A contribution on motile aeromonas septicemia caused by *Aeromonas sobria* in common carp (*Cyprinus carpio*) with trials for disease prevention

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Abstract

Along one year, clinically diseased cultured common carp showing hemorrhages on different parts of the body surface and fins, fin and tail rot with septicemic lesions in internal organs were exposed to bacteriological examination. On the basis of biochemical characteristics and API 20 E system, the bacteria were identified as *Aeromonas sobria, A. jandaei* and *A. veronii*. 70% of examined fish were affected with motile aeromonas species. *A. sobria* was the most prevalent bacteria 98% among other isolated motile aeromonas species and was recorded in all seasons except winter. All examined isolates of *A. sobria* (9) were highly pathogenic to carp at 0.2 ml of 10^8 cells/ ml. PCR indicated that 6 isolates of *A. sobria* showed the aerolysine gene at 462 base pare and not detected in *A. jandaei* or *A. veronii*. Trials for disease prevention by feeding on live yeast and vaccination were done. The using of live yeast *Saccaromycis castelli* at 5 g per Kg commercial diet increased fish resistance against *A. sobria* infection through decreasing the mortality from 100% to 10% and increasing respiratory burst and serum lysozyme activities compared to control. Also, vaccination with Formalin Killed vaccine of *A. sobria* by injection and oral routes gave protection against experimental infection by *A. sobria* than control. Antibody titre was higher in vaccinated group by injection than orally vaccinated group. Live yeast and vaccine increased carp resistance against *A. sobria*.

Introduction:

Motile aeromonas septicemia is considered one of the most common diseases of cultured warm-water fish in freshwater environments caused by motile *Aeromonas* species *(Francis-Floyd, 2002)*. These bacteria have a broad host spectrum, with both cold- and warm-blooded animals, including humans, and are known as psychrophilic and mesophilic. In fish, these bacteria cause hemorrhagic septicemia, fin rot and soft tissue rot. It was reported that epizootic ulcerative syndrome (EUS) caused by *Aeromonas sobria* resulted in great damage to fish farms (*Rahman et al., 2002). A. sobria* was also the causative agent of fish

disease in a farm of perch, *Perca fluviatilis* L, in Switzerland (*Wahli et al., 2005*). In humans, *Aeromonas* species cause diarrhea, gastroenteritis, and extraenteritic conditions such as septicemia, wound infection, endocarditis, meningitis, and pneumonia (*Buckely and Howard, 1999*). They secrete many extracellular proteins, including amylase, chitinase, elastase, aerolysin, nuclease, gelatinase, lecithinase, lipase and protease (*Chacón et al., 2003*). These proteins are known as virulence factors that cause disease in fish and humans. Aerolysin is a representative virulence factor of *Aeromonas* and was reported to function as hemolysins and cytolytic enterotoxins (*Buckley and Howard, 1999*). The detection method of aerolysin gene was recently proposed as a reliable approach by which one can identify a potential pathogenic *Aeromonas* strain (*Heuzenroeder et al., 1999*). The detection of pathogenensis depends on genes carried on DNA genome (*Wogar, 1996*). The traditional methods employed for the control of diseases include prophylaxis and antimicrobial therapy for treatment. The rationale for the development of fish vaccines parallels that of other aspects of veterinary and human medicine (*Austin and Austin, 2007*). The use of vaccination and probiotics reduce antimicrobial use in aquaculture (*FAO, 2010*). This work was concerned with isolation and identification of bacterial causative agents of common carp diseases with trial of prevention using live yeast and vaccination. Besides, using PCR for detection aerolysin gene among pathogenic bacterial isolates.

Material and Methods:

Investigation of farmed common carp

Over a period of one year, total number of 100 common carp (*Cyprinus carpio*) were collected randomly from Abbassa Fish Farm (ICLARM) for subjection to clinical and bacteriological examinations. Macroscopic alterations and postmortem findings were recorded. For bacteriological analysis, sterile loops were used to collect samples from the liver, spleen and kidney, skin lesion and ascetic fluid. These samples were used to inoculate on Tryptic Soya Agar (TSA) and incubated at 30°C for 24 hours. Bacterial culture restreaked on TSA with previous incubation temperature and time until separated colonies were observed. Separated colonies were picked up and inoculated into TSA slant for further identification by biochemical tests according to *Austin and Austin, (1993)* and API-20E strips (BioMerieux) were used for confirmatory identification. In addition, the prevalence of infection was recorded.

Pathogenicity test:

480 apparently healthy common carp (20±5 g average body weight) were collected randomly from Abbassa Fish Farm (ICLARM) to study the experimental infection of some isolates of *Aeromonas sobria*, *A. jandaei* and *A. veronii* which were isolated from naturally infected common carp. The fish were acclimated for two weeks, and then divided into 16 equal groups,

each group in three replicates (each 10 fish). First group was injected 0.2 ml sterile saline as control group. Groups from 2 to 10 were injected IP with 0.2 ml of 10^8 cells of *Aeromonas sobria*. Groups from 11-13 was injected IP with 0.2 ml of 10^8 cells of *A. jandaei*. Groups from 14-16 were injected IP with 0.2 ml of 10^8 cells *A. veronii*. Aeromonas strains were grown overnight on TSA at 18°C and cell suspensions were then prepared in sterile phosphate-buffered saline (PBS), pH 7.4. The bacterial concentration was determined from the optical density at 600 nm and confirmed by plate count.

All fish groups were checked for fish mortality and macroscopic alterations for 14 day and exposed to bacteriological examination for bacterial re-isolation.

Detection of aerolysin gene of pathogenic Aeromonas species using Polymerase Chain Reaction PCR:

PCR was used to detect the presence of the aerolysin gene in the same bacterial isolates (*A. sobria*, *A. jandaei* and *A. veronii*) w was examined for its pathognicity previously. The primers used were (Aero F: 5`-CTC AGT CCG TGC GAC CGA CT-3` and Aero R: 5`-GAT CTC CAG CCT CAG GCC TT-3`) and targeted a 462 bp fragment of the aerolysin gene. PCR amplification was performed using DNA thermal cycler (Perkin - Elmer, USA) in a total volume of 25 µl. The reaction mixture concentrations used 6.2 µl of master mix was taken which consisted of (2.5 µl TAE 10X buffer + 2 µl dNTPS + 1.5 µl Mgcl2 + 0.2 µl Taq polymerase) and put in each tube. 2 µl of each primer (F & R) was added, 2 µl DNA templates were added, 8.8 µl distilled water was added to reach 25 µl total volumes. Thirty five PCR cycles were carried out on a Minicycler 95°C for 30 sec 57°C for 30 sec and 72°C for 45 sec, followed by 7 min final extension at 72°C. PCR products were examined by electrophoresis in 1.5% agarose gel in TBE buffer. The gel was stained with Ethidium bromide and viewed under UV light.

Trials for Disease prevention:

1- Using live yeast:

Saccaromyces castelli (kindly supplied from Fish Diseases Dept. Central Lab for Aquaculture Research in Abbassa) live culture at 48 hrs in 400 ml corn-meal medium was inoculated in 4 liters corn-meal medium and incubated at 25°C. It was harvested at 4th day by centrifugation at 3000 rpm for 15 minuets then washed with saline. Commercial basal diet (crude protein 30%) was crushed, and divided into two parts. The first part was basal diet mixed with sterile saline as a control group. The second part of basal diet mixed with 5 g of live yeast / kg. The diets were reformed into pellets; dried in air and stored at 4°C for the feeding experiment.

Sixty clinically healthy common carp were collected randomly and acclimated for two weeks. Fish were divided into two groups, each group had three replicates (each 10 fish). First group was fed manually twice daily with diet containing live yeast at satiation (T1). The second group fed with

commercial diet free from yeast as control (T2). After two weeks of feeding, fish were challenged IP with 0.2 ml of 10^8 24 hr live *A. sobria*. Fish were examined for 14 day for mortalities or abnormalities. Re-isolation of injected bacteria was occurred.

Respiratory burst (NBT Activity by Spectrophotometric Assays):

Values of the extinction were transposed according to a standard curve into mg of NBT/1 ml of blood according to *Siwicki (1989)*.

Lysozyme activity:

The lysozyme content was determined based on the calibration curve and the extinction measured according to *Schaperclaus et al., (1992)*.

2. Vaccination:

Formalin-killed bacterial cells of *A. sobria* were prepared as described by **Cardella and Eimers, (1990).** A part of Formalin killed bacteria was used as IP injection and the other part was added to commercial diet (containing 30% protein) for feeding to give 1×10^8 bacterial cells g^{-1} of diet. The commercial diet was mixed well, pelleted, and left for complete drying. Treated diet was kept in refrigerator at 4 ◦C.

One hundred and thirty five apparently healthy common carp were collected randomly from Abbassa Fish Farm (ICLARM) and acclimated for two weeks. Fish were divided into three groups; each group in three replicates, each replicate contained 15 fish. Fish of the first group was injected IP with 0.2 ml of the formalin-killed vaccine in the beginning of experiment and a poster dose after one month. Fish of second group were fed with diet containing formalin-killed vaccine for satiation daily for one week. After that, fish were fed with commercial diet free from vaccine for one month. The fish were fed again with vaccinated diet for another one week. Fish of third group (control) was not vaccinated by injection or through feeding treated diet. Fish were fed with commercial diet (30% protein) for satiation daily during the period of experiment. All fish were held for six weeks, serum samples were collected at 2 - 4 and 6 weeks and stored at -20°C for serum antibody titer measurement. Thirty common carp from vaccinated and control groups were challenged IP with 0.2 ml of 10^8 cells live *A. sobria* strain. Fish were examined for 14 day for any mortality or any abnormality. Re-isolation of injected bacteria was occurred.

Agglutinating antibody assays:

Serum samples collected from vaccinated and control groups were tested for agglutinating antibody level as previously described by *Schaperclaus et al. (1992)*.

Results:

Clinical signs:

The naturally diseased *C carpio* suffered from a variety of clinical signs including emaciation, erratic fins, fin and tail rot, hemorrhages in abdominal part, gill cover, fins and hemorrhages in ventral part (Photo 1). Pale gills in some fish and hemorrhages in others were observed. The Postmortem findings revealed pale to yellow liver and

peticheal hemorrhage in some cases with enlarged spleen (Photo 2).

Causative bacterial identification:

The most causative bacterial isolates were identified as *Aeromonas sobria.* It is Gram-negative, motile, short rods. The phenotypic and biochemical characteristics of *Aeromonas sobria* were illustrated in table (1). API 20E confirmed the identification of *A. sobria* with 98%. *A. sobria* were isolated from liver, kidneys, spleen, ascetic fluid and skin lesions. Other Aeromonas species were isolated from the same infected samples in less prevalence and identified as *A. jandaei* and *A. veronii* Fig (1).

Bacterial prevalence:

98% of examined carp that showed clinical alteration were infected with *A. sobria*. The high prevalence of *A. sobria* in naturally infected carp occurred in summer (70%) while, It was not recorded in winter (Fig 2).

Pathogenicity test:

All examined isolates of *Aeromonas sobria* (9) were pathogenic to carp with different percentage (20-100% mortalities) as shown in table 2 and figure 3. Some experimentally infected carp showed clinical signs and postmortem finding nearly similar to those of naturally infected fish. The mortality rates of *A. jandaei* and *A. veronii* were 90% and 20% respectively fig (4). *A. sobria* was isolated from all freshly dead and moribund experimentally infected common carp.

Disease prevention trials:

1- By yeast treated diet:

The fish group fed diet containing live yeast (*Saccaromycis castelli*) was resistant against *A. sobria*. The common carp group fed diet containing live yeast (*Saccaromycis castelli*) for tow weeks had survival rate 100%, while the second group fed with diet free from yeast had 10% fig (5) .

Respiratory burst (NBT) Activity:

Respiratory burst activity increased in the fish that fed diet containing live yeast in comparson with control fish (Fig 6).

Lysozyme activity:

The serum lysozyme activity was increased in fish fed diet containing live yeast in comparson with control group (Fig. 7).

2- By vaccination:

Mortality rate was lower in vaccinated carp either by IP injection or oral route where it was 0.0 and 30% respectively compared to control group 100% fig. (8).

Antibody titer using direct hemagglutination inhibition assay:

Antibody titer had not been detected at the second week of vaccinated serum fish by feeding, while at the forth week appeared at cut point 1/80 and was not detected at the sixth week of feeding vaccine. Serum antibody titer of vaccinated fish by injection was high than that in vaccinated carp by feeding. Antibody titer in serum of vaccinated fish by injection was high at the second week where, the cut point was at 1/80 and decreased at the

forth week to 1/20. Antibody titer increased again after the poster dose to reach at the cut point (1/320). Antibody titer was not detected in the non vaccinated fish serum (control) as shown in table (3).

Detection of Cytolytic aerolysin gene of pathogenic *A. sobria* identification using PCR:

The results observed in fig (9) revealed that PCR amplification detected the Aerolysine primer set, amplicons with six isolates of *A. sobria* at 462 bp and was not detected with *A. jandii* or *A. veronii.*

Discussion

Without doubt the carps are the most largely cultivated species throughout the world. Fish may display many behavioral and physical changes, some of which give valuable clues as to the nature of the disease, that many signs are common to a multitude of bacterial diseases. The clinical signs of infected common carp with bacteria were hemorrhages allover the body surface and fins, which may be due to the bacterial multiplication inside the intestine causing a hemorrhagic mucous disquamative catarrh. Toxic metabolites of *Aeromonas species* are absorbed from the intestine and induce toxemia. Capillary hemorrhage occurs in the dermis of fins and trunk and in the submucosa of the stomach *(Miyazaki and Kaige, 1985)*. Fin and tail rot was also recorded. It was found that the gross lesions of bacterial diseases in affected carp were congestion of internal organs. Pale liver with peticheal hemorrhages in some cases was observed. The toxic metabolites of *Aeromonas species* induce hepatic cells and epithelia of renal tubules to show degeneration. Glomeruli are destroyed and the tissue becomes hemorrhagic, with exudates of serum and fibrin *(Miyazaki and Kaige, 1985)*. The present results are similar to that recorded by *Loch and Faisal (2010)*. On the other hand, *Stojanov et al (2010)* isolated pathogenic *Aeromonas hydrophila* strains from fishes with no clinical signs.

The present study revealed that the isolated bacteria from naturally infected fish were identified as *Aeromonadaceae* (*Aeromonas sobria, A. veronii* and *A. jandaei*). API 20E rapid identification system has been the most widely used for identification of fish pathogenic bacteria. API 20 E strips gave a confirmation to *A. sobria* with 98%. *A. veronii* and *A. jandaei* were not confirmed with API 20 E because the API 20 E analytical profile index does not contain *A. veronii* or *A. jandaei* identification. These results are in agreement with *Popovic et al. (2007)* who reported that the biochemical protocols proposed for the API 20E strips are of limited importance for identification and differentiation of ichthyopathological bacterial species. Also, some strains are wrongly identified because they are not included in the database of API 20E system. While, *Kozińska (2007)* identified a strain of *A. veroniii* as *Ch. violaceum* by the API 20E test and *Santos et al. (1993)* mentioned that motile *Aeromonas* strains in the API 20E system gave false positive or

negative reactions for some biochemical reactions. Regardless of these false reactions, the API system identified 65% of motile *Aeromonas* isolates. The prevalence of Aeromonas species was 70% with common carp in summer, while *Nam and Joh (2007*) recorded the prevalence of Aeromonas species was100% in trout fish, that is mean may be carp resistance to aeromonads than trout.

Our results revealed that *A. sobria* was highly prevalent of motile aeromonas species and recorded in all seasons except winter. These results are in agreement with *Wahli et al. (2005)* who reported that the pathogenic *A. sobria* has been identified as a causative agent in diseased *Perca fluviatilis*; *Kozińska (2007)* common carp; *Orozova et al. (2009)* and rainbow trout *Loch and Faisal (2010).*

Our results revealed that all examined isolates of *A. sobria* was highly pathogenic to common carp causing (90 to 100 %) mortalities. These results are in agreement with *Min and Ying (2005) and Wahli et al (2005)* where *A. sobria* contained haemolysine and aeolysine genes. PCR-based method is rapid, sensitive, and specific for the detection of virulence factors of *Aeromonas* spp. It overcomes the handicap of time-consuming biochemical and other DNA-based methods. Aerolysine gene was detected by PCR, which had the pathogenicity factor of Aeromonas species and this gene was detected in six isolates from nine while, the experimental infection showed mortalities in the all isolates. The later results mean another gene causing the pathogenicity than the aerolysine gene as haemolytic, cytotoxicity casinase, collagenase, protease, lipase or other factor combine with earolysin gene for pathogenic effects *(Chacón et al., 2003).*

The nonspecific immune system is the first line of defense against invading organisms while specific immune system is second line of immune system and also affords protection re-exposure to the same *pathogen (Rombout et al., 2010)*. Chemotherapeutics and antibiotics are less favored due to bioaccumulation and emergence of resistant strains *(Harikrishnan et al., 2009).* So we used live yeast (*Saccaromycis castelli*) for disease prevention and it increased fish resistant against *A. sobria* experimentally. Our results agree with *Huttenhuis et al. (2006)* which demonstrated that live yeast in diets improved survival and reduced malformation in sea bass larvae. Also (*Saccaromycis castelli*) increased immune parameters by increasing NBT activity and lysozyme level in common carp serum, these results are in agreement with *Reyes-Becerril et al. (2010*). Increased respiratory burst activity can be correlated with increased oxygen radical production and increased killing activity. Whole cell yeast enhanced the leucocytic degranulation, peroxidase, respiratory burst, phagocytic and nonspecific cytotoxic cells' activity *(Cuesta et al., 2008)*. Lysozyme is a cationic enzyme that breaks the b- 1,4-glycosidic bond between N-acetylmuramic acid and N-acetyl glucosamine in the peptidoglycan of

bacterial cell walls. This action is known to attack mainly Gram-positive bacteria and also complement some Gram-negative bacteria. Also the cell wall of yeast contains mano protein, glucans and chitin in minor amounts (*Cabib et al., 1982*) as well as nucleic acid (*Ellis, 1999*).

Vaccination against bacterial diseases has been established as successful prophylactic treatments. The vaccinated fish with formalin killed vaccine of *A. sobria* by IP rout had highly protection than the feeding rout against the pathogenic *A. Sobria.* But oral vaccines can easily be administered to large numbers of fish without inducing stress and can even be used for vaccination of young fish. *Lillehaug et al. (2003)* recorded that vaccination gave good protection against fish diseases and reduced the risk of disease outbreaks. Also IP injection rout of vaccination secured high serum antibody responses than oral rout as mentioned by *Silva et al (2009*). That may be due to local immune response of oral rout where, all immune cells necessary for a local immune response are abundantly present in the gut mucosa as lymphoid cells, macrophages, eosinophilic and neutrophilic granulocytes (*Rombout et al., 2010).*

Conclusion:

Bacterial fish pathogens were the main cause of heavy mortalities and low production. MAS was the most prevalent disease. PCR was the most specific method for detection of aerolysin gene in *Aeromonas* sp. also Pathogenicity can be caused by other than aerolysin gene as haemolytic, cytotoxicity casinase, protease, lipase or other factor combines with earolysin gene for pathogenic effects. The use of yeast as feed additive and vaccination are valuable methods for prevention of bacterial fish diseases.

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Table (1): Morphological and Biochemical characters of suspected *Aeromonas sobria* isolates from naturally infected common carp.

Items	1	2	3	4	5	6	7	8	9
Gram-stain	ve–	ve–	ve–	ve–	ve–	ve–	ve–	ve–	ve–
Shape	Short rod	Short rod	Short rod	Short rod	Short rod	Short rod	Short rod	Short rod	Short rod
Motility	+	+	+	+	+	+	+	+	+
Cytochrom oxidase	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	F	F	F	F
Growth at 5°C	+	+	+	+	+	+	+	+	+
Growth on 0% NaCl	+	+	+	+	+	+	+	+	+
3.5% NaCl	+	+	+	+	+	+	+	+	+
6% NaCl	-	-	-	+	+	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+
H_2S (TSI)	+	+	+	-	-	+	+	+	-
Indol	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	-	+	+	+
Methyl red	+	+	+	-	+	+	+	+	+
Vogaus proskauer	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	-	-	+	+	+	–
Heamolysis	β	γ	β	β	β	γ	β	β	β
Acid production from:									
Arabinose	-	-	-	-	-	-	-	-	-
Slicin	-	-	-	-	-	-	-	-	-
Scrose	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+	+	+
Ornithen decarboxylase	±	±	+	+	+	+	±	±	.
Lysin decarboxylase	±	±	+	+	+	+	+	+	.
Arginie dehydrogenase	-	-	-	-	-	-	-	-	-

Table (2): Mortality rate of common carp due to intra-peritoneal injection (I/P) with 0.2 ml × 10^{10} cells/ml of *A. sobria*.

group	Number of fish	*Origen of A. sobria*	Mortality %
1	30	Sterile saline	0
2	30	Kidney	100
3	30	Liver	100
4	30	Spleen	90
5	30	Spleen	100
6	30	Liver	60
7	30	Liver	100
8	30	Kidney	20
9	30	Kidney	90
10	30	Kidney	40

Table (3): Antibody titer of vaccinated common carp with formalin killed vaccine by I/P injection and feeding route.

Serum Dilution	Period	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Vaccinated group By feeding	2 weeks	---	---	---	---	---	---	---	---
	4 weeks	+++	++	+	+	-	---	---	---
	6 weeks	---	---	---	---	---	---	---	---
Vaccinated group By injection	2 weeks	+++	++	++	+	---	---	---	---
	4 weeks	++	+	---	---	---	---	---	---
	6 weeks	+++	++	++	++	+	+	---	---
Non vaccinated group (control)	2 weeks	---	---	---	---	---	---	---	---
	4 weeks	---	---	---	---	---	---	---	---
	6 weeks	---	---	---	---	---	---	---	---

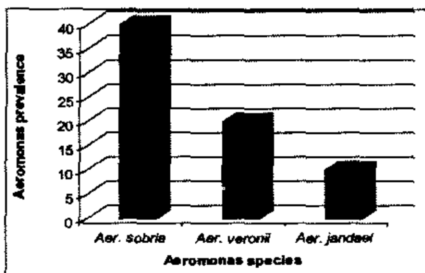

Fig (1) Aeromonas species prevalence isolated from naturally infected *C carpio*

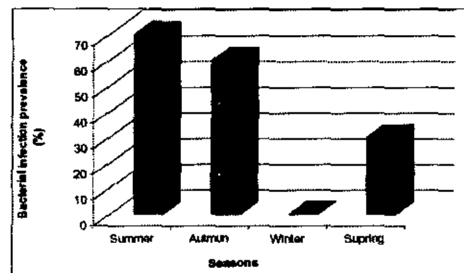

Fig (2): *Seasonal* prevalence of *Aeromonas sobria* isolates in naturally infected *C carpio*

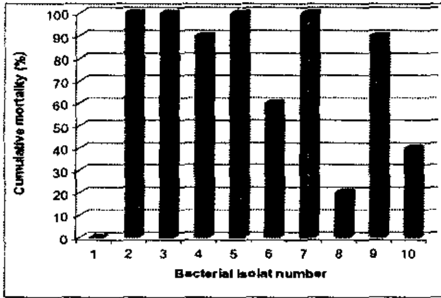

Fig (3): Mortality rate of common carp due to IP experimental infection with different isolates of *A. sobria* (2-10) and IP with sterile saline as control group (1).

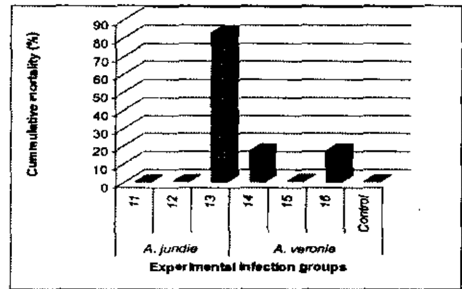

Fig (4): Mortality rate of common carp due to IP experimental infection with different isolates of *A. jandaei* (11-13) and *A. veronii* (14-16) and IP withsterile saline as control group.

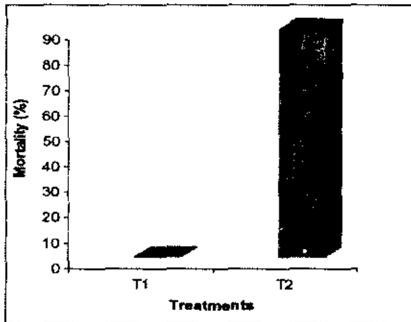

Fig (5): Mortality rate of common carp fed with diet containing 5 g of yeast /kg diet (*Saccaromycis castelli*) and challenged I/Pwith *A. sobria.* (T_1) and fishes fed with diet free from yeast (T_2).

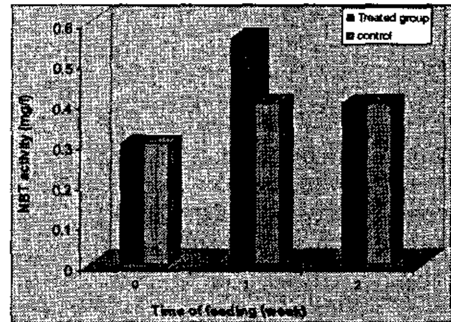

Fig (6): Respiratory burst (NBT) Activity of common carp serum fed by diet containing yeast (*Saccaromysis castelli*).

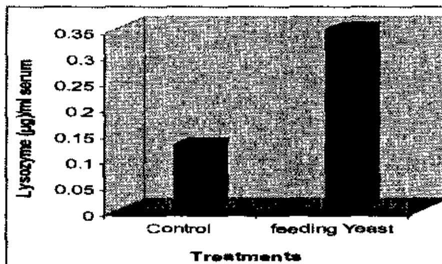

Fig (7): Lysozyme activity of common carp serum after feeding with diet containing *Saccaromysis castelli* for two weeks.

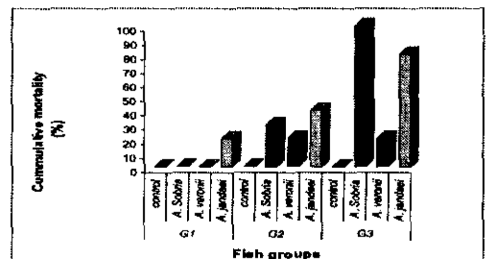

Fig (8): Mortality rate of vaccinated common carp by I/P injection and feeding with formalin killed vaccine of A. sobria after challenged I/P with 0.2 ml × 1010 cells/ml of live A. Sobria, A. veronii and A. jandaei; G1 fish groups Vaccenated I/P with formalized A. sobria; G2 fish groups vaccinated orally with formalized A. sobria ; G3 fish groups without vaccines.

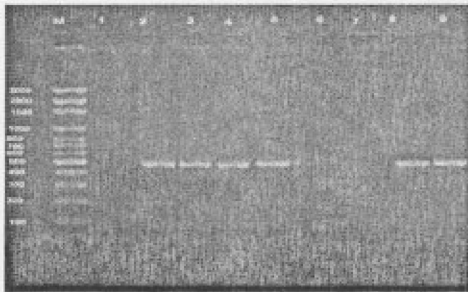

fig (9) Showing one band of Aerolysine gene of nine isolates of pathogenic *A. sobria* (1-9). M= Marker, (2, 3, 4, 5, 8, 9) isolates is *A. sobria* have a band at 462 bp,while 1, 6, 7 have not band of the aerolysine gene.

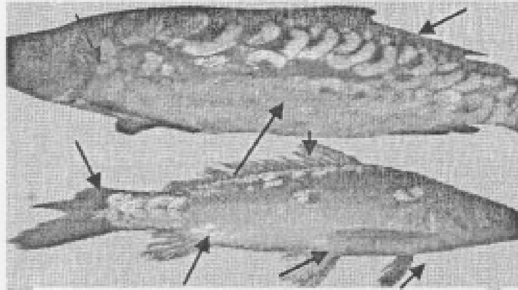

Plate (1): Showing hemorrhages in external surface, tail and fin rot and erratic fin of naturally infected common carp with *A. sobria*.

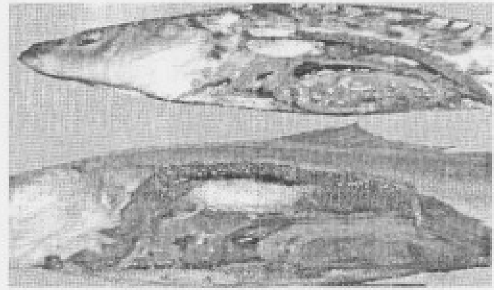

Plate (2): Showing septicemic lesions of internal organs with pale liver in naturally and experimentally infected common carp with A.sobria .

الملخص العربي

تعاني أسماك المبروك العادي المستزرعة من أعراض النزف في مختلف أجزاء الجسم و تاكل الزعانف والذيل مع وجود أعراض تسمم الدموي في الأعضاء الداخلية. وقد تم تشخيص البكتريا المعزولة من أسماك المبروك المصابة على أنها ايروموناس سوبريا , ايروموناس جاندي و ايروموناس فيروني و كانت نسبة الإصابة الكلية بالايروموناس ٧٠% بينما كانت نسبة الإصابة بالايروموناس سوبريا من الأسماك المصابة ٩٨% حيث كانت أعلى نسبة إصابة وقد سجلت في جميع المواسم ماعدا موسم الشتاء. وكانت عزلات ايروموناس سوبري (٩) كلها مرضية للمبروك المصاب معملي حيث تم حقنه بروتونيا بالايروموناس سوبريا وقد تم التعرف على الايرولايسين جين باستخدام إنزيم البلمرة المتسلسل الخاص بهذا الجين وقد وجد جيين الايرولايسين في ٦ عزلات فقط من الايروموناس سوبريا ولم يوجد في عترتي ابروموناس فيروني و جاندي. تم استخدام الخمائر و اللقاح لمنع الإصابة بهذا المرض وذلك باضافة خمائر السكاروميسين كاستيللي الى العلائق لزيادة مقاومة السمك ضد عدوى ايوموناس سوبريا حيث قلت نسبة النفوق من ١٠٠% الى ١٠% وزيادة مقياس التنفس و انزيم الليزوزيم و باسخدام اللقاح عن طريق الحقن البريتوني أو إضافته الى العلائق حيث رفع مقاومة السمك ونسبة الأجسام المضادة للايروموناس سوبريا في الدم .