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PREVALENCE OF SOME POTENTIAL PATHOGENS IN NILE FISHES IN UPPER EGYPT

(With 3 Tables)

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مدى تواجد بعض الميكروبات الممرضه في السمك النيلي بمصر العليا

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تم فحص البكتريولوجي لعدد خمسون عينة ممثلة لخمسة أنواع من الأسماك النيلية المتداولة في مدينة أسيوط وهي البلطي ، القرموط ، البياض ، الشال ، والشلبة. بالفحص الظاهري تبين صلاحية هذه الأسماك للاستهلاك الادمي وبالفحص البكتريولوجي تم عزل ٢=٢ عترة بكتيرية مختلفة من لحوم وأمعاء وكبد هذه الأسماك مع عزل الميكروبات التالية بنسب متباينة:

Strept faecalis, Strept faecium, Staph aureus, Staph epidermidis, Micrococci, E.coli, klebsiella aerogenes, Enterobacteraerogenes, Citrobacter freundi, Shigella dysenteriae, Shigella flexeneri 6, Proteus rettegeri, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Providencia, Edwardsiella tarda, Serratia marscens, Alcaligenes faecalis and Achromobacter.

كما لم يستدل على وجود السالمونيلا في أى من العينات التي تم فحصها وبتصنيف عترات الايشاريشيا كولاى المعزولة وعددها ١٤ وجد إن خمسة عترات منها من النوع الممرض EEC شملت أربعة أنواع هي : *0 55, 0 86 a, 0 119 and 0 128 a 0 128 b* . أثبتت النتائج ان سمك البلطي يحتوى على العديد من الميكروبات المختلفة كما أن الأمعاء هي أكثر الأجزاء التي تحتوى على أعداد كبيرة من الميكروبات . تم مناقشة العلاقة بين الميكروبات المختلفة المعزولة ونوع السمك وكذا الأهمية الصحية ومدى خطورة هذه الميكروبات على صحة المستهلك.

SUMMARY

Bacteriological examination was performed on fifty fresh Nile fishes. A total of 212 bacterial strains were isolated from muscles, livers and gastrointestinal tracts of some common fishes namely Tilapia nilotica, Clarias lazera, Bagrus bayad, Synodontis schall and Schilbe mystus in Assiut City. All the examined samples were accepted organoleptically. Strept faecalis, Strept faecium, Staph aureus, Staph epidermidis, Micrococci, E. coli, klebsiella aerogeness, Enterobacter aerogenes, Citrobacter freundi, Shigella dysenteriae, Shigella flexneri 6, Proteus rettegeri, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Providencia, Edwardsiella tarda; Serratia marscens, Alcaligenes faecalis and Achromobacter were isolated in different percentages. No Salmonella species

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could be recovered in the present investigation. Four strains of entero-pathogenic E. coli: O 55, O 86 a, O 119 and O 128 a O 128 b could be identified. Tilapia nilotica was found to harbour large number of bacterial species more than the other types of fish examined. On the other hand, the gastro -intestinal tracts of the fish examined were found to contain large numbers of microorganisms. Correlation between the bacterial isolates and kinds of fish as well as the public health significance of the isolated organisms were discussed.

INTRODUCTION

Fish and fish products are one of the most important feed stuffs as they are the cheapest source of animal protein and trials were made during the last years by governmental authorities in Egypt for increasing fish production (LOTFI et al., 1972 and OSMAN et al., 1980).

Numerous investigations concerning bacterial flora of fish has been increasingly carried out, in this respect BRUNS (1909) was the first to prove that the flesh and internal orgns of healthy, freshly caught fish are microbiologically sterile. However, the flesh and body fluids of freshly caught healthy fish are generally considered to be sterile (SHEWAN, 1962), although some investigators have recorded the presence of bacteria in fish muscles (SEDIK, 1971; LOTFI et al., 1972 and YOUSSEF et al., 1985).

Fish, molluscs and crustaceans can acquire pathogenic microorganisms or toxins from the natural aquatic environment, from sewage - contaminated harvesting areas, and from contamination by workers, utensils and equipment during harvesting, processing, distribution, and food preparation (NATIONAL ACADEMY OF SCIENCES, 1985).

Foodborne illness may result in human beings from ingestion of fish food products contaiing bacterial or parasitic pathogens. The bacterial pathogens include Salmonella, Shigella, streptococcus, Leptospira, Clostridium, Staphylococci, Mycobacterium, Erysipelothrix, Francisella and Vibrio species (BROWN and DORN, 1977).

Fish may be passive carriers of human pathogens in water environments polluted by human sewage or diseased animals (CHITTINO, 1972). A fish can retain in its digestive tract or on its integument many human pathogens such as E. coli, Salmonella sp., shigella sp., staphylococcus sp. and C. botulinum without becoming ill (LOTFI et al., 1972; BROWN and DORN, 1977; OSMAN et al., 1980 and YOUSSEF et al., 1981). Fish may also be carriers of water-borne pathogenic bacteria of several genera as erysyelothrix, Leptospira, Pasteurella, Aeromonas, Pseudomonas, Vibrio and Mycobacterium (BROWN and DORN, 1977).

Nile fishes are predominant in local markets in Upper Egypt therefore this study has focused on the occurrence of some potential pathogens in fresh fish obtained from the retail level.

MATERIALS and METHODS

The microbiological quality of five species of fresh water fish purchased from local retail fish markets at Assiut City were investigated. Types and numbers of fish obtained were Tilapia nilotica -17; Clarias lazera -10; Bagrus bayad -9; Synodontis schall -9; and Schilbe mystus -5. All the collected samples were examined organoleptically.

Specimens of flesh, liver and intestines were obtained from the collected fish. Muscle and liver samples were taken under sterile condition after disinfection by flaming. To determine the flora of the intestinal tract, the entire gut from stomach to anus was removed and the contents were stripped out with sterile forceps.

All prepared samples were subjected for microbial analysis as follows: Mannitol salt agar was used for isolation of staphylococci and selected colonies were tested for their coagulase reaction.

Enterococci were isolated and identified by using Enterococcus Selective Differential (ESD) medium (EFTHYMIOU and JOSEPH, 1974).

Samples were studied for the presence of Salmonella sp. by adding the prepared samples to selenite cystine broth and incubated for 24 h. at 37°C. Loopfuls from the incubated broth were streaked onto Xylose lysine Deoxycholate (XLD) and Salmonella-shigella agars. Typical colonies were checked for biochemical characteristics.

Brilliant green agar (Oxoid) was used for isolation of enteric group organisms. Further, Pseudomonas agar base supplemented with SR 103 (Oxoid) was used for isolation of Pseudomonas aeruginosa.

Serogrouping of E. coli was carried out using Bacto E. coli O Antisera (Difco).

The isolated organisms in this investigation were identified according to the standard methods recommended by BAILEY and SCOTT (1974), CRUICKSHANK et al. (1975) and WILSON and MILES (1975).

RESULTS

From the different types of Nile fishes examined, numerous types of bacteria were isolated and identified. Percentages of the different bacteria isolated from muscles, livers and intestines from each of Tilapia nilotica, Clarias lazera, Bagrus bayad, Synodontis schall and Schilbe mystus were recorded in Table (1). Most of the bacterial isolates were recovered from Tilapia nilotica. The isoalted organisms were enterococci, staphylococci, micrococci, coliforms, providencia, serratia, shigella sp., E. tarda, proteus sp., Pseudomonas aeruginosa, Alcaligenes faecalis and Achromobacter.

Incidence of different types of microorganisms recovered from muscles, livers and intestinal contents was shown in Table (2). The majority of the organisms were isolated from the gastro-intestinal tract. Table (3) illustrates the serogroups of enteropathogenic E. coli. E. coli serotypes O 55, O 86 a, O 119 and O 128 a O 128 b could be isolated from the gastro-intestinal tract of Tilapia nilotica.

Table (1): Frequency of isolated microorganisms from different fresh water fish examined.

	Tilapia nilatica			Clarias lazera			Bayrus bayad			Synodontis schall			Schilbe mystus		
	M	G	L	M	G	L	M	G	L	M	G	L	M	G	L
Enterococci	12 (37.5%)	17 (30.36%)	13 (46.43%)	4 (40%)	5 (55.56%)	5 (41.67%)	5 (41.67%)	4 ((50%)	3 (30%)	2 (25 %)	6 (66.67%)	4 (57.14%)	0	2 (66.67%)	1 (20%)
Staphylococci	3 (9.38%)	4 (7.14%)	10 (35.71%)	2 (20%)	2 (22.22%)	6 (50 %)	5 (41.67%)	1 (12.5)	4 (40%)	5 (62.5%)	1 (11.11%)	3 (42.86%)	2 (66.67%)	0	3 (60%)
Micrococci	2 (6.25%)	0	0	0	0	0	1 (8.33%)	1 (12.5%)	1 (10%)	0	0	0	0	0	0
Coliforms	8 (25 %)	13 (23.21%)	2 (7.14%)	1 (10%)	1 (11.11%)	0	0	1 (12.5%)	0	1 (12.5%)	1 (11.11%)	0	0	0	0
Providencia	1 (3.13%)	4 (7.14%)	0	0	0	0	0	0	1 (10%)	0	0	0	0	0	0
Serratia	1 (3.13%)	3 (5.56%)	1 (3.57%)	0	0	0	1 (8.33%)	0	0	0	0	0	1 (33.33%)	0	1 (20%)
Shigella sp.	0	3 (5.56%)	0	0	0	0	0	0	1 (10%)	0	0	0	0	1 (33.33%)	0
E. tarda	0	4 (7.14%)	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteus sp.	3 (9.38%)	0	1 (3.57%)	2 (20%)	1 (11.11%)	0	0	1 (12.5%)	0	0	1 (11.11%)	0	0	0	0
P. aeruginosa	2 (6.25%)	0	1 (3.57%)	1 (10%)	0	1 (8.33%)	0	0	0	0	0	0	0	0	0
Achromobacter	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Alcaligenes faecalis	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	32	56	28	10	9	12	12	8	10	8	9	7	3	3	5

Table (2): Frequency of isolated microorganisms from muscles, livers and intestinal contents of examined fish.

Organisms	Number and percentage of isolates		
	Muscle	Gut	Liver
Enterococci :	23 (35.38%)	34 (40 %)	26 (41.93%)
S. faecalis	20 (86.96%)	34 (100%)	26 (100 %)
S. faecium	3 (13.04%)	0	0
Staphylococci :	17 (26.15%)	8 (9.41%)	26 (41.93%)
S. aureus	14 (82.35%)	7 (87.5%)	20 (76.92%)
S. epidermidis	3 (17.65%)	1 (12.5%)	6 (23.08%)
Micrococci	3 (4.6 %)	1 (1.18 %)	1 (1.6 %)
Coliforms :	10 (15.38%)	16 (18.82%)	2 (3.08%)
E. coli	3 (30 %)	10 (62.5 %)	1 (50 %)
K. aerogenes	2 (20 %)	1 (6.25 %)	0
Ent. aerogenes	1 (10 %)	2 (12.5 %)	1 (50 %)
Cit. freundi.	4 (40 %)	3 (18.75%)	0
Shigella sp. :	0	4 (4.71 %)	1 (1.6 %)
Shig. dysenteriae.	0	3 (75 %)	1 (100 %)
Shig. flexeneri 6	0	1 (25 %)	0
Proteus sp. :	5 (7.7 %)	3 (3.53 %)	2 (3.08 %)
P. rettegeri.	2. (40 %)	2 (66.67%)	2 (100 %)
P. mirabilis	1 (20 %)	0	0
P. vulgaris.	2 (40 %)	1 (33.33%)	0
Pseud. aeruginosa.	3 (4.6 %)	0	1 (1.6 %)
Providencia	1 (1.5 %)	4 (4.71 %)	1 (1.6 %)
Edwardsiella tarda	0	4 (4.71%)	0
Serratia marscens	3 (4.6 %)	3 (3.53 %)	2 (3.08 %)
Alcaligenes faecalis	0	5 (5.88%)	0
Achromobacter	0	3 (3.53%)	0
Total	65	85	62

Table (3): Enteropathogenic Escherichia coli (EEC) recovered from fresh water fish

Type of fish	No of strains	Serotypes of EEC
Tilapia	1	O 55
nilotica	1	O 86 a
	2	O 119
	1	O 128 a O 128 b

DISCUSSION

Fish and fishery products could be the vehicle for many important types of bacterial food poisoning which include in addition to Salmonellae, Shigellae, Staphylococci and C. botulinum, the so-called non specific group of microorganisms such as E. coli, Proteus sp., S. faecalis, C. perfringens and B. cereus (SHEWAN, 1962 and LOTFI et al. 1972).

Although organoleptic examination showed no any abnormalities, yet, bacterial cultures were obtained from most of the fish species investigated in the present work.

In relatively hot weather specially in summer time the predominant different organisms during storage will penetrate from slime through the skin into the fish flesh and from the gills via the blood into the blood channels (LOTFI et al., 1972).

Tilapia nilotica was found to harbour large number of bacterial species more than the other types examined. In such fish species the bacterial isolates were enterococci, staphylococci, micrococci, coliforms, providencia, serratia, shigella sp. E. tarda, proteus sp., Pseudomona aeruginosa, Achromobacter and Alcaligenes faecalis.

Generally, the gastro-intestinal tracts of fish were found to be heavy loaded with large numbers of microorganisms. The higher number of bacterial isolates were found in Tilapia nilotica (56 strains). On the other hand, Schilbe mystus revealed lower incidence of bacterial isolates. Such findings agree with those reported by OSMAN et al. (1980). Moreover, SHEWAN (1972) recorded that bacterial flora of fish differed with different seasons and environmental conditions.

GELDREICH and CLARKE (1966) studied the occurrence, distribution and persistence of coliforms, faecal coliforms and faecal streptococci in the intestinal tract of fresh water fish and reported that the composition of the intestinal flora is related in varying degrees to the level of contamination of water and food in the enviroment.

Concerning the distribution and frequency of E. coli in different kinds of fish studied, it was found that a total of 14 E. coli strains were isolated from the gastro-intestinal tract (10 strains), muscles (3 strains) and livers (one strain). Enteropathogenic E. coli were 5 strains and their serogrouping were O 55, O 86 a, O 128 a O 128 b (one strain of each) and O 119 (2 strains).

It is evident in many reviews that almost invariably there has been full agreement among differet investiagtors regarding the bacterial genera which are usually associated with and are responsible for fish spoilage.

The importance and epidemiology of shigellosis as a foodborne disease has been reviewed by BRYAN (1979) and MORRIS (1984). Further, certain biotypes of E. coli cause gastro-intestinal illness in man and in several other animals.

Illness results from eating fish which have become toxic after having undergone some microbial decomposition, although overt signs of spoilage may not be evident. Under certain environmental conditions, bacteria convert the amino acid histidine

to histamine. These bacteria include members of the Enterobacteriacae (Eacherichia, Citrobacter, Enterobacter, Klebsiella, Salmonela, Shigella, Proteus, serratia and Hafina), C. perfringens, Bacillus cereus, Pseudomonas aeruginosa, Strept faecalis and Strept faecium (BRYAN, 1980 and NATIONAL ACADEMY OF SCIENCES, 1985).

Edwardsiella tarda is a serious pathogen known to affect a diverse range of fish species including Tilapia nilotica, Mugil cephalus and Anguilla Japonica (HUMPHREY et al., 1986). E. tarda occurs naturally in the intestine of a range of fish, reptiles, birds and mammals and the organism has been implicated as a cause of human gastroenteritis, septicaemic infections and meningitis (WYATT et al., 1979).

The data presented clearly indicates that the microflora of fresh fish varies considerably. Faulty harvesting, processing and distribution methods can result in microbiological activities leading to loss of quality. As fish and fishery products are susceptible to all food poisoning organisms so the basic principles of foodborne disease prevention and sanitation should be followed to protect the consumers against the public health hazard.

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