





DETECTION OF SOME BACTERIAL ZOONOSIS IN MARKET FISH IN QALYOUBIA PROVINCE AND THEIR CONTROL

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ABSTRACT

In this study, 200 fish samples of Oreochromis niloticus and Clarias gariepinus (100 of each) were collected from different fish markets at Qalyoubia province. In addition, 100 skin swabs were collected from fish sellers (60) and house wives (40) from the same localities. The objective of this study is to detect the occurrence of some bacterial zoonotic microorganisms from market fish such as Staph. aureus, Salmonella spp., E. coli, and Streptococci and to detect the effect of heat treatment as frying and grilling on survival of inoculated $(10)^4$ Staph.aureus ,(10)⁵ Salmonella typhimurium and $(10)^6$ E.coliO₁₅₇H₇ in both fish spp. In this study, it was evident that C. gariepinus samples had a significantly higher bacterial isolates (9.8%) than O. niloticus samples (6%). Among the isolated bacteria Streptococci was detectable at higher percentage (13.5%) followed by Salmonella spp (11.5%), then Staph. aureus (4.5%) and the lowest isolates were E. coli (2%). The higher percentage of bacterial isolates was recovered from surface samples than those isolated from muscle samples of both of the examined fish spp. Hand swabs of fish handlers revealed that Staph. aureus ,Salmonellae , E. coli and Streptococci were isolated at percentages of 35%, 35%, 20% and 35% respectively from fish sellers compared to 37.5%, 25%, 37.5% and 50% respectively from house wives .Also these results showed that frying of fish lead to total destroying of inoculated pathogens in both fish spp. at different weights . While grilling not efficient as frying as it kill all the inoculated pathogens in O. niloticus but not in large sized C. gariepinus. The public health importance of isolated microorganisms and suggested hygienic measures were discussed.

Keywords: Bacterial zoonosis, Market fish, Control, Qalyoubia province.

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1.INTRODUCTION

ish is considered one of the most nutritive and highly desirable food stuffs as fish meat has excellent nutritional value being rich in proteins, vitamins and unsaturated fatty acids. It is also extremely perishable and the safe consumption requires adequate sanitary conditions from the moment of catch, through preparation, sale and consumption (Franco and Landgraf, 1996). In countries, which keep adequate records of diseases transmitted through food. eating contaminated fish is responsible for a significant number of disease out breaks (Hatha mohamed and Lakshumanaperumalsamy, 1997). The most popular

fresh water fish in Egypt are Oreochromis niloticus, Bagrus bayad and Clarias lazera (Abd El Shahid et al., 2009). The fish flesh, which is the main edible part, is generally sterile immediately after catching, how ever, it may become contaminated with different microorganisms during subsequent handling as these microorganisms can penetrate from skin and the gut to the flesh (FAO, 1983). Most fish related food borne illness are traced to Salmonella. Staphylococcus spp., Escherichia coli., Vibrio parahemolyticus ,*Clostridium* perfringens, Clostridium botulinum E and Entero viruses (Center for food safety and applied nutrition in Washington,2001). Enterococcus spp, Aeromonas spp, Fecal and total Coliform, Listeria spp and Salmonella spp from the external surface of Tilapia were shown by Morales (2004). Human infection by fish pathogens is usually through contact with abraded skin with infected fish while handling or with water or other constituents of an aquatic environment (Acha and Szyfres, 2003). Moreover, people use some ways for preparation of fish to be eaten such as rapid frying, smoking and pickling and such methods have been proved to be insufficient kill to all harmful microorganisms which may be present in raw fish prior to preparation (Mansour et 1997). The consumption al.. of contaminated fish give rise to intestinal disorders ranging from diarrhea and vomiting to fever (Naglaa et al., 2002). In Egypt, Salmonellae, E. coli and Staph. aureus are widely recognized as the principle causes of food poisoning out breaks occurring because of consumption of contaminated fish and fish products (Hassan and Fatin, 2003). Therefore, this work was carried out to detect some bacterial zoonotic microorganisms from market fish such as Staph. aureus. Salmonella spp., E. coli and Streptococci and the effect of heat treatment as frying and grilling on some microorganisms as Staph. aureus, Salmonella spp. and E. coli.

2. MATERIAL AND METHODS

2.1. Sampling:-

2.1.1. Fish samples:-

200 random fish samples of *O. niloticus* and *C. gariepinus* (100 of each) were collected from different fish markets at Qalyoubia province. The collected fish samples were packed separately in sterile plastic bags and transported to the laboratory in an icebox immediately to be examined.

2.1.2. 2.1.2. Human samples:-

100 skin swabs were collected from fish sellers (60) and house wives (40) from the same localities in the same province.

2.2. preparation of samples:-

2.2.1. Fish samples:-

Surface swabs were aseptically taken from each fish and were separately inoculated into separate sterile tube containing peptone water. In addition, 5 gm of fish flesh of each samples was desiccated in a sterile flask, under complete sterile condition, and then 45 ml sterile peptone water were added and thoroughly mixed using sterile blender for 1-1.5 minutes.

The prepared samples were subjected to bacteriological examination.

2.2.2. Human samples:-

From each fish seller and house wife, hand swabs were taken by rubbing swabs in the inter- digital spaces, nails, palms and on the back of the hands and were separately inoculated into separate sterile tubes containing peptone water.

- 2.3. Bacteriological examination of samples :-
- 2.3.1. Detection of Staphylococcus aureus : according to (Macfaddin, 1980)
- 2.3.2. Isolation and identification of Salmonellae: according to the method described by (ISO, 2002).
- 2.3.3. Isolation and identification of pathogenic Escherichia coli: according to (ICMSF, 1996):

2.3.4. Isolation and identification of *Streptococci*: according to (Macfaddin, 1980)

2.3.5. Experimental study on the effect of heat treatment on control of inoculated microorganisms:

Standardization of bacterial counts of $(10)^4$ Staph aureus, $(10)^5$ Salmonella typhimurium and $(10)^6$ E. coliO₁₅₇H₇ were carried out by "Welcome Opacity tubes (BURROUGH WELLCOME CO. LONDON) . 1 ml of each mentioned bacteria inoculated into the anterior dorsal region of left hand side of fish samples

which were 36 samples from O.niloticus and C.gariepinus fish (18 samples from each type) which then divided into three groups (6 samples of each) according to size. Group I (small size: above 100 gm and less than 200 gm) group II (medium size : above 200 gm and less than 300 gm) and group III (large size : above 300 gm) and these groups were subjected to heating by frying in hot ordinary oil. Another 3 similar groups also inoculated with these microorganisms and were subjected to grilling. Fish samples for frying were put in frying oil at 192°c for 8 minutes and the fish core temperature was determined. It was 62 -70°c according to size of fish. While grilling of fish was done at temperature of 145°c for 8 minutes and the temperature of fish core was 55 - 60°c. After that, the inoculated bacteria were detected by isolation and biochemical reactions as mentioned above.

3. RESULTS

- 3.1. The total percentage of isolated bacteria was higher in *C. gariepinus* fish (9.8%) than that of *O.niloticus* (6%). Staphylococci, Salmonellae, *E.coli* and Streptococci were isolated at percentages of 3%, 8%, 1% and 12% respectively from *O. niloticus* and at percentages of 6%, 15%, 3% and 15% respectively from *C.gariepinus* fish.
- 3.2. The previously mentioned microorganisms were isolated from surface samples at higher percentages than from muscle samples of all examined fish.
- 3.3. The most predominant serotypes of the isolated microorganisms were *Staph.aureus, S. typhimurium, S. enteritidis, E. coli O55:k59, O111:k58* and *O128:k67,* for Streptococci the most predominant serotypes were *Strept. faecalis, Strept. Pyogenes, Strept. Intermediate, Strept. faecium, Strept. viridans ,Strept. mutans* and *Strept. mitis.*

- 3.4. Human swab samples revealed that Staphylococci ,Salmonellae , *E.coli* and Streptococci were isolated at percentages of 35% , 35% , 20% and 35% respectively from fish sellers ,and at percentages of 37.5% , 25% , 37.5% and 50 % respectively from house wives .
- 3.5. The most predominant serotypes of the isolated microorganisms from human swab samples were Staph.aureus, S.typhimurium, S. enteritidis, E.coli O₁₁₉:k₆₉, O₁₂₅:k₇₀ $,O_{26}:k_{60}$, O55:k59, O111:k58 and O124:k72, for Streptococci the most predominant serotypes were Strept.faecalis ,Strept. Angiosus, Strept. Faecium, Strept. mitis and Strept. sanguis.
- 3.6. Frying of fish is more efficient than grilling as frying lead to complete destruction of the inoculated microorganisms in *O.niloticus* and *C.gariepinus* fish at different weights, while grilling not able to destruct these microorganisms in large sized *C.gariepinus* fish.

4. DISCUSSION

It is evident from (table 1) that the total percentage of the isolated bacteria was 6% and 9.8% in O.niloticus and C.gariepinus respectively. The higher bacterial isolates were detected in C.gariepinus than in O.niloticus this may be due to feeding habits of C. gariepinus as they are carnivorous fish and tend to eat more animal material than plant food (Radwan, 1992). The result recorded in table (1) indicates that Staph. aureus could be detected in 4.5% (9 samples were positive out of 200 fish samples). The higher incidence of Staph. aureus were found in C. gariepinus samples which reach 6% (6 fish samples were positive out of 100 fish samples), while the incidence of Staph. aureus in O. niloticus was lower

Examined	O. niloticus (100)		C. gariepi	inus (100)	Total (200)	
Isolates	No	%	No	%	No	%
Staphylococci	3	3	6	6	9	4.5
Salmonella spp	8	8	15	15	23	11.5
E.coli	1	1	3	3	4	2
Streptococci	12	12	15	15	27	13.5
Total	24	6	39	9.8	63	7.9

Table (1): Number and percentage of isolated microorganisms from examined fish

Table (2): Frequency distribution of isolated microorganisms in surface and muscle samples of examined fish.

Examined fish Parts	O. niloticus			C. gariepinus						
	Total	Surf	face	Mus	scle	Total	Surf	ace	Mus	scle
	isolates	No	%	No	%	isolates	No	%	No	%
Isolates										
Staphylococci	3	3	3	0	0	6	6	6	0	0
Salmonella spp	8	4	4	4	4	15	8	8	7	7
E. coli	1	1	1	0	0	3	2	2	1	1
Streptococci	12	7	7	5	5	15	11	11	4	4
Total	24	15	3.8	9	2.3	39	27	6.8	12	3

Table (3). Types	s of isolated	l microorganisms	from	examined fish
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Number	O. niloticus	C. gariepinus
Staph. aureus	3	6
S. typhimurium	3	7
[O(1,4,5,12) H (i:1,2)]		
Salmonella enteritidis[O	3	6
(1,9, 12) H(g,m:1,7)]		
Salmonella chester[O(1, 4,	0	1
5, 12) H (e,h:e,n,x)]		
Salmonella Virchow[O(6,7,	1	0
14) H(r:1,2)]		
Salmonella anatum[O(3, 10	0	1
, 15 , 34) H (e,h:1,6)]		
Salmonella muenster[O(3,	1	0
10, 15, 34) H (e,h:1,5)]		
E.coli O111:k58		1
E. coli 055:k59	1	1
E. coli 0128:k67	0	1
Strept. faecalis	5	7
Strept .pyogenes	4	1
Strept. Intermediate	2	1
Strept. faecium	1	3
Strept. viridans	0	1
Strept. mutans	0	1
Strept. mitis	0	1

Examined human	Fish se	ellers (60)	House wives (40)		
	No	%	No	%	
Isolates	_				
Staphylococci	21	35	15	37.5	
Salmonella spp.	21	35	10	25	
E. coli	12	20	15	37.5	
Streptococci	21	35	20	50	
Total	75	31.3	60	37.5	

Table (4) Number and percentage of isolated microorganisms from human samples (hand swabs of fish handlers)

Table (5): Types of isolated microorganisms from human samples.

Number	Fish sellers	House wives
Types of isolates		
Staph. aureus	21	15
Salmonella enteritidis	18	10
Salmonella typhimurium	3	0
E. coli		
O26:k60	3	0
O 125: k70	3	5
O 119 :k69	3	0
O 124:k72	3	0
O 55:k59	0	5
O 111 : k58	0	5
Strept. faecalis	9	10
Strept. angiosus	3	5
Strept. faecium	3	5
Strept. mitis	3	0
Strept.sanguis	3	0

Table (6): Effect of heat treatment on the inoculated microorganisms in O. niloticus

The inoculated M.O.	Frying			Grilling			
	Small size	Medium size	Large size	Small size	Medium size	Large size	
(10) ⁴ Staph.aureus	_	_	_	_	_	_	
(10) ⁵ S. typhimurium (10) ⁶ E.coliO157H7	_	-			_	_	

(-): absent

The inoculated M.O.		Frying			Grilling	
	Small size	Medium size	Large size	Small size	Medium size	Large size
(10) ⁴ Staph.aureus	_	_	_	_	_	+
(10) ⁵ S. typhimurium	_	_	_	_	_	+
$(10)^6 E.coliO_{157}H_7$	_	_	_	_	-	+

Table (7): Effect of heat treatment on the inoculated microorganisms in C. gariepinus

(-): absent (+): present

than that in *C.gariepinus* and reach 3% (3 fish samples were positive out of 100 fish samples). The obtained results agreed to certain extent with those reported by Tamarapu et al. (2001) and Haeghebaert et al. (2002) while higher incidence were reported by Saito et al.(2011). The high incidence of Staph. aureus in the examined could samples indicate unhygienic conditions because the product contamination could be the result of a combination of improper handling, improper storage and cross contamination (Simon and Sanjev, 2007). Salmonellosis can manifest in a number of disease gastroenteritis. syndromes including bacteremia. typhoid fever and focal infections (Darwin and Miller, 1999). Results recorded in table (1) revealed that Salmonellae were isolated from Tilapia & C.gariepinus at percentage of 8% and 15% respectively, the most predominant serotypes were S. typhimurium and S. enteritids and that evident in table (3). Almost similar results were obtained by Darwish, 1991 (6.6%) and Fernandez and Torres 1996 (16%) while lower results obtained by Abd Alla, 1989 (4%) and Youssef et al., 1993(3.9%). E. coli has been differentiated from total coliforms, as more specific indicators of fecal pollution (leclerc et al., 2001). Meanwhile, presence of different virulent strains of E. coli constitutes public health hazards to consumers and make these contaminated fish unfit for consumption. Table (1) indicated that E. coli could be isolated at 2% from totally examined fish (1% from O.niloticus and 3% from C.gariepinus). These findings are nearly similar to those obtained by El- Gohary and Samaha (1992) and Lobna and El-Newishy(2010). These contamination of fresh water fish with E. coli is indicator of faecal pollution of fresh water from which fish were harvested and or from subsequent un sanitary handling during catching. distribution and marketing practices of fish (Abd El Shahid et al., 2009). The identified serotypes of E. coli in examined fish samples were shown in table (3), the most predominant serotypes were O55:k59 ,0111:k58 and O128:k67. such serotypes have a great pathogenicity in the intestinal tract and cause gastro enteritis and implicated in food poisoning out breaks (Bryan, 1980). In this study Streptococci could be isolated from Tilapia &C.gariepinus at percentages of 12% and 15% respectively (Table 1). Table (3) showed that the most predominant strains are Strept.faecalis, Strept.pyogenes, Strept. Intermediate and Strept. facium. The obtained results agreed to certain extent with those reported by Salvador et al. (2005), and Safinaz (2006) while, lower results obtained by Badran and Eissa (1991) and Eissa et al.(2000) and higher results obtained by El- Gohary and El-Ghanam (1999). The presence of Enterococci in any food is indicative of fecal contamination (Morshdy, 1992). Varga and Anderson(1968) stated that the presence of Enterococci in fish originated

from improperly sanitized working surface and their numbers reflect plant sanitation rather than faecal contamination and the difference in results may be due to the difference in season or environment from which the fish were harvested (Shewan, 1977). Table (2) showed the distribution of isolated microorganisms in surface and muscle samples of examined fish and revealed that the higher number of bacterial isolates were recovered from skin surface while muscles showed lower number of isolates at percentages of 3.8% and 2.3% for O.niloticus and 6.8% and 3% for *C.gariepinus* respectively. This may be attributed to exposure of the surface to of different sources contamination (Lobna& El- Newishy, 2010). In addition, FAO (1983) reported that the muscle of living fish is the main edible part is normally sterile. The spoilage bacteria penetrate only slowly from the skin and the gut, although when spoilage is well advanced their presence may be all too obvious in the term of unattractive bacterial slime and repulsive odors, respectively Just below the skin. Table (4) bacteriological showed results of examination of fish handlers. It was found that out of sixty hand swabs from fish sellers 21 (35%) gave positive results for Staph. aureus, whereas, 21 (35%) gave positive for Salmonellae, 12 (20%) gave positive for E. coli and 21 (35%) gave positive for Streptococci. It also found that out of fourty house wives hand swabs, 15 (37.5%) gave positive for *Staph. aureus*. 10 (25%) gave positive for Salmonellae, 15(37.5%) gave positive for *E.coli* and 20 (50%) gave positive for Streptococci. Table (5) showed that the most predominant serotypes of Salmonellae were S. typhimurium & S.enteritidis, for E. coli were O26:k60, O125:k70, O119:k69, 0124:k72, 055:k72, 0111:k58 and for Streptococci, most predominant the serotypes were Strept. Faecalis, Strept. Angiosus, Strept. faecium, Strept. mitis, Strept. sanguis. These results are supported by (Bercovier et al., 1997, Esaki et al.,

Lobna& El- Newishy, 2010), who isolated the same organisms from fish handlers at different percentages.Moreover Jay (1997) stated that Staph. aureus is present on the skin and mucosa of humans and animals as well as in the environment and a bout (30-35%) of healthy humans have Staphylococci in the nasopharynx and on the skin (Pedro and Boris, 1994). Besides, about 20% of normal individuals harbor the organism in their intestinal tract (Stewart, 1973). Also Thaikurea et al. (1995) stated that laboratory examination of food and food handlers indicated heavy growth of Staph. aureus which lead to Staphylococcal food poisoning with symptoms of nausea (93%), vomiting (88%) and abdominal pain (81.5%) after incubation period 3 hours. Regarding to the presence of E. coli, Salmonellae and fecal Streptococci in hand swab samples; it significant emphasized fecal а contamination and indicate that food handlers were not taking enough care in hand hygiene (Landeiro et al., 2007 and Morshdy, 1992). Tables (6,7) revealed that $(10)^4$ Staph aureus were inoculated into fish samples of O.niloticus & C.gariepinus and couldn't be detected at any size of both fish species after frying of the fish. But could be detected only in large sized C. gariepinus after grilling. As in grilling of large C. gariepinus the temperature in core (muscle) did not reach high enough to kill Staph aureus while frying temperature is high and reach high degree in core of fish so is sufficient to kill the microorganisms. This result agreed with those obtained by Gonzalez- Fandos et al. (2004), Mariappan et al. (2004) and Novotny et al .(2004). Tables (6,7) showed that frying lead to destroying of (10)⁵Salmonella typhimurium which inoculated at different sizes of both fish spp. While grilling only destroy $(10)^5$ Salmonella typhimurium which inoculated in O.niloticus and small& medium sized C.gariepinus but in large sized C.gariepinus, Salmonella could be detected in this fish after grilling as

2004 , Maysa&Abd-Ellal, 2009 and

C.gariepinus used was larger in thickness of it's muscle than that of Tilapia so the latter reach higher degree than in C.gariepinus so destrov the microorganisms. These results are similar to those obtained by Rahimifard et al.(2008) and Abeer (2010). Tables (6,7) showed the result of heat treatment on survival of $(10)^6 E$. coli O₁₅₇H₇ it was found that frying is efficient method of cooking could destroy the totally inoculated $(10)^{6}E$. coli O_{157} : H_7 in both fish species at varying sizes. While grilling less efficient than frying and could destroy the inoculated $(10)^6 Ecoli O_{157}: H_7$ in O. niloticus and small & medium sized C. gariepinus only but not destroy inoculated *coli* O_{157} : H_7 in large $(10)^{6}E.$ sized C.gariepinus spp. This results are similar to those obtained by Canadian food inspection (2005) and Abeer (2010). In addition, the obtained results in the present allow conclusion that the C. studv gariepinus fish had a higher bacterial isolates than O.niloticus fish. In the other side, the total isolates from the surface of both fish species was higher than those isolated from muscles of both fish species. Frying of fish is safer than grilling as frying lead to destroying of inoculated Staph. aureus, S. typhimurium and E. coli while after grilling these *O* 157H₇ microorganisms could be detected in large sized C. gariepinus muscle only. So, we recommend the could clarify and preventive and following corrective hygienic measures, to be employed, to avoid enteropathogens contamination and ensure safety of freshwater fish to be fit for human consumption: Prevention of sewage drain into fresh water, to prohibit the main source of contamination - application of obligatory hygienic training programs, personal hygiene and good hygienic practices, for fishermen and fish sellers periodical hygienic inspection of fish markets to ensure perfect cleaning and removal of waste, continual upgrading of hygienic certificates for sellers - awareness of customers for hygienic practices in

handling and efficient cooking of freshwater fish to ensure getting red off possible risk of enteropathogens and others.

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الكشف عن بعض الميكروبات البكتيرية المشتركة في أسماك السوق في محافظة القليوبية ومقاومتها

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الملخص العربي

في هذه الدراسة، تم جمع عدد مائتين (200) عينه عشوائيه من أسماك البلطي النيلي والقرموط الأفريقي (بمعدل 100 لكل نوع) من مختلف أسواق الأسماك بمحافظة القليوبيه بالاضافه الى ذلك تم أخذ عدد مائه (100) مسحه جلدية من أيدى بائعى الأسماك (60 مسحه) وربات البيوت (40 مسحه) من نفس الاماكن التي تم جمع عينات الأسماك منها بنفس المحافظ بتم فحص هذه العينات لوجود بعض الميكروبات البكتيرية المشتركة المنقولة من اسماك السوق مثل الميكروب المكور العنقودي الذهبي، السالمونيلا، الايشريشياكولاي والميكروب المكور السبحي . وايضا تم الكشف عن تأثير المعالجة الحرارية مثل القلي و الشوى على بقاء الميكروب المكور العنقودي الذهبي(10)4، السالمونيلا (10)5، الايشريشياكولاي O157:H7(10)⁶ في كلا نوعي السمك المستخدم وقد تبين من هذه الدراسة أن اسماك القرموط الأفريقي أعلى في العزل البكتيري (8 9%) من اسماك البلطي النيلي (6%). و أن الميكروب المكور السبحي كان متواجدا بنسبة عالية (13.5%) يليه السالمونيلا بنسبة (11.5%) ثم يليه الميكروب المكور العنقودي الذهبي بنسبة (4.5%) وجاءت الايشريشياكو لأي بنسبة (2%) . وقد تم عزل هذه الميكروبات من على السطح الخارجي لهذه الاسماك بنسبة اعلى من عضلات هذه الاسماك وكذلك عينات المسحة الجلدية من ايدى بائعي الأسماك وربات البيوت أظهرت أنه تم عزل الميكروب المكور العنقودي الذهبي والسالمونيلا والايشريشياكولاي والميكروب المكور السبحي بالنسب الأتية: 35 %،20،%35،% من أيدى بائعي الاسماك على التوالي و37.5%25%25% و50% من ايدى ربات البيوت على التوالي. ايضا اوضحت النتائج ان قلى السمك أكثر كفاءه في التخلص من (10)⁴ من الميكر وب المكور العنقودي الذهبي، $(10)^5$ من السالمونيلا و $(10)^6$ من الايشريشياكولاي O157:H7 بنسبة 100% في كلا نوعي السمك المستخدم وعند اوزان مختلفه ولكن الشوى لم يؤدي الي التخلص من هذه الميكروبات في سمك القرموط الأفريقي الكبير وزناً فقط وقد تم دراسة ومناقشة الاهميه الصحيه للميكروبات المعزوله وكذلك اقتراح التوصيات اللازمه للحماية من هذه الميكر وبات.

. (مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):126- 136, يونيو 2014)