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PREVALENCE AND HISTOPATHOLOGICAL CHANGES OF *Staphylococcus aureus* INFECTION OF CATFISH (*Clarias garipeneaus*)

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Abstract

Bacteriological examination was performed on 150 catfish (*Clarias garipeneaus*) with a body weight ranged from 200 – 250g suffered from ulcerated and eroded skin. Fish were collected from Bahr ELbaker in Sharkia Government and fish market in Abu-Hammad, Sharkia, in summer season of 2016 for *staphylococcus aureus* isolation. The prevalence of *staphylococcus aureus* isolaties was 100% for all examined fish. The prevalence of *S. aureus* lesions, gills, skin, liver, kidney and intestine infection were 100, 100, 40, 30 and 17% respectively. Histopathology of experimentally infected fish was done. The results showed that, the histopathological changes induced in the gills were represented by mononuclear cell infiltration in gill lamellae and gill arch with focal epithelial desquamation. The skin exhibited vacuolar and ballooning degeneration in the epidermis. The musculature displayed edema, hemorrhage, hyaline degeneration and Zenker's necrosis. The liver showed congestion in the central vein and portal vessels. It could be concluded that, fish can be contaminated by organisms that may be found in either water, or post harvest or either during marketing. This microbial contamination may affect the fish quality. So the basic principles for prevention of food borne disease and production of fish of high quality should be implemented.

INTRODUCTION

The history of freshwater aquaculture, particularly fish farming has been characterized by a great deal of health problems which have often led to loss in production and major economic loss. Infectious diseases have a major impact on freshwater fish farming. Therefore, the target organs in which the bacteria

reside and cause degenerative changes need to be studied. An important epidemiological tool used for disease diagnosis is histopathological studies (Ajithund *et al*., 2014). Fish possess bacterial populations on or in their skin, gills, digestive tract and light-emitting organs. Internal organs as kidney, liver and spleen of healthy fish may contain bacteria, but there is debate about whether or not muscle is actually sterile. These bacteria present as normal microflora (Duğenci and Candan, 2003). More than 92 bacterial genera reported as pathogen of freshwater and marine fishes such as *Aeromonas, Pseudomonas, Mycobacterium, Edwardsiella, Streptococcus, Staphylococcus, Losteridium* (Austin and Austin, 1993). *Staphylococcus spp*. are not a part of fish microflora (Herrero*et al.,* 1999), fish acquired infection by *Staphylococcus* spp. from polluted water by fecal contamination or post harvesting contamination through contaminated equipments or infected workers (Mousa and Mahmoud, 1997). *Staphylococcus aureus* has been reported as the third major causative agent of foodborn illness by fish and fish products in the European Union (Le-loir *et al*., 2003). *Staphylococcus aureus* is belongs to the family Micrococcaceae which contains more than 30 species but, *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermis* and *Staphylococcus haemolyticus* are consider the most virulent and pathogenic strain (Winn Washington *et al.,* 2006). Staphylococcus species are one of the most important food borne opportunistic bacteria in fishes and some are potential pathogens and the high population of these bacteria indicates the degree of the spoilage (Albuquerque *et al.,* 2007). *Staphylococcus aureus* were isolated from *Tilapia zillii* and *Solea vulgaris* live fish were collected from Lake Qarun, El Fayoum Governorate, during mass mortality at summer season (Younes *et al*., 2016).

The present study aimed to isolate *Staphylococcus aureus* from clinically infected catfish (*Clarias garipeneaus*) survived in Bahr ELbaker or fish market and examined pathological changes of infected tissues and organs.

MATERIALS AND METHODS

Fish samples:

A total of 150 Catfish ***(****Clarias garipeneaus****)*** with a body weight ranged from 200 – 250g suffered from ulcerated skin and erosions were collected from sewage canal (Bahr ELbaker) in Sharkia Government. In summer season in 2016, fish were transferred a live to the laboratory and subjected to full clinical and postmortem examination according to Noga (1996).

Bacteriological examination of samples:

Cultivation:

Swab was taken from each sample and streak into nutrients agar, blood agar, Baird parker agar, Mannitol salt agar. All plates were incubated at 37^0C for 48 hours and examined daily for bacterial growth.

Identification of *S. aureus*:

The suspected colonies were examined for their colonial character, haemolytic activity on 5% Sheep blood agar, microscopical examination and biochemical character according to (Quinn *et al.,* 1994).

A- The morphological characters: Smears from single colonies were prepared, stained with Gram stain and examined microscopically to observe the morphology, arrangement and staining reaction according to (APHA, 1992).

B- Biochemical identification:- Using haemolytic activity on 5% sheep blood agar, coagulase test, Catalase test, mannitol fermentation, salt tolerance using mannitol salt agar. Fermentation of sugar (maltose, sucrose), urease and nitrate reduction test were carried out and obtained result were recorded according to (MacFaddin, 2000).

Prevalence of *S. aureus* isolates to the different tissues and organs:

The prevalence of *S. aureus* isolated from different tissues and organs through this study was occurred and discussed.

Experimental studies:

It was done according to the methods described by Finlay and Falkaw (1989), a total 40 apparently healthy cat fish (150±5 g) body weight were divided into two groups. First group was injected i/p with 1ml of 1.5 x 10^8 CFU/ml of *S. aureus* at 24 hr live and second group was injected with 1 ml sterile saline as control group. Fish were fed with commercial pelleted feed. Fish were reared for 14 days in water at 20-25ºC and mortality was recorded. Infection was confirmed by re-isolation of bacteria from the internal organs (kidney, liver, gills, and intestine) of recently dead fish, using Baired parker agar medium.

Histopathological investigation:

Histopathological studies were carried out on tissues from naturally infected catfish**.** Samples of liver, kidney, intestine, gills and musculature were fixed in 10% buffered formalin. Dehydration in ascending grades of Ethanol and cleared in xylene. Tissues were embedded in parafin and processed routinely for light microscopy. Slides were prepared as mentioned by Bancroft *et al.* (2008) and stained by Hematoxylin and Eosin stains.

RESULTS AND DISCUSSION

Clinical and postmortem findings:

The examined fish suffered from anorexia, lethargies, pale gills, disorientation, skin lesion with discoloration of skin associated with the development of different patches of ulceration and hemorrhage and excessive secretion of mucus on the skin and gills (photo 1). Postmortem finding revealed the presence of septicemia in the internal organs, liver was enlarged and congested with necrosis in some fishes, kidney (photo 2) was congested, enlarged and hemorrhagic enteritis in some fishes. Infected fish by staphylococcus species initially showed no clinical signs or abnormalities and only a few cases revealed exophthalmia, or lesions in the epidermis and fins (Shih-Ling *et al.,* 1999). In contrast, Kusuda and Sugiyama (1981) showed that

typical signs in yellowtail (*S. quinquiradiata*) and red sea bream (*C. major*) caused by *S. epidermidis* included exophthalmia, congestion and ulcerations on the tail.

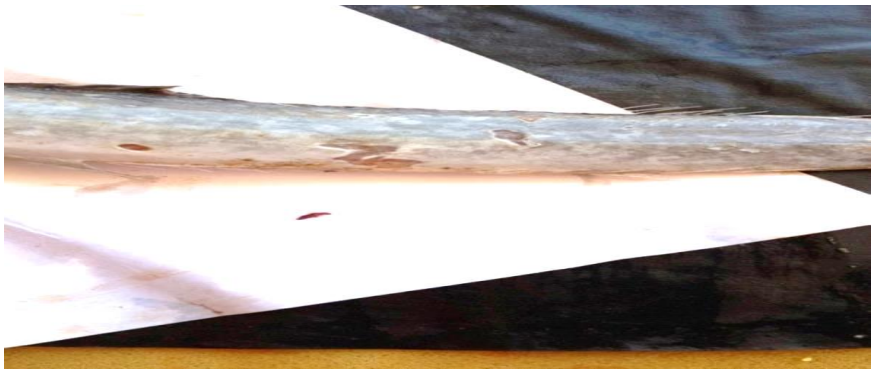

Photo1. Catfish naturally infected by Staphylococcal spp., showing skin ulcers.

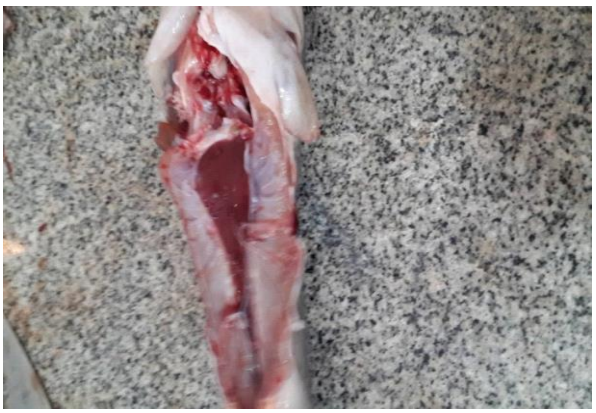

Photo 2. Catfish, infected by Staphylococcal spp., showing congested kidney.

Bacterial identification:

Isolated samples were cultivated on Baird Parker agar (BPA) characteristic appearance of black shining convex colonies of 1-1.5 mm in diameter with narrow white margin and surrounded by a clear area extended into opaque medium were suspected *Staphylococcus aureus* (photo3). It was

Gram +ve cocci arranged in cluster which agreed with Da Silva and Da Silva (2005) and Aslantas *et al.* (2007).

Photo 3. *Staphylococcus aureus* on Baired parker agar medium which appeared as black shining convex colonies surrounded by halo zone.

Morphological characteristic:

The smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes. In the present study, 430/750 isolates from fresh water Catfish samples (*Clarias garipeneaus*) identified as *Staphylococcus aureus* by colony characters, microscopical examination and biochemical tests (table 1) according to Quinn *et al.* (1994).

Table 1. Biochemical characteristic of isolated *Staphylococcus aureus*.

Tests	Reaction
Gram stain	Gram positive
Catalase	Positive
Coagulase	Positive
Oxidase	Negative
Shape	Spherical cocci
Arrangement	Grapes
Motility	Negative
Haemolysis	Beta haemolysis
Anaerobic utilization Glucose	Positive
H2S production	Positive

Prevealence of *Staphylococcus aureus* among *isolaties* examined organs and tissues in catfish:

Results in Table (2) revealed that, 430 out of 750 samples from infected catfish (*Clarias garipeneaus*) give characteristics colonies on BP agar, which identified as *Staphylococcus aureus*, Total prevalence of *Staphylococcus aureus* isolates among different examined organs and tissues were 100% from gills and skin but 40% in liver and 30% in the kidneys. The percentage of *Staphylococcus aureus* isolaties reported in the present study was relatively higher than those of freshwater fishes reported by Ali (2014) and El-Olemy *et al.* (2014). These results disagreed with Athanassopoulou *et al.* (1999) who reported that, the total prevalence of *Staphylococcus epidermis* among diseased Puntazzo in marine aquaculture systems in Greece was 10%. The high incidence of *Staphylococcus aureus* in the examined samples could indicate unhygienic conditions because the product contamination could be the results of combination of improper handling, improper storage and cross contamination (Simon and Sanjev, 2007).

Table 2. Prevalence of *Staphylococcus aureus* among isolaties examined different organs and tissues of infected Catfish.

Organ	No. of examined samples	Positive / 150		Negative / 150	
		No.	%	No.	%
Gills	150	150	100	0	0
skin	150	150	100	0	0
Liver	150	60	40	90	60
Kidney	150	45	30	105	70
Intestine	150	25	17	125	83
Total	750	430	57	320	42.6

Histopathological Results:

Catfish infected by *S. aureus* showed some pathological changes in various tissues. The gills showed mononuclear cell infiltration in gill lamellae and gill arch. The secondary gill lamellae showed either focal desquamation or epithelial hyperplasia as shown in photo (4). The skin exhibited vacuolar and ballooning degeneration in the epidermis. In some cases the epidermis showed

either erosion and ulceration. The under laying dermis suffered marked edema, hemorrhage as well as proliferation of melanomacrophage cells and mononuclear cells as shown in photo (5). The muscle bundles displayed edema, hemorrhage, hyaline degeneration and Zenker's necrosis. Melanomacrophage and mononuclear cells infiltration were seen around the necrotic muscles as shown as in photo (6). A former histopathological study performed on the tissues of fish infected by *Staphylococcus epidermidis* where electron-dense particles were observed in the internal tissues; the nuclear ultrastructural reactions and the particles were suspected to be expression of apoptosis by Kerr *et al*. (1972). Moreover, Chen *et al*. (1983) revealed severe nuclear changes with many electron-dense particles in the affected cells.

The liver showed congestion in the central vein and portal vessels. Some of hepatocytes showed vacuolar degeneration with pyknotic nuclei and others were necrotic and lacked their nuclei (keryolysis) as shown in photo (7). Few mononuclear cells were evident perivascular and infiltrated the necrotic area which gives characteristic of Staphylococcal infection, early granuloma while central liquifactive necrosis surrounded by mononuclear cells as well as melanomacrophages cells were evident photo (8). Shih-Ling *et al*. (1999) studied the epizootiology and Pathogenicity of *Staphylococcus epidermidis* in tilapia where focal necrosis with multiple diffuse granulomas of necrotic centers was observed in spleen parenchyma and kidney hematopoietic tissue together with focal necrosis and granuloma in the affected pancreas, liver, mesentery and gonad.

The kidney revealed tubular nepherosis in the renal tubule mainly vacuolar degeneration, however some renal epithelium were necrotic. Activation of melanomacrophages and hematopoietic tissue was evident in the renal tissue as shown in photo (9). The intestine showed mucinious degeneration in epithelial lining with focal epithelial desquamation and mononuclear cells infiltration in the lamina propria and submucosa. Vaslentine *et al*. (2016) examined the histopathology of staphylococcus infection in sturgeon where renal distrophy and catarrhal enteritis were seen.

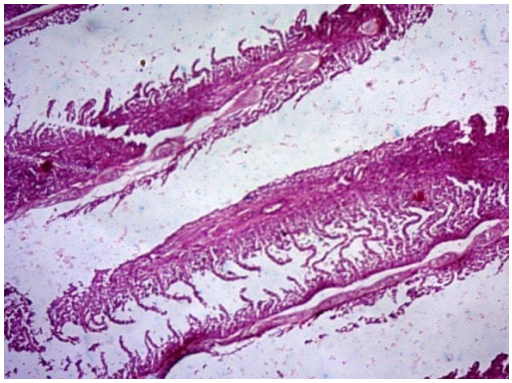

Photo 4. Gills, of naturally Catfish infected by *S. aureus* showing, focal desquamation or epithelial hyperplasia of the secondary lamellae. H&E stain, x 100.

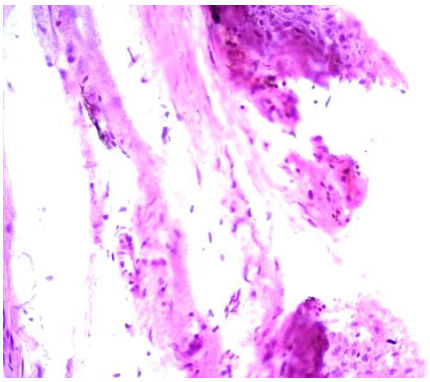

Photo 5. Skin, of naturally Catfish infected by *S. aureus*, showing epidermal ulceration and edema, hemorrhage as well as proliferation of melanomacrophage cells and mononuclear cells in the dermis. H&E stain, x 100.

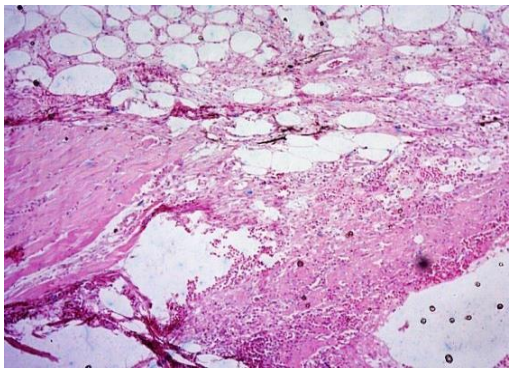

Photo 6. Muscles, of naturally Catfish infected by *S. aureus*, showing edema, hemorrhage, hyaline degeneration and Zenker's necrosis and melanomacrophages and mononuclear cells infiltration. H&E stain, x 100.

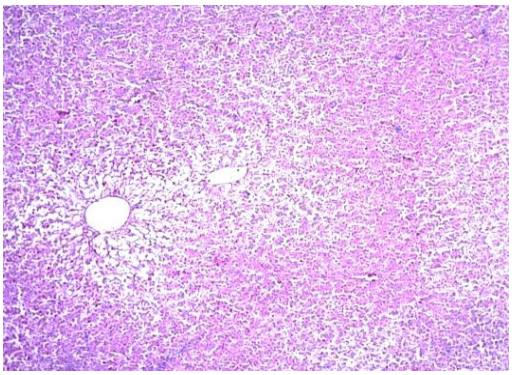

Photo 7. Liver, of naturally Catfish infected by *S. aureus*, showing vacuolar degeneration with focal necrosis. H&E stain, x 100.

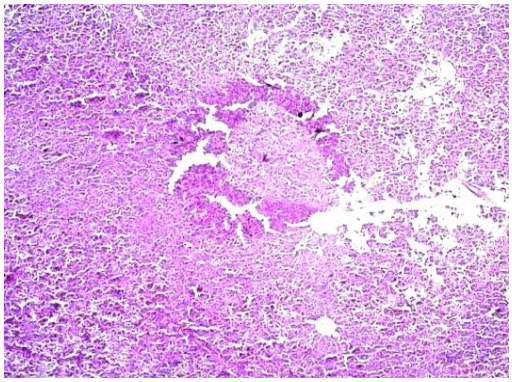

Photo 8. Liver of naturally Catfish infected by S.aureus showing granuloma with liquifactive necrosis surrounded by mononuclear cells and melanomacrophages cells. H&E stain, x 100.

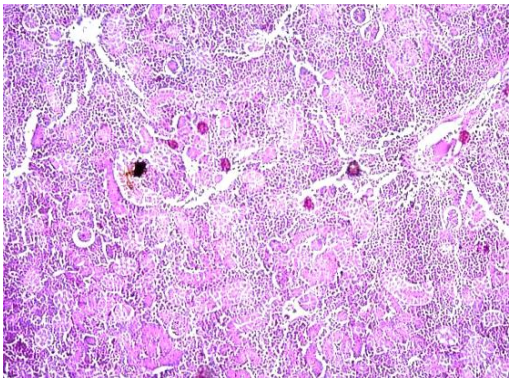

Photo 9. Kidney of naturally Catfish infected by *S. aureus*, showing tubular nepherosis in the renal tubule with activation of melanomacrophages and hematopoietic tissue. H&E stain, x 100.

Results of experimental studies:

The mortality results from Table (3) revealed that I/P Injection of Cat fish with virulent strain of *Staphylococcus aureus* from fish origin) 1ml of 24 hours live culture) were 50% in all groups except control group 0 .% the moribund catfish showed dark body, fin and skin necrosis to shallow ulcers infected fish may showed no clinical sign except pale liver and congested kidney which agree with Siti-Zahrah *et al.* (2008).

Table 3. Mortality patterns 7 days post i/p injection of cat fishes with virulent strains of *Staphylococcus aureus* from fish origin (1ml of 24 hours live culture).

Fish group	Fish No.	No. of diseased fish 7 day post-injection							Dead fish		Survived fish	
		1	2	3	4	5	6	7	No.	%	No.	%
Group 1	**10**	-	1	2	-	1	1	-	5	50	5	50
Group 2	**10**	1	2	1	-	1	-	-	5	50	5	50
Group 3	**10**	-	1	2	1	1	-	-	5	50	5	50
Control	**10**	-	-	-	-	-	-	-	0	0	10	100

CONCLUSION

Fish are susceptible to some microbial contaminants including *Staphylococcus aurous* that may be found in water, during harvest or marketing. This microbiological activity could minimize the fish quality as a consequence of the recorded hisopathological alterations or induce food borne disease with public health hazard.

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التغيرات الهستوباثولوجية فى اسماك القرموط الأفريقى المصابة بميكروب العنقودى الذهبى

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الملخص العربى

تناولت الدراسة عزل وتصنيف بكتريا المكورات العنقودية فى عينات أخذت من أسماك مصابه إكلينيكيا 150(سمكة من القرموط الأفريقى) التي تم تجميعها عشوائياً من المياه الصرف الصحى ببحر البقر بالشرقية خلال موسم الصيف لعام 2016 والتي ظهرت عليها علامات التقرحات الجلديةوتم عزل وتصنيف بكتريا المكورات العنقودية بنسبه 100% من العينات المجمعة. وتم عمل دراسة عدوى سمك القراميط بعترات الميكروب العنقودى الذهبى على الاسماك واظهرت التهابات فى الجلد، فقر دم فى الخياشيم، انتفاخ فى القناة الهضمية. تم عزل الميكروب من الخياشيم والتقرحات والكبد والكلى والامعاء نسبة 100%، 100%، 60%، 45% و 25% على التوالى. وايضا تم عمل فحص الهستوباثولوجى للعينات للاسماك المصابه بالميكروب الذى اوضح تغيرات هدامة ونخر واحتقان وتدرن فى الاحشاء الداخليه للاسماك المصابة. وقد لخص البحث الى ان التلوث الميكروبى قد يؤثر سلبا على جودة الاسماك.