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Market fish hygiene in Kenya

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SUMMARY

Vibrio parahaemolyticus was isolated from 53 out of 584 samples (9·1 %) of market fish. All strains were Kanagawa negative and were distributed as follows: sea fish 5 out of 370 samples (1·4 %), shellfish 48 out of 214 samples (22·4 %). Other fish spoilage microflora recovered were: *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aeromonas* spp. and *Vibrio alginolyticus*. Total aerobic counts and coliform counts per gram for the lake fish ranged from $2{\cdot}6 \times 10^2$ to $6{\cdot}6 \times 10^7$ and 10 to $1{\cdot}0 \times 10^2$, respectively. Those from marine fish ranged from $1{\cdot}0 \times 10^5$ to $8{\cdot}8 \times 10^6$ and $2{\cdot}0 \times 10^3$ to $1{\cdot}6 \times 10^4$, respectively. Counts for marine fish gills alone ranged from $1{\cdot}4 \times 10^5$ to $3{\cdot}4 \times 10^8$ and $7{\cdot}2 \times 10^2$ to $1{\cdot}4 \times 10^7$, respectively. No high-temperature (44°) coliforms were recovered from either lake or marine samples.

INTRODUCTION

Fish and shellfish are an important protein supplement in many parts of the world and in some areas they are a favourite delicacy. Consumers and health workers must therefore be concerned about the hygienic quality of the marketed fish. Although most people believe that freshly caught fish are free of bacteria, Ulrich (1906) has shown the presence of bacteria in the muscles of freshly caught fish. Shewan (1949) has shown otherwise, that fish which are free of bacteria upon catching acquire bacteria due to unhygienic handling during either transportation or marketing. Trawled fish have 10 to 100 times more bacterial loads than line-caught fish (Shewan, 1949; Fujino *et al.* 1953).

Whereas spoilage bacteria render the fish unfit for human consumption, there are specific bacterial gastrointestinal pathogens which may be fishborne including *Vibrio parahaemolyticus*, *Vibrio cholera*, various salmonella and shigella species and *Clostridium botulinum*, the latter only producing an intoxication whereas the rest cause infections. Unhygienic handling and processing of fish undoubtedly introduces, in addition, secondary bacterial contaminants such as *Escherichia coli*, *Staphylococcus aureus*, *C. perfingens* and many others. Most infections follow the consumption of raw or undercooked fish and crustacean or molluscan shellfish.

V. parahaemolyticus is one of the most recent organisms to be associated with

gastrointestinal food poisoning among the specific bacterial pathogens. The first implication with food poisoning was reported in Japan in 1950 (Fujino *et al.* 1953) where, among 272 persons affected, 20 died. The significance of involvement of *V. parahaemolyticus* was not realized until Takikawa (1956) described another episode in 1956 involving 120 persons (including 47 hospital personnel and 33 in-patients). It took many years for the rest of the world to recognize and report on the occurrence of this pathogen. India (Chatterjee, Neogy & Gorbach, 1970), Netherlands (Kampelmacher *et al.* 1972), Vietnam (Neumann *et al.* 1972), Britain (Peffers *et al.* 1973) and many other countries have now recorded the occurrence of this organism.

In Africa, some authors (Bockemuhl, Amedone & Triemer, 1972; Bockemuhl & Triemer, 1974) have attributed some of the cholera-like diarrhoeal cases to *V. parahaemolyticus* and were able to recover the organism from seafoods, water and sediment from waters off the West African coast (Togo). There is no mention of this organism in the East African medical literature.

The aim of the investigation reported in this communication was to test the hypothesis that the hygiene of market fish in Kenya was within acceptable levels as measured by (i) the total aerobic and coliform counts, and (ii) the isolation of specific gastrointestinal pathogens, particularly *V. parahaemolyticus*.

MATERIALS AND METHODS

Source and types of fish

Fresh lake and marine fish were obtained from fish markets at the coast and up country. The marine fish being sold in up-country markets were caught at the coast and transported to these markets in frozen or ice-cold compartments. Some dry lake fish were also sampled. Shellfish were obtained from the same markets as enumerated above for the other fish. Fish samples were obtained by swabbing the gills, the skin and intestinal contents of the various fishes examined. Homogenates of skin, gills and intestines were also made. The fishes examined were: Bareface, Rockcod, Rainbow, Rabbit, Kingfish, Mullet and Seabream; lake fish were: Tilapia and Nile perch. The crustacean species sampled included crabs, prawns and lobsters, whereas molluscan species were oysters only.

Microbiological examination

To recover *V. parahaemolyticus*, swabs were placed in 100 ml of glucose–salt–Teepol broth (GSTB – made up of 3·0 g beef extract, 10·0 g tryptone, 50·0 g glucose, 30·0 g sodium chloride, 0·02 g methyl violet and 4·0 ml Teepol; all ingredients were dissolved in distilled water made up to one litre). The shellfish were homogenized, and a portion was inoculated into GSTB and incubated overnight at 37 °C. Loopfuls of the overnight cultures were streaked on tetrathionate citrate bile sucrose (TCBS) agar and incubated at 37 °C for 18–24 h.

Colonies on TCBS which were 2–4 mm in diameter with a dark green centre after 18–24 h incubation were picked provisionally as *V. parahaemolyticus* for further identification. On further characterization, such colonies had an alkaline slant and

acid butt on triple sugar iron (TSI), were positive for lysine decarboxylase, negative for Voges–Proskauer and grew in 8 % sodium chloride. These colonies were identified further using standard biochemical tests for *V. parahaemolyticus* (Colwell, 1970). Dr G. I. Barrow and Mr D. C. Miller of Truro Hospital, Cornwall, England and Professor R. Sakazaki kindly serotyped the isolates, the results of which appear in Table 2. Other bacteria, like *V. alginolyticus*, were isolated on TCBS agar plates as well.

Culturing for other organisms

Samples were cultured on MacConkey agar, Salmonella–Shigella agar and Bromothymol blue agar in the search for salmonellas, shigellas and *E. coli*, and marine spoilage micro-organisms.

Total aerobic plate counts

A homogenate of the whole fish, gills, intestines or a mixture of the latter two was obtained by blending 50 g of the material in 450 ml sterile physiological saline in a Waring blender. Decimal dilutions were subsequently made, after which 1 ml from each dilution was put into duplicate petri dishes correspondingly labelled. Twelve to fifteen ml of plate count agar were poured into the petri dish and the contents thoroughly mixed by rotating and tilting the dishes. The agar was then allowed to solidify, after which the petri dishes were inverted and incubated at 37 °C for 48 h. All colonies appearing on plates showing between 1 and 300 colonies were counted and the average counts per dilution were recorded. Subsequently, geometric mean counts were calculated for each sample and reported as aerobic plate counts per g fish material.

Coliform count

Duplicate violet–red–bile–dextrose (VRBD) agar plates were inoculated with 1 ml of the decimal dilutions 10^{-1} to 10^{-6} made for the aerobic plate count above. One set of plates was incubated at 37 °C for 24 h while the other set was incubated at 44 °C for 24 h in order to determine presence of *E. coli*. Coliform colonies were counted and the number recorded. Calculations were further done as for the total plate counts.

RESULTS

No salmonella, shigella or *V. cholerae* was isolated. All samples yielded *V. alginolyticus*; spoilage bacteria isolated were *Proteus vulgaris*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa* and *Aeromonas* species. Most of these organisms contributed to the high total aerobic plate counts. Table 1 shows the incidence of *V. parahaemolyticus* among the sea fish and the different shellfish. Most isolates came from shellfish. Thirty-one of the 53 strains of *V. parahaemolyticus* isolated were serotyped. Fifteen strains isolated from prawns, lobsters and sea fish were type O3:K37; 10 strains isolated from prawns, lobsters, crabs, oysters and sea fish

Table 1. *Incidence of* V. parahaemolyticus *from sea fish and various shellfish sold in open fish markets in Kenya*

Type of sample	Total number	Number positive (%)
Sea fish	370	5 (1·4)
Prawns	130	29 (22·3)
Lobsters	44	13 (29·5)
Crabs	20	5 (25·0)
Oysters	20	1 (5·0)

Table 2. *Incidence of serotypes of* V. parahaemolyticus *from sea foods collected in Kenya*

Sea foods sampled	Serotype	Number of strains serotyped
Prawns	O3:K37	8
	O3:K4	4
	O11:K40	2
	O8:K39	2
Lobsters	O3:K37	6
	O11:K40	1
Oysters	O11:K40	1
Crabs	O11:K40	3
Sea fish	O11:K40	3
	O3:K37	1

Table 3. *Total aerobic plate count and coliform count per g tissue as indices of the hygienic quality of dry lake fish sold in Nairobi fish markets, Kenya*

Sample number	Total aerobic plate count per g	Coliform counts per g (37 °C)
1	$1{\cdot}5 \times 10^{1}$	—*
2	$1{\cdot}0 \times 10^{5}$	—
3	$7{\cdot}0 \times 10^{3}$	—
4	$2{\cdot}0 \times 10^{6}$	—
5	$2{\cdot}6 \times 10^{3}$	—
6	$5{\cdot}0 \times 10^{5}$	—
7	$6{\cdot}0 \times 10^{6}$	$1{\cdot}0 \times 10^{1}$
8	$6{\cdot}0 \times 10^{7}$	$1{\cdot}0 \times 10^{2}$
9	$6{\cdot}7 \times 10^{6}$	$2{\cdot}0 \times 10^{1}$
10	$1{\cdot}5 \times 10^{7}$	$2{\cdot}0 \times 10^{1}$
11	$1{\cdot}0 \times 10^{5}$	$4{\cdot}0 \times 10^{1}$

* Indicates absence of coliform colonies on the VRBD agar plates.

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Table 4. *Total aerobic plate counts and coliform counts per g fresh marine fish tissue as a hygienic index of Kenya market fish*

Type of fish	Total aerobic count/g	Coliform/g (37 °C)
Rockcod	$8{\cdot}8 \times 10^6$	$1{\cdot}6 \times 10^4$
Mullut	$1{\cdot}4 \times 10^5$	—*
Kingfish	$3{\cdot}8 \times 10^5$	$3{\cdot}6 \times 10^3$
Rainbow	$1{\cdot}3 \times 10^6$	$2{\cdot}0 \times 10^3$
Seabream	$2{\cdot}6 \times 10^6$	$2{\cdot}0 \times 10^3$
Changu	$5{\cdot}9 \times 10^5$	$4{\cdot}9 \times 10^3$

* No coliform colony was observed on VRBD agar plates.

Table 5. *Counts per g gill tissue as a hygienic index for fresh marine fish sold in Kenya fish markets*

Type of fish	Total aerobic plate counts	Coliform counts
Rockcod	$3{\cdot}0 \times 10^7$	$7{\cdot}9 \times 10^3$
Mullet	$2{\cdot}6 \times 10^7$	$1{\cdot}6 \times 10^4$
Kingfish	$1{\cdot}8 \times 10^7$	$1{\cdot}2 \times 10^4$
Rainbow	$2{\cdot}5 \times 10^6$	$2{\cdot}5 \times 10^3$
Seabream	$3{\cdot}4 \times 10^5$	$4{\cdot}6 \times 10^3$
Changu	$1{\cdot}9 \times 10^7$	$4{\cdot}0 \times 10^5$

were type O11 : K40 four strains of O3 : K4 and two strains of O8 : K39 were isolated only from prawns (Table 2).

Six of the eleven dried fresh-water fish samples examined did not show any coliforms, whereas all samples had total plate counts ranging from $1{\cdot}5 \times 10^1$ to $6{\cdot}0 \times 10^7$ per gram. Fresh fish had higher coliform counts than dry fish (Tables 3, 4 and 5).

DISCUSSION

V. parahaemolyticus was isolated from 47 out of 194 (24·2 %) of samples examined from crustaceans (crabs, prawns, lobsters); from one out of 20 (20 %) of oysters, but from only 5 out of 370 samples (1·4 %) of sea fish examined (Table 1). The high incidence in crustaceans is explicable in that crabs, prawns and lobsters live and feed mainly on the seabed and are thus exposed to contamination from organisms contained in bottom deposits. Oysters are immobile and feed by filtering organisms from the sea water, and sea fish are mobile and only exposed to organisms suspended in sea water. In general, the results show that shellfish (crustaceans and molluscs) present a greater hazard to health than sea fish in Nairobi. These findings are in sharp contrast to those of Kampelmacher *et al.* (1972), who isolated *V. parahaemolyticus* from only 1 out of 407 samples (0·25 %) of fish and shellfish

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from the North Sea. As to the hazard to health from sea fish, Clark (1977) expressed the opinion that a contamination rate of 1·4% would not present a major health hazard.

From the results it is not clear whether drying contributed to the absence of coliforms although it appears to result in a reduced coliform count (Tables 3 and 4), Total aerobic counts were higher for gills than for a whole fish (Table 5) probably because gills act as a filter. However, more important are the high counts for both total and coliform counts which indicate gross contamination and previous encounter with faecal material. Although bacteriological standards for foods for human consumption are in the making in Kenya, these levels are definitely high.

In the light of the foregoing, it is felt that consumption of raw or undercooked fish, shellfish or their products poses a potential health hazard to consumers. Proper refrigeration during transport and proper subsequent good hygiene during marketing would ensure protection to consumers.

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