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Occurrence of bacterial pollution indicators in Boulti (*Tilapia nilotica* Linn.) fish

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It has been well established that the coliform test is one of the most valuable aids for estimating the sanitary quality of water. Many workers have reported that the coliform index is highly variable and often fails to detect faecal pollution of such foods (Raj et al., 1961).

As coliforms constitute a group of organisms of diverse origin, they do not necessarily represent faecal organisms in human or animal gut. In view of this, *E. coli* type I of all the coliforms has recently received much attention as a singular representative of faecal pollution. However, the reliability of faecal *E. coli* itself as a true pollution indicator organism has been detected by many investigators (Buttiaux, 1958, and Raj et al., 1961).

Fish have been considered a conveyor of microorganisms (Potter and Baker, 1961) and may therefore influence the coliform index of their habitat. This influence could be of significant magnitude, if multiplication of *E. coli* occurs in the fish intestine as suggested by Johnson (1904), and if the organisms are retained in the fish for any length of time. The prevalence of coliform organisms has been reported to increase when fish inhabited polluted water (Guelin, 1954, and Huggins and Rast, 1963). This observation is supported by studies in which coliforms were found in marine fish taken from polluted coastal waters, but were not detected in fish taken offshore (Jones, 1960, and Ewing and Davis, 1961).

Informations as to possible sources of faecal pollution may be desirable in special investigations and can be obtained through supplemental examinations for faecal streptococci. This group encompasses a wide spectrum of strains that have diverse survival rates and specific faecal origins and also includes several biotypes that are of limited sanitary significance (Geldreich and Kenner, 1969). Organisms of streptococcal group have been isolated from the intestinal tract of various species of fresh water fish (Venkataraman and Sreenivasan, 1953, and Evelyn and Dermott, 1961).

Once fish have acquired pollution or pathogenic organisms, or both, in their gut, it is conceivable that they could become unsafe from the public health point of view.

This study was developed to include comparison between the two indicators for correct judging the sanitary quality of fish.

Nasser's Lake in Aswan represents the main source of fish catches which reach about 30,000 tons in 1980. Boulti fish was selected for this study, because it constitutes over 95 % of the previously stated amount and also because it is the well preferable species by the Egyptian consumers.

Material and methods

Boulti (*Tilapia nilotica* Linn.) fresh water fish was used in this investigation. Fish was caught from Nasser's Lake (Aswan, February 1981), transferred to Cairo under refrigeration by the Egyptian General Organization for Food Stuffs. Fish samples were transferred directly to the laboratory under refrigeration and sanitary conditions.

Swab method was used for skin and gills sampling as referred to in the studies of microbiological conditions of surfaces (Angelotti et al., 1964). Swabbing solution consisting of 0.013 M phosphate buffer, pH 7.0, was dispersed in 25-ml quantities in sterile test tubes. The solution was sterilized by autoclaving at 121 °C for 15 min. Sterile cotton swabs were moistened in this solution before use for swabbing fish surface or gills and returned to the test tube, which contained the sterile buffer solution, and shaken vigorously.

32 fish samples were divided into 4 groups. Each group was deheaded and eviscerated under aseptic conditions. Bones were removed and the flesh of each group was well mixed under aseptic conditions. Fish intestines of the same groups were removed aseptically and well mixed. 20 grams sample of fish flesh or intestines was transferred to a sterile waring blender. After the addition of 180 ml of the sterile buffer solution, the mixture was blended for 5 minutes.

All samples, i.e., skin, gills, fish flesh and fish intestines, were shaken vigorously and tested for:

I Detection of the coliform group

MacConkey broth in test tubes was inoculated with 1 ml of suspension (1:10) in sterile phosphate buffer solution from swabbing fish surfaces or gills, raw fish flesh and fish intestines. The results were recorded after 24-hr incubation at 37 °C. Acid and gas-positive tubes were streaked onto eosin methylene blue agar (EMB) medium and incubated at 37 °C for 24 hr. Confirmatory test was carried out for typical nucleated colonies with metallic sheen according to A.P.H.A. (1965) with complete identification for three pure strains from each positive sample of raw fish flesh and for two strains in the other tested cases.

II Detection of the streptococcus group

The detection of this group was carried out using the growth in azide-dextrose broth as a presumptive test (Litsky et al., 1953), followed by a confirmatory test in ethyl violet azide broth (E.V.A.) (A.P.H.A., 1965). Positive E.V.A. tubes were streaked on the standard M. enterococcus medium (A.P.H.A., 1965). Plates were incubated at 37 °C for 48 hr, after which typical colonies for the enterococci were picked up and inoculated in nutrient broth for 24 hr at 37 °C. After microscopic examination, pure strains were identified using a set of sugars for fermentation test, and also tested for heat resistance (60 °C for 30 min), starch hydrolysis, 2,3,5-triphenyltetrazolium chloride reduction (TTC), tolerance to 1:2500 K. tellurite, blood haemolysis, gelatine liquefaction, citrate utilization, growth in 40 % bile salt and in 6.5 % NaCl.

Results and discussion

The results as presented in tables 1–4 for the bacteriological survey of skin surface, gills, intestines and raw fish flesh of Boulti fish (*Tilapia nilotica* Linn.) could be evaluated as follows:

Members of coliform group were isolated from roughly 14 samples out of 32 skin swabs. The same result was obtained from testing the swabs of gills. *E. coli I* and untyped 10 represented the dominant strains for the isolates from gills or skin. The distribution of the other types, untyped and irregulars of coliform group was clear in tables 1 and 2. Although the four tested intestinal tract samples showed positive results for the possibility of coliform group detection, one sample was positive for *E. coli* type III, while untyped 10 represented the dominant strain of the isolates. All the tested composite raw fish flesh samples showed positive results for coliform group, two samples out of four were positive for *E. coli I*. Six untyped strains and one *K. aerogenes I* were also isolated (table 2).

Such previous data suggest that the detection of *E. coli I* in slime layer and gills reflect the possibility of faecal contamination. Fish may pick up microorganisms during subsequent handling on ship or on shore. The fish often coming on board may lie on deck for varying periods, sometimes in the sun before marketing (Shewan, 1962). Gelderich and Clarke (1966) reported that the presence of different bacterial indicators in the intestinal sections adjacent to the anus was detected with high counts. Since fish do not have an indigenous bacterial flora (Potter and Baker, 1961), these pollution organisms must have been introduced into the fish by contaminated food and water. During the present study, samples of water taken from Nasser's Lake showed total bacterial counts, total coliform, faecal coliform, and faecal streptococci were 10^4/ml, 23, 30 and 39 per 100 ml, respectively. Water temperature during this period was 17–19 °C.

Of 32 fish surface swabs tested for streptococcus group, 13 samples were positive. When the same number of swabs from gills were tested for the same indicator, 11 samples were positive. Finally all the tested samples from intestines and raw flesh were positive for the streptococcus group (table 4). It is of interest that the typical and atypical strains of streptococcus group (7 strains) were isolated from two samples of each of fish surface swabs, gill swabs and intestinal tract. There were great differences in the biochemical reactions and fermentative types of streptococcal isolates from other positive samples (table 3). Table 4 gives information on the correlation between the incidence of coliform group and streptococcus group in the tested samples. It was possible to note that the significance of streptococcus group as an indicator of pollution was equal to that of coliform.

Organisms of the coliform and streptococcal groups have been isolated from the intestinal tract of various species of fresh fish caught in relatively clean and moderately polluted waters (Evelyn and McDermott, 1961). Although reports of faecal streptococci outbreaks are occasionally made, the significance of streptococci in causing foodborn disease is still in doubt, and evidence to prove its pathogenicity is questionable (Deibel and Silliker, 1963).

The incidence of faecal contamination is especially important in river and lake fisheries. Thus cases of salmonellosis, resulting from the inges-

Table 1. Distribution of coliform types on fish surface, gills, intestine and fish flesh.

No of samples	Surface		Gills		Intestine		Fish flesh	
Coliform types	+	−	+	−	+	−	+	−
E. coli I	6	26	7	25	0	4	2	2
E. coli III	0	32	2	30	1	3	0	4
Cit. freundii I	0	32	1	31	1	3	0	4
Cit. freundii II	0	32	0	32	0	4	0	4
K. aerogenes I	1	31	0	32	0	4	1	3
K. aerogenes II	0	32	1	31	1	3	0	4
Untyped 1	2	30	0	32	0	4	1	3
Untyped 2	1	31	1	31	0	4	0	4
Untyped 3	0	32	1	31	0	4	0	4
Untyped 7	2	30	1	31	0	4	1	3
Untyped 8	0	32	0	32	1	3	0	4
Untyped 10	5	27	4	28	3	1	2	2
Irregular II	2	30	1	31	0	4	0	4
Irregular V	0	32	1	31	0	4	0	4
Irregular VI	1	31	0	32	0	4	0	4
Irregular VII	1	31	0	32	0	4	0	4

Table 2. Classification of coliform strains isolated from fish surface, gills, intestine, and fish flesh.

Coliform types	No of strains isolated from positive samples				Total no of strains
	Surface	Gills	Intestine	Fish flesh	
E. coli I[1])	9	10	0	4	23
E. coli III	0	2	1	0	3
Cit. freundii I	0	1	1	0	2
Cit. freundii II	0	0	0	0	0
K. aerogenes I	2	0	0	1	3
K. aerogenes II	0	1	1	0	2
Untyped 1[2])	2	0	0	1	3
Untyped 2	1	1	0	0	2
Untyped 3	0	1	0	0	1
Untyped 7	2	2	0	2	6
Untyped 8	0	0	1	0	1
Untyped 10	7	7	4	3	21
Irregular II[3])	2	1	0	0	3
Irregular V	0	1	0	0	1
Irregular VI	1	0	0	0	1
Irregular VIII	1	0	0	0	1
Total no	27	27	8	11	73

[1]) Report of the coli aerogenes (1956)
[2]) Ramadan and Moussa (1960)
[3]) Wilson et al. (1935)

Table 3. Fermentative types and distribution of faecal streptococci strains isolated from fish surface, gills, intestine, and fish flesh.

Biochemical reactions of isolates														No and source of solates				
60 °C H. R.	Growth in		Starch hydro-	Fermentation:							K.tel- lurite 1:2500	T.T.C. reduc- tion	Cit- rate	Sur- face	Gills	Intes- tine	Fish flesh	Total no
	40 % bile	6.5 % NaCl	lysis	Frac- tose	Su- crose	Man- nitol	Sor- bitol	Glyc- rol	Raf- finose	Inu- lin								
+	−	+	−	+	+	+	+	±	±	∓	+	∓	−	0	0	0	2	2
−	−	+	−	∓	+	∓	∓	∓	∓	−	−	−	−	3	2	1	2	8
−	−	±	−	−	+	−	−	−	−	−	−	p÷p	−	1	2	2	1	6
−	+	+	−	−	−	∓	−	−	−	−	±	−	−	4	1	0	1	6
−	±	−	−	+	+	+	∓	+	−	−	−	−	−	1	1	0	0	2
+	+	+	−	+	±	+	∓	+	∓	±	±	p.p	−	3	2	0	1	6
+	+	+	−	+	+	+	+	+	±	+	+	−	−	0	0	0	0	0
+	−	+	−	+	+	+	±	+	±	−	−	−	−	0	0	0	0	0
+	+	+	+	+	+	+	+	−	−	+	+	p	−	0	0	0	0	0
−	−	+	−	+	+	±	±	+	±	±	−	p.pp	−	0	0	2	0	2
+	−	+	+	+	+	+	+	+	±	±	±	−	−	0	0	0	0	0
+	−	−	−	+	+	±	∓	∓	−	−	−	−	−	0	0	0	0	0
+	−	−	−	+	+	+	+	+	±	±	+	p	−	0	0	0	0	0
+	+	∓	−	−	+	−	−	−	−	−	+	p	−	0	0	0	0	0
±	−	±	+	+	+	+	±	+	±	−	±	−	−	1	0	0	0	1
−	−	+	−	+	+	+	±	±	±	−	−	−	−	0	2	0	1	3
−	−	−	−	+	∓	+	−	±	±	−	±	p	−	0	1	0	0	1
[1]) +	+	+	−	+	+	±	−	∓	∓	−	−	p	∓	0	0	1	0	1
[2]) ∓	∓	−	−	−	±	−	−	−	∓	∓	∓	p	∓	2	0	2	0	4
[3]) −	+	+	−	+	+	+	∓	∓	−	−	∓	p.pp	∓	0	2	0	0	2

[1]), [2] and [3]) Strains classified as *Str. faecium, Str. equinus* and Atypical faecalis I, respectively. All tested strains were negative for blood haemolysis and liquefaction of gelatine.
p = pink
p.p = pale pink

Table 4. Detection of streptococcus and coliform groups in fish surface, gills, intestine, and fish flesh.

Source and No of tested samples	Strepto-coccus (+)	Coliform (+)	Coliform (+) Strepto-coccus (+)	Coliform (+) Strepto-coccus (−)	Strepto-coccus (+) Coliform (−)	Strepto-coccus (−) Coliform (−)
Fish surfaces (32)	13	14	12	2	1	17
Gills (32)	11	14	11	3	0	18
Intestine (4)	4	4	4	0	0	0
Fish flesh (4)	4	4	4	0	0	0

tion of fish, have been recorded from the Nile valley, South America, and the great lakes in central Africa (Shewan, 1962).

Bacteriological standards have often been associated with the safety to the consumer. According to Griffiths and Stansby (1934), fish quality was stale and inedible at the level of 10^6/g as total viable aerobes. Shewan (1961) recorded that 10^7/g at 37 °C have usually been associated with food-poisoning outbreaks. According to El-Zanfaly and Ibrahim (1980), Boulti fish showed somewhat high figures and reached 10^9/g of raw fish flesh. There are many problems associated with the viable count concept alone as a standard. It is not always safe to assume that a product with low count is otherwise safe (Silliker, 1963). Returning to the proposed standards (Longrée, 1967), coliforms and enterococci are usually present in varying numbers in the natural flora but never more than 10^2/g and 10^3/g, respectively, in newly caught fish (Shewan, 1970). It should be added that the standard for products that are not likely to undergo a full cooking treatment at home should be more rigorous than for the raw products cooked at home. The risk of the utilization of contaminated fish must be low, because Boulti fish is not eaten raw.

Zusammenfassung

Es wird über das Auftreten von coliformen Bakterien und Bakterien der Streptokokkengruppe auf der Haut (32 Proben), den Kiemen (32 Proben), im Verdauungstrakt (4 Proben) sowie im frischen Fischfleisch (4 Proben) von *Tilapia nilotica* Linn., einem Frischwasserfisch aus dem Nassersee in Aswan berichtet.

Streptokokken wurden in 13 Proben der Fischoberfläche, 12 Proben der Kiemenabstriche sowie in allen Darmproben und den Proben aus rohem Fischfleisch gefunden. Coliforme Organismen befanden sich in fast 43 % der Haut- und Kiemenproben sowie in allen Proben des Darmtraktes und des rohen Fischfleisches.

Schlüsselwörter: coliforme Bakterien, Streptococcus, Kiemen, Indikatoren

Summary

A study was made for the occurrence of coliform and streptococcal groups on the skin surface (32 samples), gills (32 samples), intestinal tract (4 samples) and raw fish flesh (4 samples) and raw fish flesh (4 samples) of Boulti fish (*Tilapia nilotica* Linn.), a fresh water fish caught from Nasser's Lake in Aswan.

Streptococcus group was detected in 13 samples taken from fish surface, 12 samples out of 32 swabs from gills. All intestine samples and raw fish flesh were positive for the streptococcus group.

Coliform organisms were detected at nearly 43 % of skin or gill samples, 100 % of intestine and raw fish flesh samples.

Key words: coliform, streptococcus, gills, indicators

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