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OCCURRANCE AND SIGNIFICANCE OF SOME AEROBIC PATHOGENS IN *OREOCHROMIS NILOTICUS* AND *LABEO NILOTICUS* FISHES IN ASSIUT

(With 4 Tables)

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أهمية ومدي تواجد بعض الميكروبات الهوائية الممرضة في أسماك البلطي والليبس النيلي بأسيوط

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أجري هذا البحث علي ٨٠ سمكة (٥٠ من أسماك البلطي النيلي , ٣٠ من أسماك الليبس النيلي) جمعت من اسواق السمك المختلفة بأسيوط , وقد تم فحصها ظاهريا وبكتريولوجيا . وبالفحص الظاهري تبين صلاحية هذه الاسماك للاستهلاك الآدمي وقد تم عزل عدد ٣٤٥ , ١٧٨ عترة بكتيرية هوائية مختلفة من خياشيم وأمعاء ولحوم أسماك البلطي والليبس النيلي علي الترتيب . ووجد أن متوسط العدد الكلي للميكروبات الهوائية ٣٢,١٣ × ١٠^{٥} / جم , ٤٠,٣ × ١٠^{٤} / جم لاسماك البلطي والليبس النيلي علي التوالي . ولقد تم تصنيف الميكروبات المعزولة , كما لم يستدل علي وجود السالمونيلا والميكروب القولوني في اي من العينات التي تم فحصها . واتضح من البحث انه يجب أن يكون الفحص البكتريولوجي مصاحبا للفحص الظاهري للحكم علي صلاحية الاسماك للاستهلاك الآدمي . تمت مناقشة العلاقة بين الميكروبات المختلفة المعزولة ونوع السمك وكذا الاهمية الصحية ومدي خطورة هذه الميكروبات علي صحة المستهلك .

SUMMARY

A total of eighty fish samples (fifty samples of *Oreochromis niloticus* and thirty samples of *Labeo niloticus*) were collected from various fish markets located in Assiut City. All sampls were examined

organoleptically and bacteriologically. All the examined samples were accepted organoleptically . A total of 345 and 178 bacterial strains were isolated from gills, intestine and muscles of *O. niloticus* and *Labeo niloticus* respectively. The average number of total aerobic colony count per gram muscles were 32.13×10^5 / gram and 40.3×10^4 / gram for *O. niloticus* and *Labeo niloticus* respectively. All the bacterial isolates were identified morphologically, culturally and biochemically. No Salmonella species and *E. coli* could be recovered in the present investigation. Bacteriological examination of fishes must be associated with organoleptical examination to give accurate judgement. Correlation between the bacterial isolates and kind of fish as well as the public health significance of the isolated organisms were discussed.

Key words: *Aerobic Pathogens Oreochromis niloticus, Labeo niloticus*

INTRODUCTION

Fish is considered as an important source of animal protein. It contains protein of high biological value, vitamins, fat, essential minerals as well as appreciable amounts of trace elements (Krause, 1966). However, public health hazards associated with their handling and consumption are bound to increase, because of the perishable nature of the fish, contamination from aquatic environment or unhygienic handling may occur.

It is generally accepted that the internal flesh of live, healthy fish is sterile, the natural bacterial flora reside primarily in the slime layer of skin, gills and intestines (Swaminathan and Sparling, 1998). Lerke et al. (1965) revealed that the diseased weakened fish contains considerable numbers of bacteria in their muscles. On the other hand, Lotfi et al. (1972); Youssef et al. (1985); Hefnawy et al. (1989) and El-Gohary and Samaha (1992), could isolate a number of bacteria from fresh fish muscles.

Fish can acquire pathogenic microorganisms from the natural aquatic environment, from sewage contaminated harvesting areas and/or from contamination by workers; utensils and equipment during harvesting , distribution and food preparation (National Academy of Sciences, 1985). Fish may be passive carriers of human pathogens in water

environments polluted by human sewage or diseased animals (Chittino, 1972), or it may also be carriers of water-borne pathogens of several genera as Aeromonas, Pseudomonas, Mycobacterium and Vibrio (Brown and Dorn, 1977).

O. niloticus and *Labeo niloticus* are predominant in local fish markets in Assiut . So, this work was conducted to determine the isolation frequencies of some aerobic potential pathogens from different sites (gills, intestines and muscles of *such fishes* which collected from various fish markets located at Assiut City.

MATERIAL and METHODS

A total of 80 fish samples were collected from various local fish markets at Assiut City. They were divided as 50 samples were *O. niloticus* and 30 *Labeo niloticus*. Each sample was put in a plastic bag and were transferred directly to the laboratory in sampling boxes with crushed ice (Temp. ≤ 4.4°C). The collected fishes were examined organoleptically.

Bacteriological examination of fishes:

Three samples (gills, intestine and flesh) were collected from each fish and subjected to bacteriological examination which was achieved as follows:

Sampling technique:

1- Gills:

Specimens from gills were aseptically taken from each fish and were cut into small pieces using sterile scissors. Each sample was divided into two parts. The first was immersed in a tube containing sterile nutrient broth. After thorough mixing, the inoculated tubes were incubated at 37°C for one hour before being cultivated. While the other part was cultured in a tube containing 10 ml selenite "F" broth and incubated at 37° C for 18 hours.

2-Muscles:

Preparation and sampling:

The skin was first wipped with gauze to remove the mucous and scales and then rubbed thoroughly with cotton wool, soaked in absolute alcohol , followed by rapid flamming of the suface to ensure complete sterilization .A small piece of the skin was carefully removed avoiding opening the belly cavity or reaching the gills . Ten grams of muscle from

each fish were transferred under aseptic condition to sterile blender jar to which 90 ml of sterile peptone water 0.1 % were added to provide a dilution of 10^{-1}. The blender was operated to give 3000 r.p.m. for not more than 2.5 minutes, then the mixture was allowed to stand for 15 minutes at room temperatrure (35±1°C). The contents of the jar were mixed by shaking before ten fold serial dilution were prepared up to 10^{-6} using sterile peptone water (ICMSF, 1978).

3- Intestine:

The intestinal tract of each fish was carefully dissected and opened by a sterile scissor and forceps and direct samples from intestinal contents were taken using sterile loope.

Enumeration of aerobic plate count:

It was carried out according to the standard plate count. Colonies were counted according to A.P.H.A. (1972).

Isolation technique:

Loopfuls from nutrient broth inoculated with samples of gills as well as homogenated muscles and a loopful from the intestinal contents were cultivated on plates of Nutrient agar, Crystal violet blood agar, Blood agar, Enterococcus selective differential agar, Mannitol salt agar and Pseudomonas selective agar media. The inoculated plates were incubated at 37°C for 24 hours. While pseudomonas selective agar plates were incubated at 25°C for 24 hours. On the other hand, approximatelly 3 loopfuls of selenite "F" broth were steaked on three selective solid media (MacConkey, Brilliant green and S.S agar plates) and incubated over night at 37°C for cultivation members of the family *Enterobacteriaceae*.

Identification of isolates:

Pure cultures of suspected growth on agar slopes were prepared . Isolates were grouped according to their staining reaction with Gram's stain, then subjected to further identification according to Koneman et al. (1994) and Quinn et al. (1994).

RESULTS

The results are tabulated in Tables 1,2,3 and 4.

DISCUSSION

Although the organoleptic examination based on organoleptic tests, its relation to skin (colour and fresh odour), scales, eyes, gills and firm flesh showed no any abnormalities and all the samples were fresh (100%) Table (1). Yet, bacterial cultures were obtained from the two fish species investigated in this study (Table, 1). Therefore, bacteriological examination must be associated with organoleptic examination to give the accurate judgement. These results substantiate what have been reported by El-Mossalami and Sedik (1973) and Youssef et al. (1985).

The summarized results reported in Table (1) showed that a total of 345 and 178 bacterial isolates were recovered from the 50 and 30 fishes of *O. niloticus* and *Labeo niloticus* respectively, of these 162 (46.96%), 77 (43.26%) were detected from the gills, 108 (31.30%) and 66 (37.08%) from intestines and 75 (21.74%) and 35 (19.66 %) from muscles samples of *O. niloticus* and *Labeo niloticus* respectively.

Counts, number of isolates and type of microorganisms recovered from fish varies significantly according to the mode of life, degree of water pollution, season and methods of sampling (Mousa et al., 1987). Moreover, Subsequent handling of fish during catching, transportation, storage time and marketing may add new contaminants which render the fish unsound constituting a public health hazard (Khalafalla, 1988).

It is worthmentioning, that *O. niloticus* was found to harbour large numbers of bacterial isolates more than *Labeo niloticus* and the bacterial isolates recovered from gills are higher than those from intestine and muscle samples of *O. niloticus* and *Labeo niloticus*. Nearly similar results were reported by Laila et al. (1986) and El-Gohary and Samaha, (1992).

Total aerobic plate count:

The results mentioned in Table (2) showed that the viable bacterial count/g fish muscle samples of *O. niloticus* were 3×10^5, 85×10^6 and 32.13×10^5 as minimum, maximun and average values respectively. The corresponding values of *Labeo niloticus* fish muscle samples ranged from 6×10^4 to 22×10^5 with average value of 40.3×10^4. This result is somewhat similar to those reported by Youssef et al. (1985). While lower count was recorded by El-Mossalami and Sedik (1973).

The high counts met within examined samples are mostly attributed to unsatisfactory sanitation during handling and distribution, excessive handling between fishing and marketing as well as the high storage temperature especially in summer. In relatively hot weather especially in summer time the predominant different organisms during storage will penetrate from slime through the skin into the flesh and from the gills via the blood channels (Lotfi et al., 1972).

The critical value of edibility of fish is 10^4 to 10^8 million bacteria/gm (Nickerson and Proctor, 1935). On the other hand, Braumuller (1958) reported that a number of 0.8 million bacteria/g could be a limit for changes in quality, beginning decomposition or for its fitness for human consumption.

Isolated and Identified bacteria:

Staphylococcus aureus was isolated from *O. niloticus* and *Labeo niloticus* at an incidence of 4.35% and 3.94% respectively (Table 3 and 4). These results were lower than those obtained by Hefnawy et al. (1989) and El-Gohary and Samaha (1992). Mousa and Mahmoud (1997) reported the occurrence of *Staph.aureus* in *O. niloticus* but they did not record the incidence of occurrence. In addition, *Staph.aureus* could be isolated in the present study from gills and intestine but this organism could not be isolated from the examined muscle samples. These findings substantiate what has been reported by El-Mossalami and Sedik (1973); Youssef et al. (1985) and El-Gohary and Samaha (1992). A contradictory finding was given by Hefnawy et al. (1989) and Mousa and Mahmoud (1997) who isolated *Staph.aureus* from muscles of *O. niloticus.*

The presence of *Staph. aureus* in fish indicated their contamination from polluted water (Brown and Dorn, 1977) or it is good indicators of the personal hygiene of food handlers from suppurating lesions or from the nostrils of carrier "usually via the hand". (Elwi, 1994).

Staph.aureus is one of the most important specific microorganism responsible for food poisoning in human-beings and staphylococcal intoxication symptoms usually appear within 2 to 4 hours following consumption of contaminated food (Bergdoll, 1979). There were few deaths reported especially in elderly or very young (Varanam, 1991).

A total of 11 bacterial isolates of *Staph.epidermidis* were recovered from the 80 examined fish from these 7 strains were detected from *O. niloticus* "5 from gills and 2 from intestine" and 4 strains from *Labeo niloticus* " 3 from gills and 1 from intestine (Table 3 and 4). The

present result is somewhat similar to those reported by Laila et al., (1990).

The *Staph.epidermidis* is one of the most important specific microorganism responsible for congestion and ulceration on the tail of fishes (Kusuda and Sugiyama, 1981).

The data presented in tables 3 and 4 revealed that haemolytic streptococci was isolated from gills of *O. niloticus* and *Labeo niloticus* at incidence of 2.47% and 1.29% respectively. Such pathogen was previously isolated by Laila et al. (1986) from the surface of *O. niloticus*. It was found to be an etiological significant in some epizootic among *O. niloticus* and other fresh water fish (Kitao et al., 1981). The affected fish are characterized by external petechial haemorrhages around the anus and ventral body surface, secretion of abnormal slime on the gills, numerous haemorrhages in the intestine and accumulation of redish asciatic fluid in the body cavity (Vgajin, 1981).

The enterococci were recovered from gills, intestine and muscles of *O. niloticus* and *Labeo niloticus*. They includes *Streptococcus faecalis* (19.71% and 23.04 %) and *Streptococcus faecium* (3.19 % and 3.93%) in *O. niloticus* and *Labeo niloticus* respectively. These results goes hand in hand with those reported by Youssef et al. (1985), Laila et al. (1986); Gohary and Samaha (1992).

The presence of detectable number of *Streptococcus faecalis* and *Streptococcus faecium* on or in the fish examined during this study is an indication of pollution of water with sewage and animal wastes. Moreover these organisms have been isolated from foods implicated in cases of food poisoning and the pathogenicity of such organisms on fish is not clearly recognized (Laila, et al., 1986).

The occurrance of *Pseudomonas aeruginosa* in the examined fish was at an incidence of 2.90% and 7.30% from *O. niloticus* and *Labeo niloticus* respectively. While, *Pseudomonas fluoresence* was isolated from *O. niloticus* and *Labeo niloticus* at an incidence of 8.11% and 8.42% respectively. These findings agree to a certain extent with those reported by Laila et al., (1986) and Younes et al. (1990).

Pseudomonas aeruginosa sometimes colonizes humans and it is the major human pathogens which produces infection of wounds and burns giving rise to blue-green pus; meningitis and urinary tract infection, it also infects eyes which may lead to its rapid destruction. The bacterium

may invade the blood stream and result in fatal spesis especially in infants and/or debilitated persons (Brooks et al., 1995).

It was mentioned that *Pseudomonas aeruginosa* has the ability to cause spoilage of foods and leads to several outbreaks of food poisoning (Pererra et al., 1977). Many strains of *Pseudomonas aeruginosa* produce exotoxin A, which inhibits intracellular protein synthesis and it is toxic for human blood macrophages (Wilson and Miles, 1983).

Pseudomonas fluoresence was recovered in this study. This organism was encountered in cases of spottiness of the skin (Duijn, 1973) and haemorrhagic bacterial septicaemia (Roberts, 1978) among different species of fish.

The current study revealed isolation of most members of family *Enterobacteriaceae* from *O. niloticus* and *Labeo niloticus* that were identified as *Klebsiella pneumoniae, Klebsiella oxytoca, Proteus vulgaris, Proteus mirabilis, Proteus rettegeri, shigella dysenteriae, Shigella flexeneria, Serratia liquefaciens, Enterobacter aerogenes, Citrobacter freundi and Edwardsiella tarda.* The percentage of incidence of these bacteria isolated from *O. niloticus* and *Labeo niloticus* were calculated in Tables (3,4).

Those bacteria are potenially present in water and are not known as classical fish pathogens, yet the oxygen depletion and high water temperature rendered fishes to be easily infected with those bacteria (Badran et al., 1994). Furthermore, domestic waste water carriers a variety of fish pathogens such as *Edwardsiella tarda* (Austin and Austin, 1987). *Edwardsiella tarda* is a serious pathogen known to affect a diverse range of fish species including *O. niloticus* (Humphery et al., 1986). *Edwardsiella tarda* occurs naturally in the intestine of a range of fish, birds, reptiles and mammals and the organism has been implicated as a cause of human gastroenteritis, septicaemic infection and meningitis (Wyatt et al., 1979).

Klebsiella pneumoniae was only detected from the gills of *O. niloticus* and *Labeo niloticus* (Table 3,4). This organism is a faecal coliform and histamine-producing bacteria (Orskov, 1984). Under certain environmental conditions, bacteria convert the amino acid histidine to histamine.

The public health importance of Klebsiella species lies in the assuumption of being a member of the food poisoning organisms and a

cause of respiratory as well as urinary affections in man (Marchant and Packer, 1975).

In this study, the overall incidence of *Shigella dysenteriae* recovered from gills and intestine of *O. niloticus* amounted to 4.94% and 4.63% respectively, concerning *Labeo niloticus, Shigella flexeneria* was isolated from two samples of gills (2.59%). Hefnawy et al., (1989) recovered Shigella species from 5.56% of the examined intestinal samples of *O. niloticus*. A contradictory finding was given by El-Monela (1981) who failed to isolate shigella species from freshly caught *O. niloticus*. It is well known that Shigella is a strict human pathogen, and the isolation from fish is doubtless of human source (Khan, 1968). The examined fish are marketed and subjected to contamination from the hands of purchasers or clients or mechanically by flies or roaches. (Marchant and Paker, 1975). Furthermore, the importance and epidemiology of shigellosis as a food-borne disease has been reviewed by Morris (1984). Moreover, there are many reports about the public health significance of other members of *Enterobacteriaceae* (Banwart, 1981 and Fraizer, 1986). Members of this family of bacteria are of potential public health importance as it causes disease for human during lowering of their resistance. Also this group contains most members of food poisoning microorganisms. Furthermore, this group is used as indicator for the degree of water pollution (Mousa and Mahmoud, 1997).

Information derived from the results reported in Table (3&4) revealed that Salmonella species and *Escherichia coli* could not be isolated from any site of examination of the *O. niloticus* and *Labeo niloticus*. These results substantiate what have been reported by Al-Wakeel et al. (1982) who foud that the bacterial examination of fish muscles was negative for *Salmonella typhimurium* and *E.coli*. Furthermore, Fernandes et al. (1997) failed to isolate *Escherichia coli* O157:H7 from the 120 samples of fresh aqua cultured Catfish filets. Moreover, *MacMillan and Santucci (1990)* observed that presence of *E. coli* was a seasonal phenomenon when they examined the intestinal bacterial flora of farm-raised channel Catfish. In addition, Lotfi et al., (1972) reported that Salmonella organisms were only revealed in the slime of *O. niloticus* and added that so they could not be accepted as being from the normal flora of fish. In Egypt, El-Mossalami and Sedik (1973) and Ahmed et al. (1986) failed to detect Salmonellae from examined fish. This could be explained in the view that Salmonellae are

unlikely to be harboured in creatures with a low body temperature especially those caught far out at sea (Hobbs and Gilbert, (1982).

The data recorded in this work proved that *O. niloticus* and *Labeo niloticus* can be a vehicle for many types of microorganisms. Environmental conditions may be a great factor for growth and multiplication of various microorganisams. Consequently fish can be a public health hazards, such hazard include fish-borne microbial infections. To improve the status of fish, strict hygienic measures should be carried out during the different steps from fishing to marketing. Fish handling should be minimized and education programmes should be imposed for producers and handlers.

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Table (1): Frequency distribution of the examined fish based on organoleptic tests and total number of isolates recovered from different parts.

Fish examined	Organoleptice ex.				No. of isolates Site of isolation						Total
	Fresh fish		stale fish		Gills		Intestine		Muscles		
	No.	%	No.	%	No.	%	No.	%	No.	%	No. of isolates
O. niloticus	50	100	-	0.0	162	46.96	108	31.30	75	21.74	345
Labeo niloticus	30	100	-	0.0	77	43.26	66	37.08	35	19.66	178
Total	80	100	-	0.0	239	45.70	174	33.27	110	21.03	523

Table (2): Results of viable total aerobic colony count/gm muscles of *O. niloticus* and *Labeo niloticus* at 35 ±1°C.

Species of fish examined	Positive samples		Minimum	Maximum	Average
	No.	%			
O. niloticus	50	100	3×10^5	85×10^6	32.13×10^5
Labeo niloticus	30	100	6×10^4	22×10^5	40.3×10^4

Table (3): Number and incidence percentage of the different species of aerobic bacteria isolated from *O. niloticus*

Organisms	Site of examination						Total	
	Gills		intestine		muscles			
	No.	%	No.	%	No.	%	No.	%
Gram +ve bacteria								
Staphylococci								
Staph.aureus	11	6.79	4	3.70	-	0.00	15	4.35
Staph.epidermidis	5	3.08	2	1.85	-	0.00	7	2.03
Sterptococcus pyogenes " Haemolytic streptococci"	4	2.47	-	0.00	-	0.00	4	1.16
Enterococci								
Steptococcus faecalis	18	11.11	29	26.85	21	28.00	68	19.71
Stept. faecium	7	4.32	6	5.56	2	2.67	15	4.35
Gram-ve bacteria								
Enterobacteriaceae								
Klebsiella aerogenes	7	4.32	5	4.63	-	0.00	12	3.48
K. pneumoniae	2	1.23	-	0.00	-	0.00	2	0.58
K. Oxytoca	4	2.47	2	1.85	-	0.00	6	1.73
Enterobacter aerogenes	6	3.70	4	3.70	1	1.34	11	3.19
Enter. liqueficens	8	4.94	5	4.63	22	29.33	35	10.14
Citrobacter freundi	5	3.08	2	1.85	4	5.33	11	3.19
Shigella dysenteriae	8	4.94	5	4.63	-	0.00	13	3.77
Shigella flexeneri	4	2.47	2	1.85	-	0.00	6	1.73
Proteus vulgaris	16	9.89	11	10.19	2	2.67	29	8.41
P. mirabilis	13	8.03	5	4.63	3	4.00	21	6.06
P. rettegeri	15	9.26	8	7.41	1	1.33	24	6.96
Serratia liquefaciens	8	4.94	3	2.78	9	12.00	20	5.80
Edwardsiella tarda	6	3.70	2	1.85	-	0.00	8	2.32
Other types of bacteria								
Pseudomonas aeruginosa	2	1.23	5	4.63	3	4.00	10	2.90
Pseudomonas fluoresence	13	8.03	8	7.41	7	9.33	28	8.11
Total	162	100.0	108	100.0	75	100.0	345	100.0

Table (4): Number and incidence percentage of the different species of aerobic bacteria isolated from *Labeo niloticus*

Organism	Site of examination						Total	
	Gills		intestine		muscles			
	No.	%	No.	%	No.	%	No.	%
Gram +ve bacteria								
Staphylococci								
Staph.aureus	5	6.49	2	3.03	-	0.00	7	3.93
Staph.epidermidis	3	3.90	1	1.52	-	0.00	4	2.25
Sterptococcus pyogenes								
" Haemolytic streptococci"	1	1.30	-	0.00	-	0.00	1	0.56
Enterococci								
Steptococcus faecalis	11	14.29	22	33.33	8	22.86	41	33.03
Stept. faecium	3	3.90	4	6.06	-	0.00	7	3.93
Gram-ve bacteria								
Enterobacteriaceae								
Klebsiella aerogenes	2	2.60	2	3.03	-	0.00	4	2.25
K. pneumoniae	1	1.30	-	0.00	-	0.00	1	0.56
K. Oxytoca	4	5.19	-	0.00	1	2.86	5	2.81
Enterobacter aerogenes	7	9.09	3	4.55	6	17.14	16	8.99
Enter. liqueficens	3	3.90	4	6.06	7	20.00	14	7.87
Citrobacter freundi	2	2.60	1	1.52	1	2.86	4	2.25
Shigella dysenteriae	-	0.00	-	0.00	-	0.00	-	0.00
Shigella flexeneri	2	2.60	-	0.00	-	0.00	2	1.12
Proteus vulgaris	5	6.49	3	4.55	1	2.86	9	5.16
P. mirabilis	4	5.19	6	9.09	2	5.71	12	6.74
P. rettegeri	6	7.79	5	7.57	-	0.00	11	6.18
Serratia liquefaciens	5	6.49	4	6.06	3	8.57	12	6.74
Edwardsiella tarda	-	0.00	-	0.00	-	0.00	-	0.00
Other types of bacteria								
Pseudomonas aeruginosa	7	9.09	4	6.06	2	5.71	13	7.30
Pseudomonas fluoresence	6	7.79	5	7.57	4	11.43	15	8.43
Total	77	100.0	66	100.0	35	100.0	178	100.0