakaguchi, G., Sakazaki, R. and Takeda, Y. Eds) p.117 Saikon Publishing Co., Tokyo.

Nanu, E. & Soman, M. (1980) Kerala J. of Vet. Sci. 11:107.

Narayanan, K.G. & Sharma, V.K. (1979) J. Com. Dis. 11:112.

Natarajan, R., Nair, B.G. & Abraham, M. (1979a) Indian J. Mar. Sci. 8:166.

Natarajan, R., Abraham, M. & Nair, B.G. (1979b) Indian J. Mar. Sci. 8:286.

Neufeld, N. (1971) Proc. Technical Conference on Fish Inspection and Quality Control. FIC/69/0/26 Halifax, Canada.

Niskanen, A. & Nurmi, E. (1976) Appl. Environ. Microbiol. 31:11.

Oberhofer, T.R. & Frazier, W.C. (1961) J. Milk Fd. Technol. 24:172.

Oda, T., Ohkubo, M., Nagai, Y., Nishimoto and Ohmaru (1979) Annu. Rep. Fukuoka City Hyg. Lab. 4:33.

Ogston, A. (1881) Report upon microorganisms in surgical disease. Brit. Med. J. 1:369.

Okabe, S. (1974) Vibrio parahaemolyticus. Fujino, T., Sakaguchi G., Sakazaki, R. and Takeda, Y. (Eds) p.5 Saikon Publishing Co., Tokyo.

Olson, J.C., Jr. & Shelton, J.R. (1973) Microbial safety of fishery products. Chichester, C.O. and Graham, H.D. (Eds.) p.87 Academic Press, New York.

Olsvik, O., Berdal, B.P., Fossum, K. & Omland, T. (1981) Acta. Pathologica. Microbiologica Scandinavica. B. 89:423.

Omori, G., Kato, K. & Lida, S. (1960) Osaka City Med. J. 6:33.

Pal, S.C. and Ghosh Ray, B. (1962) J. Indian. Med. Assoc. 38, 456.

Pal, S.C. (1972) J. Com. Dis. 14:219.

Papavassiliou, J. & Obliger, G. (1959) Uber das Vorkommen und das phage nbild von koagulase-positiven staphylokokken in Milch. Zentr. Bakteriol. Parasitenk, 175:505.

Park, C. and Szabo, R. (1986) Can. J. Microbiol. 32, 723.

Pasteur, L. (1880) De l'extension dala thorie des germes a' letiologie de quelques maladies communes, C.R. Acad. Sci. (Paris) 90:1033.

Payne, D.N. & Wood, J.M. (1974) J. Appl. Bact. 37:319.

Peffers, A.S.R., Bailey, J., Barrow, G.I. and Hobbs, B.C. (1973) Lancet. 143.

Peterson, A.C., Black, J.J. & Gunderson, M.F. (1962) Appl. Microbiol. 10:16.

Phillips, F.A. & Peeler, J.T. (1972) Appl. Microbiol. 24:958.

Pillai, V.K., Lekshmy, A., Govindan, T.K., Mathen, C. and Iyer, H.K. (1965) Fish. Tech. 2, 115.

Pillai, V.K. and Rao, K. Krishna (1969) FAO Technical Conference on Fish Inspection and Quality Control, Halifax.

Pradeep, R. & Lakshmanaperumalsamy, P. (1984) Indian J. Mar. Sci. 13:113.

Pradeep, R. & Lakshmanaperumalsamy, P. (1985) Fish. Technol. 22:135.

Pullen, M.M. & Genigeorgis, C.A. (1977) J. Food. Prot. 40:704.

Puncochar, J.F. & Pottinger, S.R. (1954) A study of Sanitary requirements of handling operation and suggestions for technological improvement. US. Dept. Interior Fish and Wildlife Service, Commercial Fisheries TL 8.

Quadri, R.B. & Zuberi, R. (1977) Pakistan J. Sci. Ind. Res. 20:183.

Raj, H. & Liston, J. (1961) Fd. Technol. 15:429.

Raj, H. & Bergdoll, M.S. (1969) J. Bacteriol. 98:833.

Rajalakshmi & Rajyalakshmi, K. (1982) Indian J. Med. Res. 76:127.

Rao, C.C.P., Khera, S.S. and Sharma, G.L. (1966) Indian. Vet. J. 43:181.

Rao, C.C.P. (1976) J. Fd. Sci. Technol. 13:264.

Rao, C.C.P. (1977a) Indian J. Microbiol. 17:106.

Rao, C.C.P. (1977b) J. Fd. Sci. Technol. 14:224.

Reali, D. (1982) J. Hygiene, 88:103.

Reay, G.A. and Shewan, J.M. (1949) Adv. Fd. Res. 11:429.

Reiser, R., Conaway, D. & Bergdoll, M.S. (1974). Appl. Microbiol. 27:83.

Ridley, S.C. & Slabyj, B.M. (1978) J. Food Protect. 41:40.

Robbins, R., Gould, S. & Bergdoll, M.S. (1974) Appl. Microbiol. 28:946.

Roland, F.P. (1970) New Eng. J. Med. 282:1306.

Rosebury, T., Gale, D. & Taylor, D.F. (1954) J. Bact. 67:135.

Rosenbach, F.J. (1884) Mikroorganismen bei den Wundinfection skrankheiten des Menschen. Wiesbaden, J.F. Bergmann.

Saha, A.L. & Ganguly, N.C. (1957) Indian J. Publ. Hlth. 1:22.

Saito, M. (1967) Vibrio parahaemolyticus II. Fujino, T. and Fukumi (Eds). p.295 Nayashoten, Tokyo, Japan.

Sakazaki, R., Iwanami, S. & Fukumi, H. (1963) Jap. J. Med. Sci. Biol. 16:161.

Sakazaki, R. (1968a) Jap. J. Med. Sci. Biol. 21:359.

Sakazaki, R., Iwanami, S. & Tamura, K. (1968b) Jap. J. Med. Sci. and Biol. 21:313.

Sakazaki, R., Tamura, K., Kato, T.. Obara, Y., Yamai, S. & Hobo, K. (1968c) Jap. J. Med. Sci. and Biol. 21:325.

Sakazaki, R. (1969) Food-Borne Infections and Intoxications. Ed. Riemann, H., Academic Press, New York.

Sakazaki, R. (1973a) The Microbiological Safety of Food Eds. Hobbs, B.C. and Christian, J.H.B., Academic Press, London.

Sakazaki, R. (1973b) The Microbiological safety of food Eds. Hobbs, B.C. and Christian, J.H.B. p.375 Academic Press, London.

Sakazaki, R., Tamura, K., Nakamura, A., Kurta, T., Gohda, A. & Kazuno, Y. (1974) Jap. J. Med. Sci. and Biol. 27:35.

Sakazaki, R., Kareshimada, T., Yada, K., Sakai, S., Asakawa, Y., Yamazaki, M., Nakanishi, H., Kobayashi, T., Okazaki, H., Doke, T., Shinada, T. and Tamura, K. (1979) Archiv fur Lebensmittell hygienic, 30, 103.

Sakazaki, R. (1983) Food Microbiology. Rose, A.H. (Ed) p.225 Academic Press, London.

Sanyal, S.C., Chowdhury, M.G., Sen, P.C. & Singh, H. (1973) Indian J. Med. Res. 61:324.

Sanyal, S.C. & Sen, P.C. (1974) International Symposium on Vibrio parahaemolyticus. Fujino, T., Sakaguchi, G., Sakazaki, R. and Takeda, Y. (Eds) p.227 Saikon Publishing Co., Tokyo.

Scheusner, D.L., Hood, L.L. and Harmon, L.G. (1973) J. Milk Food. Tech 36:249.

Sen, D., De, S.P., Ghosh, S.N., Chanda, D.K., Ghosh, A. and Pal, S.C. (1977) Indian J. Med. Res. 65:628.

Shelton, L.R., Leininger, H.V., Surkiewiez, B.F., Bear, E.F., Elliott, R.P., Hyndman, J.B. & Kramer, N. (1962) A bacteriological survey of the frozen precooked food industry. Dept. Hlth. Edn. and Welfare, Food and Drug Admn. Washington D.C.

Shewan, J.M. (1949) J.R. Sanit. Inst., 69, 394.

Shewan, J.M. (1961) Fish as Food (Ed. Borgstrom, G.) Vol.I, p.487 Academic Press, New York.

Shewan, J.M. (1962) Fish as Food (Ed. Borgstrom, G.) Vol.2, p.443 Academic Press, New York.

Shewan, J.M. (1977) Handling, processing and Marketing of Tropical Fish. p.51 Trop. Prod. Inst., London.

Shingaki, M., Igarashi, H., Fujiwara, H., Ushioda, H., Trayma, T. and Sakai, S. (1981) Annu. Rep. Tokyo. Metrop. Res. Lab. Public Health. 32(1) 128.

Shivananda, P.G., Vikineswary, S. & Achyutha Rao, K.N. (1978) Indian J. Microbiol. 18:215.

Shrivastava, K.P. and Floodgate, G.D. J. Mar. Biol. Ass. India. 8, 1.

Simkovicova, M. & Gilbert, R.J. (1971) J. Med. Microbiol. 4:19.

Skerman, V.B.D. (1967) A guide to the identification of the genera of bacteria. The William and Wilkins Co., Baltimore.

Sreenivasan, R. and Joseph, K.C. (1966) Fish. Technol. 3, 103.

Stephen, E.D. (1959) Staphylococcal pyogens and its relation to disease. p.153 E and Livingston Ltd. Edinburgh.

Stojanow, I. & Meeser, G. (1970) Staphylokokken - Nahrungsmittel-Enterotoxikosen, Alimenta, 9:228.

Straka, R.P. & Combs, F.M. (1952) Fd. Res. 17: 448.

Summer, W.A., Moore, S.T., Bush, M.A., Nelson, R., Molenda, J.R., Johnson, W., Garber, H.W. & Wentz, B. (1971) Morbidity. Mortality Weekly Rept. 20:356.

Sumner, J.L., Samaraveera, I., Jayaveera, V. & Fonseka, G. (1982) J. Sci. Food Agric. 33:802.

Sundaram, S.P., Shanthakumari, S.L. and Murthy, K.V. (1982) Indian. J. Microbiol. 22, 222.

Surendran, P.K. and Iyer, K.M. (1976) Fish. Tech., XIII, 139.

Surkiewiez, B.F. (1966) Appl. Microbiol. 14:21.

Surkiewiez, B.F., Hyndmann, J.B. & Yaneey, M.V. (1967) Appl. Microbiol. 15:1.

Surkiewiez, B.F., Johnston, R.W., Elliott, R.A. and Simmons, E.R. (1972) Appl. Microbiol. 23:515.

Sutton, R.G.A. (1973) The Microbiological safety of food (Ed. Hobbs, B.C. and Christian, J.H.B.) p.153 Academic Press, London.

Sutton, R.G.A. (1974) International symposium on Vibrio parahaemolyticus. Fujino, T., Sakaguchi, R., Sakazaki, R. and Takeda, Y. (Eds.) p.83, Saikan Publishing Co., Tokyo.

Takeuchi, T., Hirose, S., Taneka, K., Yamawaki, M., Dei, S. & Sakaguchi, S. (1957) Bull. Tokyo. Med. Dent. Univ. 4:359.

Takikawa, I. (1958) Yokohama. Med. Bull. 9:313.

Task Force (1977) The Staphylococcal enterotoxin problem in fermented sausage. Rep. to U.S. Dep. Agric. Washington, D.C.

Tatini, S.R., Jezeski, J.J., Morris, H.A., Olson, J.C. & Casman, E.P. (1971) J. Dairy, Sci. 54:815.

Tatini, S.R., Wesala, W.D., Jezeski, J.J. & Morris, H.A. (1973) J. Dairy, Sci. 56:429.

Tatini, S.R. (1973) J. Milk Food Technol. 36:559.

Taylor Windle Edwin (1958) The Examination of waters and water supplies. p.104 J and A. Churchill Ltd. Gloucester Palace, W.I., London.

Tenmei, R., Yanagisawa, F., Yamaguchi, M., Tanaka, K. & Hirose, S. (1961a) Publ. Hlth. (London) 25:340 (in Japanese)

Tenmei, R., Osemura, S., Funabashi, E. & Aric, K. (1961b) Shokuhin Eisei. Kenkyu, 11:31.

Thompkin, R.B., Ambrosino, J.M. & Stozek, S.K. (1973) Appl. Microbiol. 26:833.

Thomson, W.K. & Trenholm, D.A. (1971) Can. J. Microbiol. 17:545.

Thomson, W.K. & Thacker, C.L. (1972) J. Fish. Res. Board Canada. 29:1633.

Thomson, W.K. & Pivnick, H. (1972) Canadian J. Publ. Hlth. 63:515.

Thomson, W.K. & Thacker (1973) Can. Inst. Food Sci. Technol. J. 6:156.

Thomson, C.A. Jr. & Vanderzant, C. (1976) J. Food. Sci. 41:204.

Thota, F.H., Tatini, S.R. & Bennett, R.W. (1973) Abstr. Annu. Meet. Am. Soc. Microbiol. 1.

Todd, E.C.D. (1978) J. Food Prot. 41:559.

Trivedi, B.P. and Sarkar, J.K. (1954) J. Indian. Med. Assoc. 23, 324.

Troller, J.A. (1971) Appl. Microbiol. 21:435.

Troller, J.A. (1972) Appl. Microbiol. 24:440.

Turner, F.J. and Schwartz, B.S. (1958) J. Lab. Clin. Med. 52, 888.

Twedt, R.M., Spaulding, P.L. & Hall, H.E. (1969) J. Bacteriol. 98:511.

Twedt, R.M., Ivins, B.E., Novelli, R.M.E., Thomson, R.G. & Hall, H.E. (1970) Bacterial Proc. p.6

Untermann, F. (1972) Zum Vorkommen Von Enterotoxin-bildenden Staphylokokkeen bei Mensehen, Zentralbl. Bakteriol (Orig) A. 222, 18.

U.S.D.H.E and W. (1969) Bacteriological analytical manual, Second Edition.

Valson, A.P., Nambiar, V.N., Damle, S.P., Garg, D.K., Iyer, T.S.G. & Vasu, N.M. (1985) Harvest and Post-harvest Technology of Fish. Society of Fisheries Technologists (India), Cochin - p.661.

Vandenbosch, L.L., Fung, D.Y.C. & Widomski, M. (1973) Appl. Microbiol. 25:498.

Vanderzant, C. and Nickelson, R. (1970) J. Milk Food Technol. 36:135.

Vanderzant, C., Nickelson, R. & Parker, J.C. (1970) J. Milk Food Technol. 33:161.

Vanderzant, C. & Nickelson, R. (1972) Appl. Microbiol. 23:34.

Vanderzant, C., Matihys, A.W. & Cobb III, B.F. (1973) J. Milk Food Technol. 36:253.

Van schouwenburg-Van Foeken, A.W.J., Stadhouders, J. & Witsenburg, W.W. (1979) Netherl. Milk Dairy, J. 33:49.

Varadaraj, M.C. & Nambudripad, V.K.N. (1982) J. Fd. Sci. Technol. 19:53.

Vargu, S. & Hirtle, W.A. (1975) J. Fish. Res. Board Can. 32:541.

Varma, P.R.G., Iyer, T.S.G. & Cyriac Mathen (1988) Fish. Technol. 25, 36.

Velankar, N.K. & Govindan, T.K. (1958) Proc. Indian. Aca. Sci. XLVIII, 202.

Venugopal, M.N., Indrani Karunasagar & Karunasagar, I. (1984) Journal of Food Sci. and Technol. 21:235.

Victor, C.B. & Fred a, C. (1976) Fish. Technol. 13:36.

Vijayalakshmi, P. and Bhaskaran, C.S. (1981) Indian J. Microbiol, 21, 96.

Virgilo, R., Gonzales, C., Mendoza, S., Avendano, S. & Munoz, N. (1970) J. Fd. Sci. 35:845.

Wagatsuma, S. (1968) Media Circle 13:159 (in Japanese)

Wallmark, G. & Finland, M. (1961) Jour. Amer. Med. Assoc. 175:10, 886.

Wasek, A., Chatterjee, B.D., Basu, A.K. and Aikaf, B.K. (1963) Bull. Inst. Post Grad. Med. Ed. and Res. Calcutta, 5, 12.

Weimann, O., Mollohan, C.S., Olson, R.L., Zimmer, G.A., Skinner, H.G. & Pook, L.H. (1971) Morbid. Mortal. Weekly Rep. 20:253.

William, R.E.O. (1963) Bacteriol Rev. 27:56

Wood, E.J.P. (1952) J. Gen. Microbiol. 6:205.

Wood, E.J.P. (1953) Aus. J. Mar. Fish. Res. 4, 160.

Yanagisawa, F. & Takeuchi, T. (1957) Shokuhin. Eisei Kenkya, 7:11 (in Japanese)

Zak, C., Jeljaszewics, J. and Stockmal, I. (1971) Zentralbl. Bakteriol. (orig), A, 218, 41.

Zen-Yoji, H., Sakai, S., Terayama, T., Kudo, Y., Ito, T., Benoki, M. & Nagasaki, M. (1965) J. Infec. Dis. 115:431.

Zen-Yoji, H., Sakai, S., Kudo, Y., Itoh, T. & Maruyama, T. (1970) Media circle, 15:82.

Zen-Yoji, H., Le Clair, R.A., Ohta, K. & Montague, T.S. (1973) J. Infec. Dis. 127:237.

PUBLICATIONS OF THE AUTHOR

1. Incidence and growth of coagulase-positive staphylococci in cooked, picked and frozen crab meat. Sanjeev, S., Arul James, M., and Mahadeva Iyer, K., (1985) Harvest and Post-harvest Technology of Fish, Society of Fisheries Technologists (India) p.494.

2. Antibiotic Sensitivity of Staphylococcus aureus strains isolated from fishery products. Sanjeev, S., Mahadeva Iyer, K., and Panduranga Rao, C.C. (1985) Fish. Tech. 22, 58.

3. Enterotoxigenic Staphylococci in dried fishery products from Cochin area. Sanjeev, S., Mahadeva Iyer, K., Arul James, M., and Panduranga Rao, C.C. (1985) J. Food. Sci. and Tech. 22, 295.

4. Occurrence of enterotoxigenic Staphylococci in frozen fishery products. Sanjeev, S., Mahadeva Iyer, K., Panduranga Rao, C.C. and Arul James, M. (1986) Fish. Tech. 23, 164.

5. Occurrence of Vibrio parahaemolyticus in marine and brackish waters of Cochin, Southwest coast of India. Sanjeev, S. and Mahadeva Iyer, K. (1986) I. J. Mar. Sci. 15, 189.

6. Carriage of enterotoxigenic Staphylococci in workers of fish processing factories. Sanjeev, S., Gopalakrishna Iyer, T.S., Varma, P.R.G., Panduranga Rao, C.C. and Mahadeva Iyer, K. (1987) I. J. Med. Res. 85, 262.

7. Antibiotic resistance of Staphylococcus aureus strains isolated from fish processing factory workers. Sanjeev, S. and Mahadeva Iyer, K. (1988) Fish. Tech. 25, 139.

8. Inactivation of Vibrio parahaemolyticus in drinking water. Sanjeev, S. and Mahadeva Iyer, K. (1989) Fish. Tech. 26, 149.

9. "Vibrio parahaemolyticus and Sea Foods" (Bulletin) Sanjeev, S. and Mahadeva Iyer, K. (1989).