A CONTRIBUTION ON BACTERIAL PATHOGENS INFECTING MULLET (*MUGIL CAPITO*) CULTURED IN FRESHWATER FARMS IN SHARKIA GOVERNORATE

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Abstract

A total number of a hundred (100) clinically diseased mullet (*Mugil capito*) were collected from various freshwater fish farms in Sharkia province and subjected to clinical, postmortem and bacteriological examinations. The common clinical signs were loss of appetite, swam near the surface of water, increased mucus secretion and congestion of gills. Hemorrhages at the base of fins, abdomen and gill cover. Necrotic areas on the gill cover were also recorded. Abdominal distention and intestinal prolaps were also observed. The postmortem findings were congestion of the internal organs and pale liver. The prevalence of the isolated bacteria were *Streptococcus iniae* (31.46%), *Aeromonas hydrophila* (26.96%). *Pseudomonas* species (10.11%), *Citrobacter fruendii* (10.11%) *Satphylococcus aureus* (8.98%), *Micrococcus* species (6.74%), *Flavobacterium* species (3.4%) and *Achromobacterium lividum* (2.24%). The prevalence of the bacteria in tissues and organs of naturally infected fish were recorded.

Ciprofloxacin and Trimethoprim +sulphamethoxazol were more effective antibiotics against *A. hydrophila* or *Pseudomonas* species, while Vancomycin was the most effective on *S. iniae*.

S. iniae was pathogenic to *Oreochromis niloticus*. At 0.5 x 10^7 cells/ml it causes 50% mortality when injected intraperitonealy. The morbidity was 100% without mortality in the I/M injected *O. niloticus*. It is anticipated that this information will help the prompt diagnosis of pathogenic agents for treatment and control.

INTRODUCTION

Global aquaculture production has been growing very rapidly for over two decades. *Mugil* species culture is one of an important aquaculture activity in Egypt and other counteries. The major factor which would hamper its successful development and sustainability would be diseases (Austin and Austin, 1993). Fish farming provide an ideal conditions for transmission of many diseases. Fish diseases represent the most important threats for rapid expansion of aquaculture industry. Cultured fishes subjected to considerable stress during collection and transportation (Roberts, 2001). Outbreaks of diseases attributed to bacterial pathogens are devasting to both culture and wild fish populations (Austin and Austin, 1993). The external clinical signs and postmortem lesions of infected fish make them unmarketable (Samaha *et al.*, 2004).

Because of great economic importance of *Mugil capito* farming, the aim of the present investigation aimed to through the light on the most prevailing bacterial diseases in a commercial aquaculture facility in Sharkia province.

MATERIALS AND METHODS

Fish:

A total of a hundred (100) live and freshly dead naturally diseased *Mugil capito* (average body weight 200 ±5 g) were collected from governomental and private freshwater fish farms and transfered to the laboratory. Clinical and postmortem examinations were carried out as described by Austin and Austin (1993).

Bacteriological examinations:

A loopful from gills, liver, spleen, kidneys, gonads and intestine were aseptically taken and cultured on tryptic soya agar (Difco), incubated at 25°C for 24 h. Purified isolats were identified by standard biochemical tests according to Bergey *et al.* (1984) and Austin and Austin (1993). In addition, API 20-E strip system (Bio Merieux) was used.

Sensitivity test:

Sensitivity of some bacterial isolates (*Streptococcus iniae, Aeromonas hydrophila* and *Pseudomonas* species) to different antibiograms, were estimated according to Carter and Cole (1990).

Experimental fish:

one handred *M. capito* (180 ± 5 g body weight) and ninty *Oreochromis niloticus* (80 ± 3 g body weight) apparently healthy were tested for susceptabilty to experimental infection with *S. iniae* isolated from naturally infected *M. capito*. The fishes were maintained in glass aquaria supplied with well-aerated de-chlorinated tap water to be acclimated. All experimental fish were fed with commercial ration at rate of 2% body weight. Four groups of *O. niloticus* were created, the first and second groups (30 fish of each) were injected at a dose of 0.5 ml of saline containing 10^7 cells/ml intraperitoneal (i.p.) and intramuscularly (i.m.) respectively. The third and fourth groups (15 fish of each) were inoculated i.p. and i.m. respectively with 0.5 ml saline as control groups. The aquaria were checked for fish mortality and any abnormal clinical signs daily and samples were seeded for bacterial reisolation .

RESULTS AND DISCUSSION

Fish diseases constitute one of the most important factor hindring the development and sustainability of aquaculture. Infectious diseases, in addition to causing mass mortalities among fishes, can reduce the value of fish as food for humans (Roberts 2001 and Samaha *et al.*, 2004)

The common clinical signs due to bacterial infections were loss of appetite, swam near the surface of water, increased mucus secretion and congested gills. Hemorrhages at the base of fins, abdominal part, gill cover and necrotic area on the gill cover. Abdominal distention and intestinal prolaps were observed. The postmortem findings were congested liver, in other cases congesion at the margins with necrosis and in others pale colour and yellowish white patches were observed. Spleen was dark in colour. Kidneys and gonads were congested and watery ascitic fluid was recorded.

Dealing with bacteriological agents isolated from the naturally infected *Mugil capito*, several bacterial isolates were obtained. The most common isolated bacteria were *Streptococcus iniae, Aeromonas hydrophila, Pseudomonas* species, *Citrobacter fruendii, Flavobacterium* species, *Achromobacterium lividum, Satphylococcus aureus* and *Micrococcus* species.

S. iniae had the highest prevalence (31.46%) among *M. capito* followed by *A. hydrophila* (26.96%). *Pseudomonas* species (10.11%), *C. fruendii* (10.11%) *S. aureus* (8.98%), *Micrococcus* species (6.74%), *Fl.* species (3.4%) and *Ac. lividum* had the lowest prevalence (2.24%) as shown in table (1).

Regarding to tissue distribution, (table, 1) showed the prevalence in gills, intestine, liver, spleen, kidneys and gonads were 28.1, 22.5, 16.85, 14.6, 8.98 and 7.86% respectively.

Streptococcal infections have increased in number during the last decade as a consequence of intensification of aquaculture and responsible for economic losses in the fish farms industry (Pasnik *et al.*, 2005). *S. iniae* are facultative pathogen that invades gills and skin tissue damaged by parastism and skin or alimentary canal damaged by environmental stress or nutritional disorders Austin and Austin (1999). *S. iniae* is the cause of disease in fish and mammals including human (Ausin and Austin, 1999). Toranzo *et al.* (2005) isolated *Streptococcus* sp. from mullet and from mari-culture. Our results showed that *S. iniae* infected *M. capito* with prevalence (31.46%) which, the cause of streptococcosis. The infected fish had eye opacity, distended abdomen, hemorrhages at the base of fins, on gill cover and congested gills. The postmortem findings were congesion of liver with pale colour aeras, congested intestine, kidneys were pale and dark spleen, watery ascitic fluid was observed (Fig 1). The prevalence of *S. iniae* among tissues was 9, 4.5, 5.6, 3.4, 4.5 and 4.5% in gills, intestine, liver, spleen, kidneys and gonads respectively. Similar results were recorded by Austin and Austin (1999) and Ahmed (2004).

The most common bacterial pathogen in freshwater fish "*A. hydrophila*" is considered a truly opportunistic pathogen, because it is relatively common in the aquaculture environment (Yanong and Francis-Floyd, 2002). *A. hydrophila* is the cause

of motile *Aeromonas* septicemia among *M. capito* as (26.96%) . The clinical signs were hemorrhages allover the body (fins, mouth, eyes, gill cover and urogenital openning), enlargment of abdomen. The potmortem findings were watery asitic fluid, severe hemorrhages of all internal organs. Petecial hemorrhages in liver with necrotic foci (Fig 2). The prevalence in the internal organs were 9, 5.6, 5.6, 3.4, 2.24 and 1.1% in gills, intestine, liver, spleen, kidneys and gonads respectively (table 1). This result was in agreement with Ahmed (2004) who isolated *A. hydrophila* from naturally diseased *Mugil* species.

Pseudomonas species has been considered as a secondary invader of damaged fish tissue as well as a primary poor and weak pathogen (Schäperclaus, 1979). *Pseudomonas* species was isolated from *M. capito* with prevalence (10.11%). The clinicl signs were petecial hemorrhage in skin, base of fins, mouth, gill cover and eyes. The postmortem findings were hemorrhages in all internal organs and in some cases the liver was yellowish white colour (Fig 3). The prevalence of *Pseudomonas* species among the internal organs were 4.5, 3.4 and 2.24% in gills, intestine and spleen respectively (table, 1). Ahmed (2004) isolated *Pseudomonas* species from naturally diseased *Mugil* species.

C. fruendii was isolated from fish suffered from hemorrahge in gill cover and different parts of the body sites and congested gills and its prevalence was 10.11%. The postmortem findings were hemorrhages with whitish coloured liver (Fig. 4). *C. freundii* had 3.4, 2.24, 2.24 and 1.1% in intestine, spleen, kidneys and gills, respectively (table, 1). Hua *et al.*, (2001) mentioned that *C. freundii* was pathogenic to different fish species causing septicemia and the mortality was 100% due to artificial infection.

Flavobacterium species and Ac. *lividum* were the Gram-negative bacteria isolated in rate of 2.24% and 1.1% from liver respectively. The liver suffered from congesion and yellowish white colour (Fig. 4). *Flavobacterium* species was isolated from congested intestine with percentage 1.1% and *Ac. lividum* from dark colour spleen 1.1%. *Diab (1997)* isolated *Flavobacterium* species from marine cultured mullet which had external wounds, erthema at the mouth and opercula with congestion of most internal organs. Figueiredo *et al.* (2005) isolated *Fl. columnare* from fingerling and adult Nile-tilapia and mentioned that *F. columnare* causing outbreak among *O. niloticus* and catfish. Madetoja, *et al.* (2006) reported on the efficacy of a mineral oil-based vaccine against *F. psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), under laboratory and field conditions.

Micrococcus species and *S. aureus* were Gram-positive bacteria isolated from different organs showing hemorrhage and anemia. The prevalence of *Micrococcus*

species was 2.24% in intestine and 1.1% from all other organs except kidneys (0.0%). While, *S. aureus* isolated at 3.4, 2.24, 1.1, 1.1 and 1.1% from gills, intestine, liver, spleen and gonads respectively (table, 2). Samaha *et al.* (2004) isolated *S. aureus* from gills of *M. cephalus* and *M. capito* by 3.81 and 6.82 % respectively. Pereira *et al.* (2006) isolated *S. aureus* with other pathogenic bacteria from different diseased fish species and Oyster.

Sensitivity test was carried out on *S. iniae, A. hydrophila* and *Pseudomonas* species which the most common bacterial diseases among cultured fishes.Table (2) shows that *S. iniae* was sensitive to Erthromycin, Streptomycin, Vancomycin and Tetracycline while it was resistant to Ampicllin, Nalidixic acid, Kanamycin, Ciprofloxacin, Trimethoprim +sulphamethoxazol and Salphonamide. *A. hydrophila* was sensitive to Ciprofloxacin, Erthromycin, Nalidixic acid, Trimethoprim +sulphamethoxazol and Tetracycline, while it was resistant to Ampicllin, Kanamycin, Streptomycin and Salphonamide. *Pseudomonas* species was sensitive to Ciprofloxacin, Kanamycin, Streptomycin and Trimethoprim +sulphamethoxazol while it was resistant to Ampicllin, Erthromycin, Nalidixic acid, Tetracycline, and Salphonamide. Ciprofloxacin and Trimethoprim +sulphamethoxazol were more effective against *A. hydrophila* and *Pseudomonas* species while; Vancomycin was the most effective on *S. iniae* that agree with Zorrilla *et al.* (2003). Ahmed (2004) who recorded that *A. hydrophila* senstive to Cipro while *Pseudomonas* species was sensitive to Rifampeine.

The results of pathogenicity of *S. iniae* among *O. niloticus* (table, 3) showed that the morbidity was 50 % in *O. niloticus* when injected I/P and 100% in *O. niloticus* when injected I/M 48 h post infection. The clinical signs were haemorrhages at the site of injection and changed to ulcer after one week of injection (Fig 5). The postmortem changes showed hemorrhages in all internal organs. The mortality was 50%. No mortality was recorded in the I/M injected *S. iniae* group. The clinical signs were disappeared from the experimentally injected I/M with pathogenic *S. iniae* within a month. While the control groups showed neither clinical signs nor lesion. Inglis *et al.* (1993) noticed that *Streptococcus* species caused mortality 50% within 3-7 days among freshwater fishes including mullet, while Ferguson *et al.* (1994) recorded that the mortality rate as 100% within 2-4 days minnows. Unfortunetly, we faild to maintain *M. capito* alive. All fish were found dead in the third day of acclimation because *M. capito* is very sensitve to environmental changes. So we can not make pathogenicity test and completed in *O. niloticus* which in polyculture system. Also, we can not make treatment trials among *M. capito* but this information will help the prompt diagnosis of pathogenic agents for treatment and control.

Table 1. The prevalence of isolated bacteria to the different organs.

Isolates	No., of isolate	%	Organs											
			Gills		Intestine		Liver		Spleen		Kidneys		Gonads	
			No	%	No	%	No.	%	No.	%	No.	%	No.	%
Aeromonas hydrophila	24	26.96	8	9.0	5	5.6	5	5.6	3	3.4	2	2.24	1	1.1
Pseudomonas species	9	10.11	4	4.5	3	3.4	0	0	2	2.24	0	0	0	0
Citrobacter freundii	9	10.11	1	1.1	3	3.4	1	1.1	2	2.24	2	2.24	0	0
Flavobacterium species	3	3.4	0	0	1	1.1	2	2.24	0	0	0	0	0	0
Achromobacterium lividum	2	2.24	0	0	0	0	1	1.1	1	1.1	0	0	0	0
Streptococcus inai	28	31.46	8	9	4	4.5	5	5.6	3	3.4	4	4.5	4	4.5
Staphylococcus aureus	8	8.98	3	3.4	2	2.24	1	1.1	1	1.1	0	0	1	1.1
Micrococcus species	6	6.74	1	1.1	2	2.24	1	1.1	1	1.1	0	0	1	1.1
Total	89	100	25	28.1	20	22.5	15	16.85	13	14.6	8	8.98	7	7.86

Table 2. Sensitivity of some bacterial isolates to different antibiograms.

Antibiotic againt	symbol	Concentration (mcg)	Susceptible zoon (mm)	*A. hydrophila*		Pseudomonas. spp.		*Streptococcus inai*	
				inhibition zoon (mm)	Sensitivity reaction	inhibition zoon (mm)	Sensitivity reaction	inhibition zoon (mm)	Sensitivity reaction
Ampicllin	AM	10	≥29	16	R	7	R	13	R
Ciprofloxacin	CIP	5	≥21	32	S	27	S	17	R
Erthromycine	E	15	≥18	20	S	8	R	22	S
Kanamycin	K	30	≥18	15	R	18	S	10	R
Nalidixic acid	NA	30	≥19	28	S	12	R	8	R
sulphonamycin	P	10	22-29	0.0	R	0.0	R	15	R
Streptomycin	S	10	≥15	10	R	16	S	16	S
Trimethoprim + sulphamethoxazol	Sxt	1.25 / 23.75	≥16	28	S	17	S	0.0	R
Tetracycline	TE	30	≥19	22	S	16	R	22	S
Vancomycin	VA	30	≥12	0.0	R	0.0	R	20	S

Table 3. Pathogenicity of *Streptococcus* species among *O. niloticus* .

Bacterial isolate	*Streptococcus* species		Control	
Rout of injection	I/P	I/M	I/P	I/M
Dose	0.5×10^9 cells/ml	0.5×10^9 cells/ml	0.5 ml of saline	0.5 ml of saline
No. of injected fish	30	30	15	15
Mortality %	50	0.0	0.0	0.0
Morbidity %	50	100	0.0	0.0

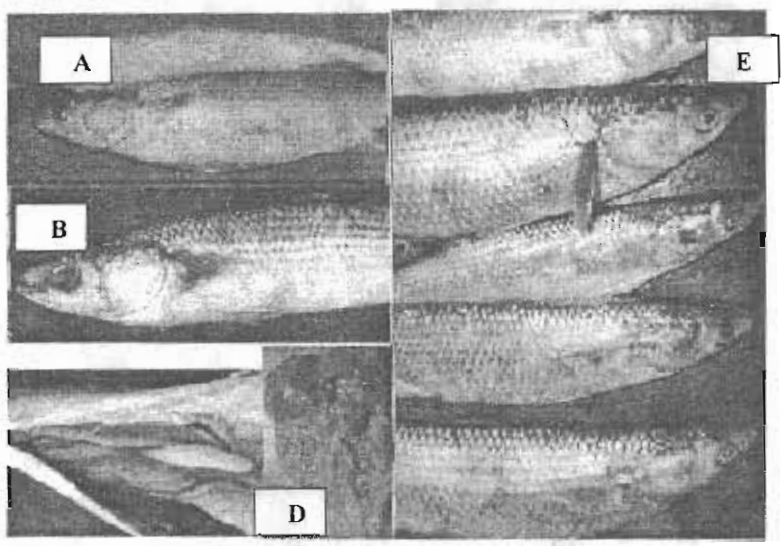

Fig. 1. *Mugil* capito naturally infected with *Streptococcus* iniae showing (A) abdominal distention (B) hemorrhages at the base of fins, (E) opercular cover, eyes and eye opacity, and (D) congestion in the internal organs.

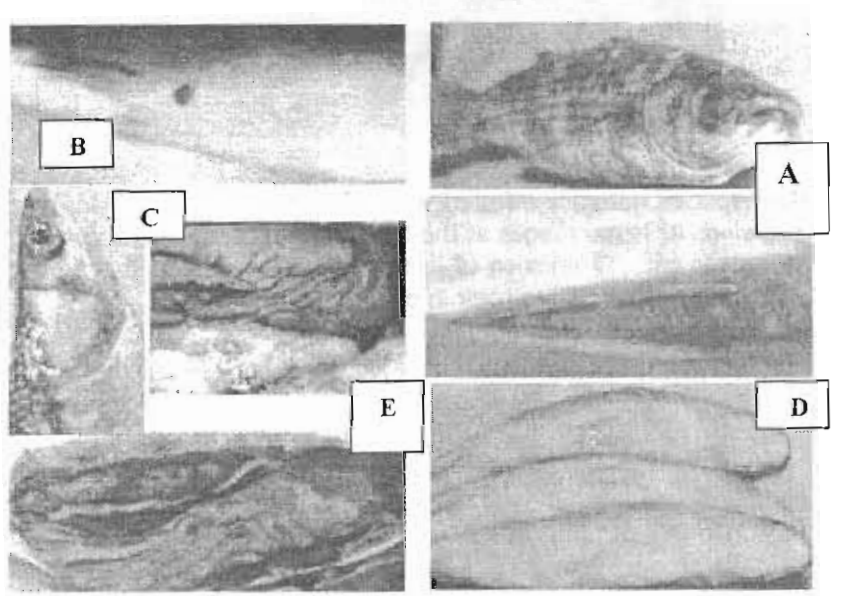

Fig. 2. *Mugil* species naturally infected with *Aeromonas hydrophila* showing (A) hemorrhages all over the fish body, fins, eyes, mouth, (B) urogenital opening, (C) opercular cover and (D) abdominal distention. (E) Sever congestion in all internal organs.

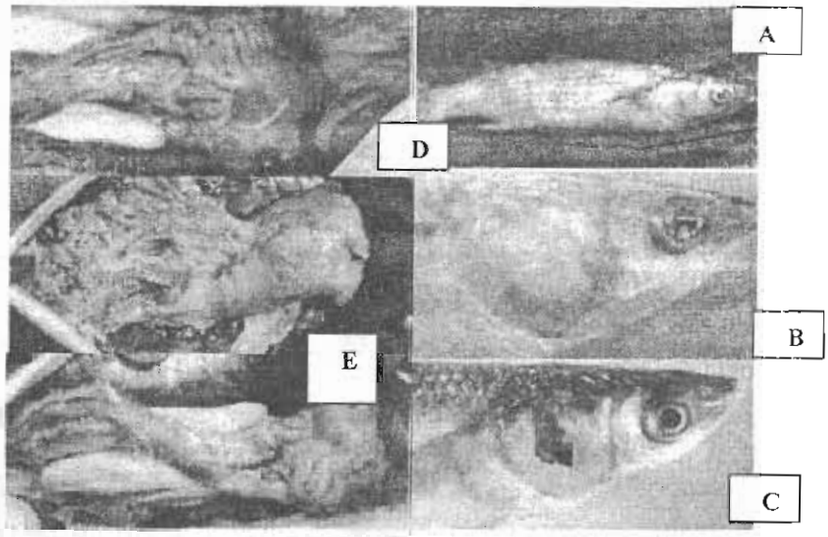

Fig. 3. *Mugil* capito naturally infected with *Pseudomonas speices* showing (A) erythemic hemorrhages on different parts of fish skin. Hemorrhages at the base of (A) fins, (B) opercular cover, (C) eyes). (D) Petechial hemorrhage in liver and (E) yellowish white colour in others.

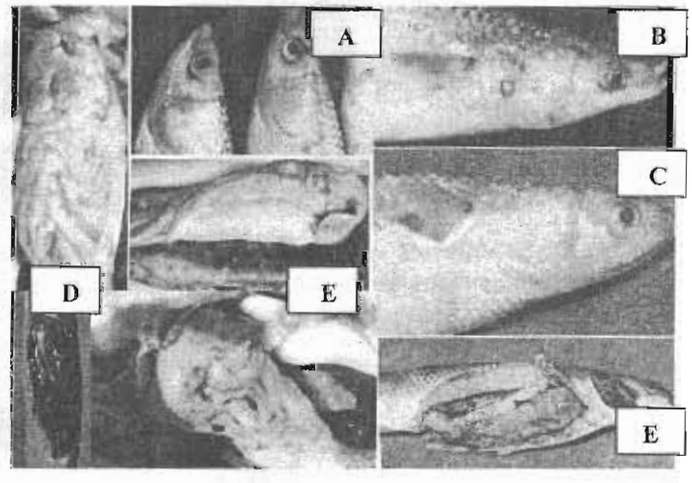

Fig 4. *Mugil* species naturally infected with Gram-negative and Gram-postive isolates showing (A) hemorrhages at the base of fins and opercular cover. (B) Ulcer on gill cover and (C) erection of lateral fin. Hemorrhages in the internal organs, yellowish white to white color in others.

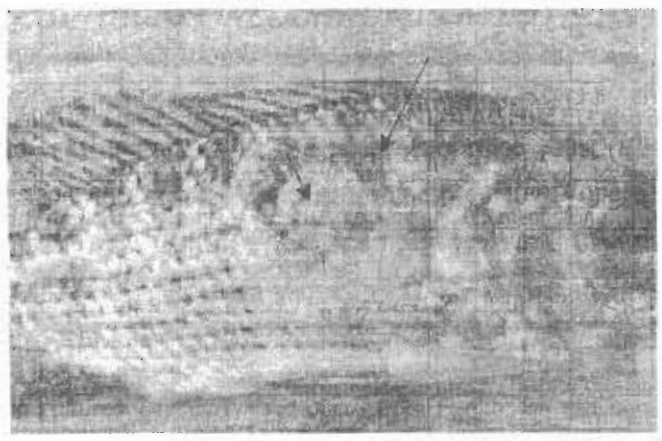

Fig 5. *Oreochromis niloticus* infected expermintally by I/M injection with 0.5 ml x10^7 cells/ml of *Streptococcus* species showing hemorrhaged at the side of injection with ulceration.

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إسهامة عن العدوى ببعض البكتيرية الممرضة لأسماك البوري المرباة في مزارع مياه عذبة في محافظة الشرقية

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تم إجراء هذه الدراسة علي عدد ١٠٠ سمكة من أسماك البوري المرباة في مزارع أسماك مياه عذبة والتي تم تجميعها في محافظة الشرقية لعمل حصر للمشاكل البكتيرية التي تواجه استزراع أسماك البوري. تم عزل بعض العترات البكتيرية السالبة والموجبة الجرام من أسماك العائلة البورية المصابة طبيعيا وتتمثل العلامات المرضية في وجود أنزفة علي الجلد وعلي أماكن متفرقة من جسم الأسماك، (علي الغطاء الخيشومي، العين، قاعدة الزعانف ، الفتحة الشرجية والخياشيم) مع تضخم البطن (استسقاء). وتمثلت الصفة التشريحية لهذه الأسماك في وجود أنزفة لكل الأعضاء والأنسجة الداخلية، وفي بعض العينات كان الكبد ذات لون أصفر مع وجود تنكرز، والبعض الآخر الكبد أبيض اللون. دكانه في لون الطحال، مع وجود سائل مائي في التجويف البرتوني. تم عزل بكتيريا ستربتوكوكس إنياي، ايروموناس هيدروفيلا، سودوموناس، ستروبكتر فرندای. ستافيلوكوكس اوريس، ميكروكوكس، فلافيبكتيريم و اكروموبكتيريم ليفيديم بنسبة ٣١,٤٦، ٢٦,٩٦، ١٠,١١، ١٠,١١، ٨,٩٨، ٦,٧٤، ٣,٤و ٢,٢٤ علي التوالي.

كلا من السبروفلكساسين و الترای‌ميثوبريم والسلفاميثكسازول لهما تأثير قوى كمضاد حيوي لكل من ايروموناس هيدروفيلا و سودوموناس، بينما فانكوميسين له تأثيره علي بكتيريا الستربتوكوكس. كانت نتيجة العدوى الصناعية لعترة ستربتوكوكس بين أسماك البلطي النيلي والمحقونة ٠,٥ ملي $\times ١٠^{٧}$ خلية لكل ملي في البروتون نسبة النفوق ٥٠% بينما الأسماك المحقونة في العضلات تعاني علامات مرضية فقط تتمثل في أنزفة مع وجود قروح ظهرت بعد ٤٨ ساعة من العدوى ثم حدث استشفاء تام لهذه القروح خلال شهر مع تحسن البيئة المائية.