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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.coli*$O_{157}H_{7}$ 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

Fish 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 Lakshumana-perumalsamy, 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 to kill all harmful microorganisms which may be present in raw fish prior to preparation **(** Mansour *et al.,* 1997**)**. The consumption 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. coli*$O_{157}H_7$ 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 O_{128}*:*k_{67}, 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* O_{119}*:*k_{69} *,* O_{125}*:*k_{70} *,*O_{26}*:*k_{60} *, O55:k59* , *O111:k58* and O_{124}*:*k_{72}, 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

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

Examined / Isolates	*O. niloticus* (100)		*C. gariepinus* (100)		Total (200)	
	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 (2): Frequency distribution of isolated microorganisms in surface and muscle samples of examined fish.

Examined fish Parts / Isolates	*O. niloticus*					*C. gariepinus*				
	Total isolates	Surface No	%	Muscle No	%	Total isolates	Surface No	%	Muscle No	%
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 of isolated microorganisms from examined fish

Number	*O. niloticus*	*C. gariepinus*
Staph. aureus	*3*	*6*
S. typhimurium [O(1, 4 , 5 , 12) H (i:1,2)]	*3*	*7*
Salmonella enteritidis[O (1,9, 12) H(g,m:1,7)]	*3*	*6*
Salmonella chester[O(1, 4 , 5 , 12) H (e,h:e,n,x)]	*0*	*1*
Salmonella Virchow[O(6,7, 14) H(r:1,2)]	*1*	*0*
Salmonella anatum[O(3, 10 , 15 , 34) H (e,h:1,6)]	*0*	*1*
Salmonella muenster[O(3, 10 , 15 , 34) H (e,h:1,5)]	*1*	*0*
E.coli O111:k58		*1*
E. coli O55:k59	*1*	*1*
E. coli O128: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*

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

Examined human / Isolates	Fish sellers (60)		House wives (40)	
	No	%	No	%
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 (5): Types of isolated microorganisms from human samples.

Number / Types of isolates	Fish sellers	House wives
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.coli$O_{157}H_7$	_	_	_	_	_	_

(-): absent

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

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 (+): 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 samples could 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 syndromes including gastroenteritis, 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* ,*O111:k58* and O_{128}:k_{67}. 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 different sources of 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) showed results of bacteriological 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, O124:k72, O55:k72, O111:k58 and* for Streptococci, the most predominant serotypes were *Strept. Faecalis*, *Strept. Angiosus*, *Strept. faecium*, *Strept. mitis*, *Strept. sanguis*. These results are supported by **(**Bercovier *et al*., 1997, Esaki *et al*., 2004 **,** Maysa&Abd-Ellal, 2009 and 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 emphasized a significant fecal 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 $(10)^5$*Salmonella 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

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 destroy 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* $O_{157}H_7$ 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 $(10)^6$*E. coli* O_{157}*:*H_7in large 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 study allow conclusion that the C. 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* $O\ 157H_7$ while after grilling these microorganisms could be detected in large sized C. gariepinus muscle only. So, we could clarify and recommend the following preventive and 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 البلطى النيلى والقرموط الأفريقى (بمعدل 100 لكل نوع) من مختلف أسواق الأسماك بمحافظة القليوبيه.بالاضافه الى ذلك تم أخذ عدد مائه (100) مسحه جلدية من أيدى بائعي الأسماك (60 مسحه) وربات البيوت (40 مسحه) من نفس الاماكن التى تم جمع عينات الأسماك منها بنفس المحافظه.تم فحص هذه العينات لوجود بعض الميكروبات البكتيرية المشتركة المنقولة من اسماك السوق مثل الميكروب المكورالعنقودى الذهبي، السالمونيلا، الايشريشياكولاى والميكروب المكورالسبحى . .وايضا تم الكشف عن تأثير المعالجة الحرارية مثل القلي و الشوى على بقاء الميكروب المكورالعنقودى الذهبى(10)4، السالمونيلا (10)5، الايشريشياكولاى $O_{157}:H_7$(10)^6 فى كلا نوعى السمك المستخدم . وقد تبين من هذه الدراسة ان اسماك القرموط الأفريقي أعلى في العزل البكتيري (9.8%) من اسماك البلطي النيلى (6%). و أن الميكروب المكورالسبحى كان متواجدا بنسبة عالية (13.5%) يليه السالمونيلا بنسبة (11.5%) ثم يليه الميكروب المكورالعنقودى الذهبى بنسبة (4.5%) وجاءت الايشريشياكولاى بنسبة (2%) . وقد تم عزل هذه الميكروبات من على السطح الخارجى لهذه الاسماك بنسبة اعلى من عضلات هذه الاسماك. وكذلك عينات المسحة الجلدية من ايدى بائعى الأسماك وربات البيوت أظهرت أنه تم عزل الميكروب المكور العنقودى الذهبى والسالمونيلا والايشريشياكولاى والميكروب المكور السبحى بالنسب الأتية: 35 %،35%،20%،35% من أيدي بائعي الاسماك على التوالي و37,5%،25%،37,5%و50% من ايدى ربات البيوت على التوالي. ايضا اوضحت النتائج ان قلى السمك أكثر كفاءه فى التخلص من (10)^4 من الميكروب المكور العنقودي الذهبي، (10)^5 من السالمونيلا و(10)^6 من الايشريشياكولاى $O_{157}:H_7$ بنسبة 100% فى كلا نوعي السمك المستخدم وعند اوزان مختلفه ولكن الشوى لم يؤدى الى التخلص من هذه الميكروبات فى سمك القرموط الأفريقي الكبير وزنا فقط. وقد تم دراسة ومناقشة الاهميه الصحيه للميكروبات المعزوله وكذلك اقتراح التوصيات اللازمه للحماية من هذه الميكروبات.

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