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ORGANISMS OF PUBLIC HEALTH SIGNIFICANCE IN FISH AND SHRIMP FROM KARACHI COASTAL WATERS – A SURVEY

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Using standard procedures, a total of 131 samples of fish and shrimp were examined for the organisms of public health significance. Samples were collected from the local retail markets, from the fish harbour, as well as from the trawlers before landing at the harbour. Samples collected from the markets appeared to be more polluted than those from the harbour. Harbour samples were also excessively polluted, if compared with those of trawler samples. 14% of the harbour samples and 5% of the trawler samples yielded *Salmonella.* All the *Salmonella* isolates were confirmed serologically as *Salmonella enteritidis* paratyphi A or B. Proposals for the possible improvement in the present situation are made.

INTRODUCTION

Pakistan exports considerable amount of 'Frozen Shrimp'. Since strict microbiological specifications have been implemented in almost all the importing countries, it is imperative to maintain sanitary conditions in the processing factories as well as during handling of shirmp before it is processed.

The joint FAO/WHO Codex Alimentarius commission [1] has outlined the general principles for the establishment and application of microbiological criteria for foods. The purpose of microbiological criteria is the protection of the consumer against health risks and to meet the requirements of international trade.

There has been substantial amount of work on organisms that are most indicative or representative of the sanitary quality of foods. A variety of different indicator organisms have been proposed and used for this purpose, [2,3]. Thus, the presence of *E. coli* has made it the classical indicator of pollution of direct or indirect faecal origin, and the corresponding potential presence of enteric pathogens [4].

In a previous study [5], the authors reported the bacterial count on fish and shirmp at the time of landing at fish harbour and at local retail markets. The objectives of the present study were to determine the organisms of public health significance in fish and shirmp landed at the harbour and those sold in various local retail markets in order to determine the percentage of landed shirmp and fish that would meet proposed quality standards and to determine the incidence of *Salmonella* in this commodity.

MATERIALS AND METHODS

Samples of fish and shrimp were collected in clean plastic bags at regular intervals from local retail markets, the fish harbour and from the trawlers before washing and landing at the harbour. The samples (1 – 3 kg), well surrounded with crushed ice were brought to the laboratory within 1 – 2 hours from collection. To determine seasonal variation in the occurrence of the organisms of public health significance, sampling for bacteriological examination was conducted from three different sources representing different handling conditions for the periods specified below:

(1). From local retail markets (March 1975 to March 1976); (2). From fish harbour (December 1976 to November 1977); (3). From trawlers before washing and landing (June 1978 to July 1979).

Samples from markets were examined for coliform and and faecal coliform MPN only while those from harbour and trawlers were examined for coliform MPN, faecal coliform MPN and *Salmonella.*

Bacteriological Examination: Total and faecal coliform most probable number (MPN) determinations were performed on each unit sample in accordance with the methods described by Thatcher and Clark [6]. Immediately on arrival, 10 g each of the samples were blended for 90 sec. in waring blender with 90 ml 0.1% sterilized peptone water and further serial dilutions were made using the same diluent.

A variety of *Salmonella* isolation procedures were used to determine the most efficient method. After comparing the efficiency of the methods tried, the following procedure was adopted for isolation of *Salmonella.* All media used

in this investigation were from E.Merck.

25 g of the homogenate was added aseptically to a flask containing 225 ml of lactose broth and incubated at 37° for 24 hr. After incubation, 50 ml of lactose broth culture was added to 50 ml double strength selenite broth and incubated at 43° for 18 hr. After shaking, the enriched broth was streaked onto the surface of brilliant green agar and bismuth sulphite agar. Brilliant green agar plates were incubated at 37° for 24 hr and bismuth sulphite agar plates for 48 hr. Typical suspected colonies (3 – 5) from each plate were isolated on nutrient agar slant. All the suspected isolates were examined for gram stain and oxidase reaction and inoculated into TSI, urea, agar and SIM medium. The cultures were subjected to further biochemical testing following American Public Health Association (APHA) diagnostic procedures, and were tested for the presence of O and H antigens by using standard serological procedures [7].

RESULTS AND DISCUSSIONS

Samples collected from market appear to be polluted at a higher level than those from the harbour, although the latter were also excessively polluted if compared with those of trawler samples. The differences in the extent of pollution are reflected by different handling conditions of the samples. It is apparent that substantial changes in pollution can occur during unhygienic handling of fish and shrimp at the harbour and markets.

Table 1 summarizes the bacteriological results of 67 samples of fish and shirmp collected from the local markets, 43 samples from the fish harbour and 21 samples from fishing trawlers. The samples neither showed any difference between the MPN values of total and faecal coliforms obtained from mackerel, pomfret or shirmp species, nor revealed any apparent seasonal variation throughout the period of investigation. The results of MPN carried out each month on different fish and shell fish species collected from market, harbour and trawler have, therefore, been averaged for the convenience of presentation. The MPN values of individual samples from the local market ranged from 200 to 1100,000 and from nil to 110,000 for total and faecal coliforms, respectively. Most of the samples were found to contain both total and faecal colifroms in excessive numbers.

Bacteriological analysis of samples of fish and shrimp collected from the fish harbour showed wide ranges of coliform and faecal coliform MPN. Total coliforms MPN values ranged from 24,000 to > 110,000 per 100 g and faecal coliform MPN values from Nil to 110,000 per 100g. *Salmonella* species were recovered from 14% of the samples examined. Total and faecal coliform MPN values for samples containing *Salmonella* ranged from 15,000 to > 110,000 per 100g and from Nil to 21,000 per 100g respectively. All the samples examined had comparatively excessive numbers of total coliforms, but no faecal coliforms could be detected in 24 out of 43 samples examined. *Salmonella* was isolated from only one sample containing no faecal colifroms.

The results of 21 samples collected directly from the fishing trawlers showed MPN values of individual samples ranging from Nil to > 110,000 and from Nil to 1,500 for total and faecal coliforms respectively. In general, the samples examined yielded a low total and faecal coliform MPN as compared with samples from harbour and markets. Many samples examined had no total or faecal coliforms. *Salmonella* species were recovered from only one sample (5%).

The comparative distribution of total and faecal coliform MPN values for samples collected from different sources is given in Table 2. Bacteriological specifications for imported fish and shrimp from different countries are not uniform and in many countries various limits have not been established. Thus, the choice of various category level is arbitary. The International Commission of Microbiological Standards for Foods (ICMFS) [8], an internationally recognized body, have established standards for certain categories of fish and its products. Some other standards have also been proposed for fish and fishery products. However, there are no statutory standards operating for any fish and fishery products anywhere in the world [9]. Shewan and Hobbs [10], suggested the following standards for fishery products:

Total viable count (at 37°)	not $> 10^5$ per g.
Presumptive coliform	< 100 per g.
Faecal coli	< 10 per g.
Salmonella absent in	50 g.
S. aureus absent in	10 g.

Pakistani exporters have to meet more or less similar specifications for their fishery products meant for export purposes. It was, therefore, of interest to compare the data obtained from the examination of fish and shrimp to these standards. If considered on the basis of presumptive coliform, 91% of the market samples, 95.2% of the harbour samples and 28.7% of the trawler samples would have been Judged unacceptable. On the basis of faecal coliform the percentage of unacceptable samples would have been 91% and 32.6% for market and harbour samples respectively. All the samples from the trawler would have been considered acceptable on MPN faecal coliform basis. Samples containing faecal coliform counts > 10,000 per 100g. were found in both market and harbour samples, but never encountered in trawler samples.

Table 1. Comparison of bacteriological analysis of market, harbour and trawler samples.

Month and year	No. of samples examined	Coliform MPN/100g (geometric mean).	Faecal coliform MPN/100g (geometric mean)	*Salmonella*
		MARKET		
March, 75	9	204,688 (6)[a]	75,966 (4)[a]	Not determined.
April, 75	6	58,000 (2)[a]	13,166	"
May, 75	9	98,255 (8)[a]	59,377 (2)[a]	"
June, 75	9	90,111 (6)[a]	81,777 (4)[a]	"
July, 75	9	126,666 (8)[a]	110,000 (9)[a]	"
August, 75	3	110,000	24,766	"
October,75	7	110,000 (7)[a]	68,285 (2)[a]	"
November 75	6	218,333 (5)[a]	32,166	"
December 75	4	725,000 (2)[a]	39,250	"
January,75	2	610,000 (1)[a]	11,000	"
March, 75	3	886,666 (2)[a]	5,500	"
		HARBOUR		
December 76	5	79,400 (2)[a]	6,260	-Ve
January 77	12	57,672 (2)[a]	583	+Ve 2
Fabruray77	6	78,000 (3)[a]	48,511 (2)[a]	+Ve 1
April, 77	2	110,000 (2)[a]	NIL	- Ve
May, 77	3	110,000 (3)[a]	8,933	+ Ve 2
June, 77	3	110,000 (3)[a]	36,600	+ Ve 1
July, 77	2	110,000	NIL	- Ve
September 77	2	110,000	350	- Ve
October 77	6	110,000 (6)[a]	NIL	-Ve
Noverm-ber 77	2	110,000 (2)[a]	NIL	-Ve
		TRAVLER		
June, 78	3	18,933	500	-Ve
September 78	2	16,000	200	+Ve 1
October 78	2	67,000	200	-Ve
November 78	4	2,075	7,500	-Ve
December 78	2	67,000	1,100	-Ve
January, 79	2	2,700	400	-Ve
February, 79	2	200	NIL	-Ve
March, 79	1	NIL	NIL	-Ve

Continued. . . . .

April, 79	1	NIL	NIL	-Ve
May, 79	1	NIL	NIL	-Ve
June, 79	1	110,000	1,500	-Ve

[a] Number in parentheses indicates the samples having MPN > 110,000 coliform or faecal coliform/100g. In these cases, the MPN value of 110,000/100g was used for purposes of calculating the geometric mean.

Table 2. Distribution of total and faecal coliform MPN values of market, harbour and trawler samples.

MPN value /100g	Percent of sample					
	Total Coliforms			Faecal coliforms		
	Market	Harbour	Trawler	Market	Harbour	Trawler
> 110,000	80.6	61.9	9.5	29.9	2.4	0
> 50,000 – 110,000	4.4	14.3	4.7	14.9	4.6	0
> 10,000 – 30,000	6.0	19.0	14.5	26.8	9.3	0
> 1,000 – 10,000	6.0	4.8	33.3	19.4	16.3	23.8
> 100 – 1,000	3.0	0.0	9.5	7.5	16.3	19.0
nil – 100	0.0	0.0	28.5	1.5	51.1	57.2

Of the 43 harbour samples examined in this study, six were found to contain confirmed *Salmonella* spp. From the trawler samples only one sample contained a confirmed *Salmonella* spp. Even though, the incidence of *Salmonella* in the samples obtained from the trawler was low, there was an approximately three fold high incidence of this pathogen in harbour samples (14%) as compared to trawler (5%). All the samples contained single serotype isolated from fish and shrimp in this study. All the isolates were confirmed as *Salmonella enteritidis,* paratyphi A or B.

A high incidence of *Salmonella* may be due to unhygienic handling of shrimp at the harbour. *Salmonella* was not isolated necessarily from the samples containing excessive faecal coliforms MPN. It must be noted that *Salmonella* was also recovered from sample containing no faecal coliforms. Thus, it is desirable to test fish and shrimp for enteric pathogens as well along with the indicator organisms. It is also worth mentioning that serologically confirmed *Salmonella* spp. were isolated when the primary nonselective enrichment step was used, samples processed simultaneously without non-selective enrichment did not yield confirmed *Salmonella* isolation. It is, therefore, important that non-selective enrichment step should be used when fish and shrimp are examined for *Salmonella.*

Salmonella do not occur normally on marine fish and shrimp caught in open sea [11], however, from the amount of data available, it is clear that fish are potential vehicle for all the most important types of bacterial food poisoning [12]. It is clear from this study that fish and shrimp harvested from our waters are generally free from pathogens or indicator organisms associated with usual bacterial indicators such as *E. coli.* During subsequent handling fish can pick up organisms such as *E. coli* [13] and even *Salmonella* [14]. The degree of contamination depends on the extent of handling and the state of hygiene during handling. This is well illustrated in this limited study.

One of the reasons of the increase in the bacteria of public health significance at the harbour is probably the use of harbour water for washing fish and in particular shrimp before landing at the harbour. It is obvious that the use of harbour water is objectionable from public health point of view. We examined harbour water samples and found faecal coliforms amounting to 100 – 1000 per ml. It is necessary that clean sea water of potable water must be used for this purpose. The increase in the bacteria in samples from the market may be attributed to the prevailing unhygienic conditions. Fish are exposed for sale in piles on dirty surfaces and are kept without ice for varying periods. Offensive odour persists in the area where fish is sold. The existing conditions could be substantially improved by applying simple sanitary measures. A high standard of cleanlines is warranted at places where fish is handled and from every one involved in this chain of handling events.

It is clear from this study that the commercial handling of fish and shrimp in the harbour and in the market results in an increase in the number of bacteria both by contamination from other sources and due to an increase in the flora already present in prevailing high temperatures. The

study also demonstrate that the handling of catch after harvest leaves much to be desired.

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