

## Studies on Edwardsiellosis in *Clarias Gariepinus* Fish at Sohag Governorate

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**Abstract:** Edwardsiellosis (Emphysematous putrefactive disease) is one of the most serious bacterial diseases affecting *Clarias gariepinus* (*C. gariepinus*) fish. This study reported the most characteristic lesions including skin depigmented areas, external hemorrhages, small cutaneous ulcers, gas filled pockets give rise offensive odour and ascitis. The disease prevalence was 5.2% in the examined fish sample and 16.7% in the clinically diseased fish. The isolated strains were identified by the conventional biochemical tests and API20-E as *E. tarda*. The isolated strain was pathogenic and its LD<sub>50</sub> was 1.5x10<sup>5</sup>CFUml<sup>-1</sup>. Combination of carvacrol and its precursor cymene (100ppm of each) for 14 days as food additives controlled the disease in *C. gariepinus* while 50ppm of them reduced mortality to 10% and 55mgkg<sup>-1</sup> body weight of Oxytetracycline reduced the mortality rate to 20%.

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### 1. Introduction

African catfish (*Clarias gariepinus*) and *Oreochromis niloticus* are two major species that are reared in the polyculture system in Egypt (Ibraheim et al., 2011). *Edwardsiella tarda* infects primary catfish causing Edwardsiellosis (Emphysematous putrefactive disease, which is a dangerous septicaemic disease with high economic losses and its prevalence in catfish was more predominant during warm seasons of the year (Dwarkin et al., -2006). Although, it is a bacterium of fish, it causes public health hazards in humans including gastroenteritis, liver abscesses, meningitis, skin abscesses and valvular endocarditis (AIDS) (Mikamo et al., 2003 and Mizunoe et al., 2006)

It was investigated in *C. gariepinus* catfish at different localities by Abd El-Azeem (2000), Mohamed (2000), Abo El-Yazeed and Ibrahem (2009) whom reported 2, 6.25 & 0.42% prevalence respectively. The affected fish were suffered from small cutaneous ulcers and hemorrhages in the skin and muscle. The lesions in the muscles often developed to large gas filled pockets and the diseased fish loss control over the posterior half of the body and continue to feed. The post-mortem lesions were abdominal distention with yellow ascetic fluid, pale anemic or yellowish to deep brown liver, distended gall bladder, congested and enlarged spleen and haemorrhagically inflamed intestinal tract (Abo El-Yazeed and Ibrahem, 2009).

There is no established successful commercial treatment, control and preventive measure available for *E. tarda* infection (Mohanty and Sahoo, 2007). Although, number of antibiotics such as

oxytetracycline, norfloxacin, ciprofloxacin, gentamicin and chloramphenicol had been proven to be successful in controlling the *E. tarda* infection (Sahoo and Mukherjee, 1997), the pathogenic *E. tarda* isolates were often found to be naturally resistant against multiple antimicrobial compounds which increases the difficulty of antibiotic-based treatment (Stock and Wiedemann, 2001; Yoo et al., 2003 and Alcaide et al., 2006). In addition to, using of antibiotics in fish farms may introduce potential hazards to public health and to the environment by the emergence of drug-resistant microorganisms and antibiotic residues. Furthermore, the normal microbial flora in the digestive tract, which is beneficial to fish are also killed or inhibited by oral chemotherapy (Gerald & Jane, 1966 and Sugita et al., 1990).

Searching for safe and effective new strategies to control emphysematous putrefactive disease in African catfish, it was found that the plant extract carvacrol in combination with its precursor cymene had strong antibacterial effects against many bacterial pathogens including *Bacillus cereus* (UItee et al., 2000), *Shigella* sp. (Bagamboula et al., 2004), *E. coli* (Kiskó and Roller, 2005), *Streptococcus* (Botelho et al., 2007) and finally *E. tarda* (Rattanachaikunsopon and Phumkhachorn, 2010) and *Aeromonas hydrophila* (Zheng, et al., 2011).

This investigation was planned to study the prevalence and diagnosis of Edwardsiellosis in African catfish (*Clarias gariepinus*) fish at Sohag governorate and a trial for its treatment by using safe new strategy plant extract carvacrol was conducted.

## 2. Material and Methods

### Fish and clinical examination

A total number of 135 *C. gariepinus* fish with body weight ranged from 120 - 235g were collected alive from fish markets at Sohag governorate during the period from May to September of 2010. The fish were subjected to clinical and post mortem examination according to Stoskopf (1993) and Kimberley (2004).

### Bacteriological isolation

Bacteriological samples were collected from the liver, spleen, kidney and lesions (if present) of the examined fish and inoculated on Tryptic soya agar then incubated at 25°C for up to 48hrs. The pure colonies were picked up and re-streaked on Salmonella Shegilla agar (SS agar) and incubated at 25°C for up to 48hrs (Muratori *et al.*, 2001).

### Identification of the bacterial isolates

Identification of the isolates was done on the basis of morphological characters, conventional biochemical tests and confirmed by the API 20E system (Koneman *et al.*, 1988 and Quinn *et al.*, 2002) and manual description of bioMérieux (69780 Marcy, l'Etoile, France).

### Pathogenicity

**Table (1):** Determination of median lethal dose (LD<sub>50</sub>) of *T. maritimum*

Fish groups	No. of fish	Dose/fish	Route of injection
Group 1	10	$1.5 \times 10^5$	I/P
Group 2	10	$1.5 \times 10^4$	
Group 3	10	$1.5 \times 10^3$	
Group 4 (control)	10	-----	

### Control of *Edwardsiellosis* by Oxytetracycline and combination of carvacrol & cymene.

#### 1- Preparation of fish diets:

The carvacrol and cymene were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and were prepared in 95% ethanol. Four fish diets were prepared, Diet – 1 and Diet – 2 were prepared according to (Rattanachaikunsonpon and Phumkhachorn, 2010), the basal fish ration (ZooControl Company, Egypt) was supplemented with carvacrol and cymene at concentration of 100ppm of each one (Diet -1) and 50 ppm of each one(Diet -2). Diet – 3, the basal fish ration was supplemented with oxytetracycline at concentration 55 mg kg<sup>-1</sup> body weight (Sahoo and Mukherjee, 1997). Diet – 4, the basal fish ration was used as control fish diets and prepared by the same process without additives.

#### 2- Experiment

Twenty *C. gariepinus* fish with body weight (200±10g) were acclimated for two weeks then divided

into two equal groups, the 1<sup>st</sup> group was I/P injected with 0.3ml of *E. tarda* suspension at concentration  $1.5 \times 10^6$  (Galal, *et al.*, 2005) and the 2<sup>nd</sup> group was injected with sterile saline and used as control group. The two fish groups were closely and daily observed for three weeks, clinical signs and mortalities were recorded. All freshly dead fish were subjected to bacteriological isolation and re-identification to verify the specificity of mortality (Austin and Austin, 1993).

### Median Lethal Dose (LD<sub>50</sub>) of the isolated *E. taeda*

A total number of 40 *C. gariepinus* with body weight (190±10g) were acclimated for two weeks then subdivided into 4 equal groups each of 10 fish, the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> fish groups were injected I/P with 0.3ml of  $1.5 \times 10^5$ ,  $1.5 \times 10^4$  and  $1.5 \times 10^3$  *E. tarda* suspension respectively and the 4<sup>th</sup> group was injected with 0.3ml sterile saline and used as control. (Rattanachaikunsonpon and Phumkhachorn, 2010). The fish groups were closely observed for 2 weeks and the number of dead fish was daily recorded in each group (Table 1).

Forty *C. gariepinus* fish with body weight (170 ± 10g) were collected alive from fish markets and acclimated for two weeks then divided into four equal groups (10 for each). The fish groups were fasted for 24hrs then each fish of all groups were infected intraperitoneally with 0.3ml of *E. tarda* suspension at concentration  $1.5 \times 10^5$  (LD<sub>50</sub>). 12hrs later, the 1<sup>st</sup> group was fed on fish diet-1, the 2<sup>nd</sup> group was fed on diet-2, the 3<sup>rd</sup> group was fed on the diet-3 and the 4<sup>th</sup> group was fed on diet -4 (control diet), all fish groups were fed at feeding rate 3% of body weight subdivided into two times daily and closely observed for two weeks, the clinical signs and mortalities were recorded. All freshly dead fish were subjected to bacteriological isolation and re-identification to verify the specificity of mortality to *E. tarda* (Austin and Austin, 1993).

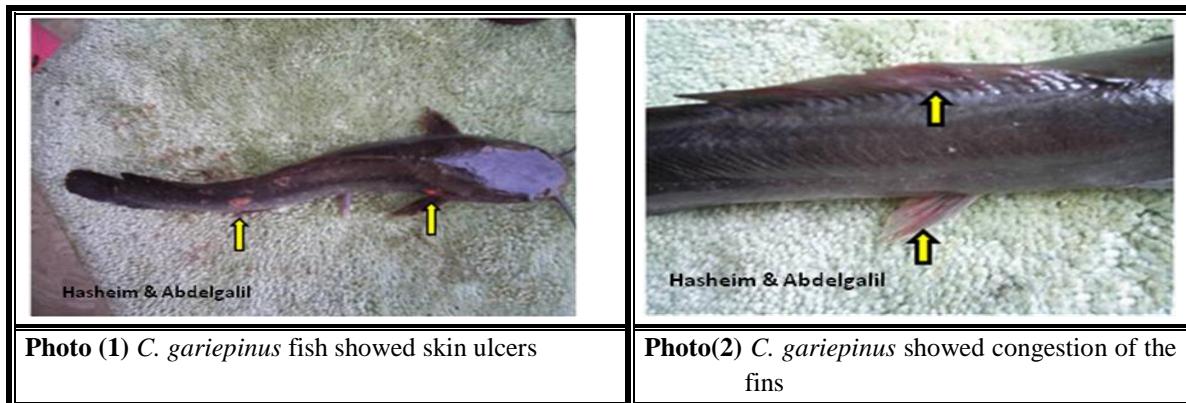
### Results

### Clinical signs and post-mortem lesions

The naturally infected *C. gariepinus* showed ulceration of the skin (photo -1), disintegration of

dorsal fin, congestion of fins (photo -2), fin and tail rot. Internally the naturally infected catfish exhibit

hyperemia, accumulation of bloody ascetic fluid and severe hemorrhagic enteritis.



#### Prevalence of clinically diseased *C. gariepinus*

Forty two out of 135 *C. gariepinus* fish were clinically diseased and exhibit disease clinical signs and their prevalence was 31.1% (Table-1).

Table (1): Prevalence of clinically diseased *C. gariepinus* fish

Fish species	Number of examined fish	Clinically diseased fish	
		Number	%
<i>C. gariepinus</i>	135	42	31.1

#### Prevalence of *Edwardsiellosis*:

A total number of 7 *Edwardsiellosis* diseased fish was reported and its prevalence in the all examined and the clinically diseased fish was 5.2 and 16.7% (Table -3).

Table (3): Prevalence of *Edwardsiellosis* in the examined and clinically diseased *C. gariepinus*:

	Examined fish (135)		Clinically diseased fish (42)
	No	7	7
	%	5.2	16.7
<i>Edwardsiellosis</i>			

#### Bacteriological isolation

A total number of 47 isolates were isolated from the investigated fish, 42 were isolated from the clinically diseased fish and 5 from the apparently healthy fish on TSA. 7 strains out of the 42 strains

isolated from the clinically diseased fish could be grow on SS agar and the growing colonies varied in size and characterized by creamy small colonies with black centre to entire black small colonies .

#### Identification of the isolate

The conventional biochemical test and API20E assay identified the 7 isolates as *E. tarda*. (Table 2 and Photo 3 ).

#### Pathogenecity of the isolated *E. tarda*:

The experimentally infected *C. gariepinus* fish showed lethargy, congestion and hemorrhages all over the fish body and at the site of injection, excessive mucus, areas of skin depigmentation (Photos 4 & 5) and offensive gas filled pockets, in association with 60% mortality rate. The post mortem findings of the experimentally infected fish were congestion of the abdominal cavity and enlarged congested kidney with putrid odour serosanguinus exudates in the abdominal cavity (Table - 4).

#### Median lethal dose ( $LD_{50}$ ) of the isolated *E. tarda*:

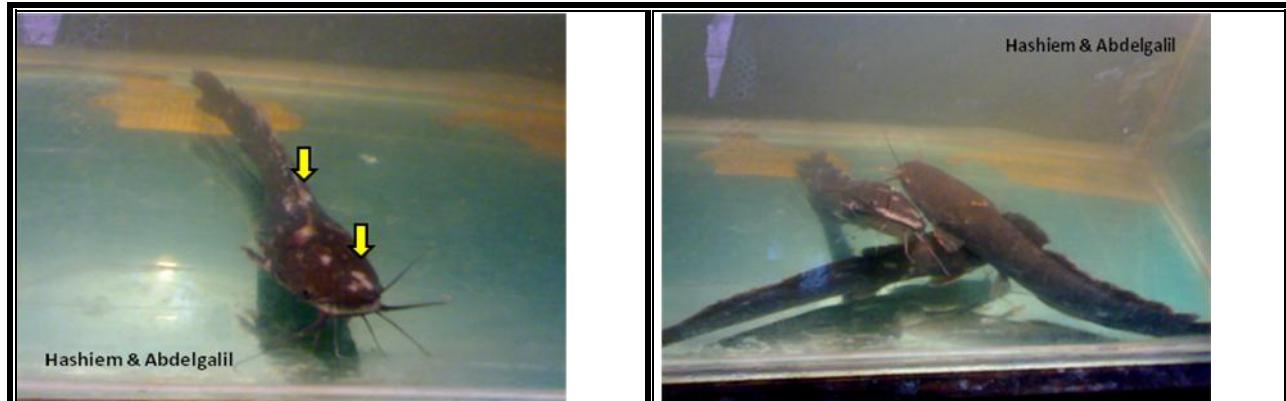
The mortalities of the *C. gariepinus* fish were observed for 2 weeks after they were intraperitoneally infected with different concentrations of *E. tarda*. The mortality of fish began at 24 hours and was 50, 40 and 20% in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> fish groups respectively. The  $LD_{50}$  of *E. tarda* for *C. gariepinus* was  $1.5 \times 10^5$  CFU/ml) (Table 5).

**Table (2):** Identification of *E. tarda* isolated from *C. gariepinus*.

Test	Result		
Colony on Salmonella Shegilla agar	Round, small, creamy in colour with black centre or whole colony is black		
Gram's stain	Gram negative short bacilli		
Cytochrome oxidase	-		
Catalase test	+		
Motility	+		
<b>API20E system</b>			
Test	Result	Test	Result
Ortho NitroPhenyle Galactopyranosidase (ONPG)	Negative	Gelatine liquefaction test (GEL)	Negative
Arginine Dehydrolase (ADH)	Negative	Glucose (GLU)	Positive
Lysine Decarboxylase (LDC)	Positive	Mannitol (MAN)	Negative
Ornithine Decarboxylase (ODC)	Positive	Inositol test (INO)	Negative
Citrate test (CIT)	Positive	Sorbitol (SOR)	Negative
H <sub>2</sub> S production test (H <sub>2</sub> S)	Positive	Rhaminose (RHA)	Negative
Urase test (URE)	Negative	Sucrose (SAC)	Negative
Tryptophane Deaminase (TDA)	Negative	D-Melibiose (MEL)	Negative
Indole test (IND)	Positive	Amygdaline (AMY)	Negative
Vogus proskauer (VP)	Negative	L-Arabinose (ARA)	Negative

**Table (4):** Pathogenicity assay of *E. tarda* in *C. gariepinus*

Isolate	Dose 0.3 ml	Fish number	No. of dead fish / day							Total no. of dead Fish	Mortality rate %
			1	2	3	4	5	6	7 to 14		
<i>E. tarda</i>	1.5×10 <sup>6</sup>	10	3	2	-	-	-	1	-	6	60
Control	Saline	10	-	-	-	-	-	-	-	-	0



**Photo (4&5)** *C. gariepinus* fish in the aquarium showed areas of depigmentation and fin disintegration

Table (5): showed the median lethal dose ( $LD_{50}$ )

Groups	Dose/fish 0.3 ml of	Fish No.	Number of dead fish / day											Number of dead fish	Mortality rate %
			1	2	3	4	5	6	7	8	9	10	11 to 14		
Group 1	$1.5 \times 10^5$	10	2	-	1		1	-	-	1	-	=	-	5	50
Group 2	$1.5 \times 10^4$	10	1	-	1	1	-	-	-	-	1	-	-	4	40
Group 3	$1.5 \times 10^3$	10	1	-	-	1	-	-	-	-	-	-	-	2	20
Control	Saline	10	-	1	-	-	-	-	-	-	-	-	-	1	10

#### Control of *Edwardsiellosis* by:

##### Oxytetracycline

55mgkg<sup>-1</sup> body weight of Oxytetracycline decreased the mortality rate to 20% where the control group reported 50% mortality and the clinical signs such as external hemorrhages, skin ulceration and fins rot were documented in both groups.

##### Carvacrol

Carvacrol and its precursor cymene at concentration 100ppm of each completely prevented the clinical signs appearance and no mortality could be reported in the treated fish, moreover the fish of these groups were alert and had good appetite. 50ppm prevented the clinical signs appearance and reduced the mortality to 10% only. The control group represented clinical signs of the disease and recorded 50% mortality.

#### 4. Discussion

The infectious diseases constitute a major constrain to aquaculture production with a consequent effect on the economic development, the bacterial agents are among the highly encountered causes of aquaculture diseases (Yunxia *et al.*, 2001).

*Edwardsiellosis* causes massive mortalities and consequently high economic losses in natural environment and fish farming worldwide (Plumb, 1993) and one of the most commonly affected fish species is catfish (Mohanty and Sahoo, 2007).

The clinical examination of diseased *C. gariepinus* showed typical signs of septicemia in the form skin ulcerations, disintegration of dorsal fin, congestion of the fins and tail rot. The PM lesions of the infected fish were hyperemia, accumulation of ascitic fluid, enlarged kidney and pale liver. Plumb and Evans (2006) described similar clinical and PM signs in *E. tarda* infected *C. lazera* catfish. These septicemic lesions of *E. tarda* infected *C. gariepinus* may be attributed to its virulence factors including extracellular products particularly haemolysine (Mathew *et al.*, 2001) and to its adherence ability to the host surface by fimbriae which is controlled by the *E. tarda* specific type 1 fimbrial gene (Sakai *et al.*, 2007).

The prevalence of *Edwardsiellosis* among the examined *C. gariepinus* fish was 5.2% and this result come in accordance with the results of Mohamed (2000) who reported that the incidence of

Edwardsiellosis in *Clarias lazera* was 6.25% and in disagreement with Abd El-Azeem (2000) who reported 2% incidence of Edwardsiellosis in *Clarias lazera* at El fayum governorate and this disagreement may be attributed to the difference in stocking density, locality, temperature and water quality.

The conventional biochemical tests identified the isolated strains as *E. tarda* and confirmed by the API 20-E assay and this identification was in accordance with the findings of Ling *et al.* (2001) and Ibrahim *et al.* (2011).

Concerning the pathogenicity assay of the isolated *E. tarda*, it revealed that the isolate is pathogenic strain and caused clinical signs similar to those of the naturally diseased fish associated 60% mortality rate comparing with 0% mortality in control group. The LD<sub>50</sub> of the isolated *E. tarda* was 1.5X10<sup>5</sup> CFU mL<sup>-1</sup> which caused 50% mortality in the tested fish within the experiment period. Galal *et al.* (2005) and Ibrahim *et al.* (2011) recorded 10<sup>4</sup> CFU mL<sup>-1</sup> LD<sub>50</sub> for *E. tarda*, and this disagreement may be attributed to the difference in fish species and surrounding environment.

Dealing with the treatment of *E. tarda* infection in *C. gariepinus*, 55mg kg<sup>-1</sup> body weight oxytetracycline succeeded to reduce the mortality in *E. tarda* infected fish to 20%, it is important to take into account that its disadvantages including development of disease-resistant strains and dose problems were documented by Mohanty and Sahoo (2007).

This study focused on the treatment by using new alternative strategies such as the plant extracts carvacrol which is a major component of oregano and thyme and has a broad spectrum antimicrobial activity against both gram-positive and gram-negative bacteria (Burt, 2004). Synergism between carvacrol and its precursor cymene against many bacterial strains including *E. tarda* in fish had been reported by Kiskó and Roller (2005) and Rattanachaikunsonopon and Phumkhachorn (2010). 100ppm of both carvacrol and cymene for 14 days as food additives succeeded to control Edwardsiellosis in *C. gariepinus*, this results were confirmed by Rattanachaikunsonopon and Phumkhachorn (2010) who controlled Edwardsiellosis in *O. niloticus* by carvacrol and cymene. Carvacrol had bactericidal activity on *E. tarda* which may be attributed to the damaging of cytoplasmic membrane which leads to bacterial cell collapse and depletion of the ATP pool (Ultee *et al.*, 2002).

In conclusion, Edwardsiellosis was diagnosed in *C. gariepinus* fish at sohag and its prevalence was 5.2% in all examined and 16.7% in the clinically diseased fish. 100ppm of both carvacrol and its precursor cymene for 14 days as food additives controlled the disease in *C. gariepinus*. Further

studies are required to reduce the treatment duration and dose and to clarify the drug effects on the intestinal and environmental beneficial bacteria.

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