Occurrence of Enterotoxigenic Staphylococci in Frozen Fishery Products

S. SANJEEV, K. MAHADEVA IYER, C. C. PANDURANGARAO and M. ARUL JAMES
Central Institute of Fisheries Technology, Cochin - 682 029

Sixty six samples of frozen fishery products consisting of frozen crab meat, frozen prawns, frozen cuttle fish and frozen mussel collected from nine cold stores situated in and around Cochin city were examined for coagulase positive staphylococci. Forty eight samples (72.72%) revealed the presence of coagulase positive staphylococci, which were then tested for enterotoxigenity, employing cellophane-over-agar method for toxin production and optimum-sensitivity-plate (OSP) method for toxin detection. Seventy seven out of one hundred and two (75.49%) coagulase positive staphylococcal isolates from the above products produced enterotoxins A, B, C, D and E either singly or in combinations. Enterotoxin A and D were detected more often than others. The occurrence of enterotoxigenic staphylococci in frozen fishery product is reported for the first time in this country.

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 a few reports of staphylococcal food poisoning, all 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 the one by Rajalakshmi & Rajyalakshmi (1982) enterotoxigenicity of the staphylococcal isolates was determined even though the only method of ascertaining the food-poisoning potentialities of staphylococcal isolates has been the test for enterotoxigencity.

Though a few studies have been carried out in recent years on the prevalence of enterotoxigenic staphylococci in milk, meats and in dried fishery products in this country (Ghosh, 1970; Rao, 1976, 1977a, 1977b; Sanjeev *et al.* 1985) no such information is available in respect of frozen sea foods. Hence the present study was undertaken so as to assess the potential health hazards to consumers from staphylococcal intoxication through frozen sea foods.

Materials and Methods

Sixty six samples of frozen fishery products consisting of 38 nos. of cooked, picked and frozen crab meat (body meat and claw meat) 19 nos. of frozen prawns, 6 nos. of frozen mussel and 3 nos. of frozen Cuttle fish collected from nine cold stores situated in and around Cochin were examined for coagulase-positive staphylococci. The samples were brought to the laboratory in polythene bags in ice chest packed with clean ice and analysed immediately on arrival. Staphylococcal counts were carried out by surface spreading 0.5 ml of the homogenate of the sample in sterile normal saline (after appropriately diluting the homogenate) on plates of pre-set Baird—Parker agar, by using sterile bent glass rod. Duplicate plates were used for each dilution and incubated for 48 h at 37°C. The colonies of staphylococci were identified as *Staphylococcus aureus* on the basis of colonial morphology and coagulase test (tube) using rabbit plasma (Difco). The strains were streaked on to nutrient agar slants for further studies.

Enterotoxigencity of the *S. aureus* isolates were tested by the method of Hallender (1965) as applied by Jarvis & Lawrence

(1970). Cellophane paper was cut and sterilized by the method of Robbins *et al.* (1974). The sterile cellophane circles were asceptically transfered to petri-dishes containing 20 ml of Brain Heart Infusion Agar (Difco). 0.2 ml of an 18 to 20 h old Brain Heart Infusion Broth Culture of the test organism was spread over the cellophane with a sterile cotton swab. The plates were incubated for 24 h at 37°C after which the growth was harvested from the cellophane with 2.5 ml of 0.01 M Na_2HPO_4. The cell suspensions were centrifuged at 10,000 r.p.m. for 30 min and the supernatent was analysed for toxin.

Optimum - Sensitivity - Plate (OSP) method of Robbins *et al.* (1974) was used for the detection of enterotoxin in the culture supernatent, wih the following modifications, Instead of 50 by 12 mm, plastic petri dishes, glass petri dishes of the same dimensions were used. The dishes were incubated in humidified boxes at room temperature (29 ± 1°C) for 48 h for the development of precipitation lines. The plates were read before and after flooding with 0.1 M phosphoric acid.

Results and Discussion

Results of examination of the frozen fishery products for enterotoxigenic staphylococci are presented in Table 1.

Table 1. *Incidence of enterotoxigenic staphylococci in various frozen fishery products*

Name of samples	No. of samples examined	No. of samples revealing coagulase-positive staphylococci	No. of samples revealing enterotoxigenic staphylococci
Frozen crab meat (Body)	23	23	21
Frozen crab meat (Claw)	15	15	14
Frozen prawns	19	10	10
Frozen mussel	6	0	0
Frozen cuttle fish	3	0	0

All the thirtyeight samples (100%) of frozen crab meat and 10 out of 19 frozen prawn samples employed in the present study revealed coagulase-positive staphylococci. None of the frozen mussel and frozen cuttle fish samples revealed coagulase-positive staphylococci. Thus it is seen that 48 (72.72%) out of a total of 66 frozen samples revealed coagulase-positive staphylococci, while only 23.2% of dried fishery products (Sanjeev *et al.* 1985) and varying proportions of samples of meat and milk (Ghosh, 1970; Nair, 1972; Gupta & Choudhury, 1975; Rao, 1976; 1977a, 1977b; Nanu & Soman, 1979) revealed coagulase-positive staphylococci in this country. These differences could be due to several factors including differences in the native flora of the different types of foods and the quantum and variety of environmental factors to which such foods are subjected to.

Seventy seven (75.49%) out of 102 coagulase-positive isolates in the present study were found to be enterotoxigenic. In other words 45 (68.19%) out of 66 frozen fishery products revealed eterotoxigenic staphylococci. In an earlier study on dried fishery products 23 (88.46%) out of 26 coagulase-positive staphylococcal isolates were reported to be enterotoxigenic (Sanjeev *et al.* 1985). In contrast to these observations on fishery products, lower proportions of coagulase-positive staphylococcal isolates from milk and meats in this country were found to be enterotoxigenic (Rao, 1976, 1977a. 1977b; Varadaraj & Nambudiripad, 1982). It appears from the above that coagulase-positive staphylococci from fishery products are more often enterotoxigenic and consequently more hazardous than similar isolates from milk and meats.

The distribution of enterotoxigenic staphylococci encountered in the present study by toxin pattern is presented in Table 2. Enterotoxin 'A' either alone or in combination with other toxins was produced by majority of strains (44 out of 77), followed by enterotoxin 'D' (33 out of 77). A similar trend was observed in respect of dried fishery products by Sanjeev *et al.* (1985). These results are very interesting when compared with the preponderance of enterotoxin 'C' producing straints in milk, meat and human carriers reported by Rao (1976, 1977a,

Table 2. *Distribution of enterotoxin (s) among enterotoxigenic staphylococcal isolates*

Enterotoxin (s) pattern	Positive isolates No.	%
A	20	25.97
B	4	5.19
C	7	9.10
D	12	15.58
E	1	1.30
AC	4	5.19
AD	9	11.69
AE	2	2.60
BD	1	1.30
CD	6	7.80
ABC	3	3.90
ABD	1	1.30
ACD	3	3.90
ACE	1	1.30
BCD	1	1.30
BCE	1	1.30
ABCE	1	1.30
Total	77	100.02

1977b) and enterotoxin 'B' producing strains in Khoa reported by Varadaraj & Nambudiripad (1982). Majority of *S. aureus* isolates recovered from cases of bacterial food poisoning were found to produce enterotoxin 'C' either alone or in combination with others, in a study reported by Rajalakshmi & Rajyalakshmi (1982).

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 surfaces with which they come in contact. Further studies are waranted on these aspects in order to assess their role in the contamination of fishery products with the hazardous enterotoxigenic staphylococci.

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