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ISOLATION AND IDENTIFICATION OF *Edwardsiella tarda* FROM INFECTED NILE TILAPIA FISH *"Oreochromis niloticus"*

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By
W. D. Saleh

Department of Microbiology, Faculty of Agriculture, Cairo University, Giza , Egypt

ABSTRACT

Edwardsiella tarda (*E. tarda*) is one of the important warm-water-fish pathogens causing serious economic loss. This work was planned to isolate and identify pathogenic *E. tarda* from naturally infected Nile tilapia fish, *Oreochromis niloticus*, by different methods. Out of 200 randomly collected fish, fifty naturally displayed the clinical symptoms of *E. tarda* infection. Seven strains, isolated from the diseased fish, were selected according to their colony characteristics. Biochemical results of these seven isolates gave the pattern of the bacterium *E. tarda*. Antibiotic sensitivity, against ten antibiotics, confirmed identifying all the seven isolates as identical *E. tarda*. The incidence of that pathogen in the liver, kidneys and spleen was 28.57% for each, while 14.29% were isolated from the intestine, whereas the deep musculature was free of *E. tarda*. Experimental infection, by intramuscular injection, exhibited typical signs of Edwardsiellosis on all injected fish and 57.5% mortality within two weeks.

Key words: *aquaculture, Edwardsiella tarda, fish disease,identification, isolation, Nile tilapia, Oreochromis niloticus.*

1. INTRODUCTION

Edwardsiella tarda (*E. tarda*) is one of the most important microorganisms causing severe economic losses among warm-water fishes all around the world, *e.g.*, North America, Japan, Taiwan, and Africa (Thune *et al.*, 1993; Baya *et al.* 1997; Muratori *et al.*, 2000 and Pressley *et al.*, 2005).

Edwardsiellar septicemia caused by the bacterium *E. tarda* is currently accepted name for the disease caused by this pathogen (Baldwin & Newton, 1993), but other synonymous ones are fish gangrene, emphysematous putrefactive disease of catfish (Meyer and Bullock, 1973) and red disease of eels (Egusa, 1976) and Edwardsiellosis in zebrafish (Pressley *et al.*,2005).

Both *E. tarda* and *E. ictaluri* are normal inhabitants in the intestine of snakes and are occasionally found in human feces. Infected fish, when consumed by man, cause gastroerities and more rarely other man diseases (Ewing *et al.*, 1965; Meyer & Bullock, 1973, Janda & Abbott, 1993; Plumb, 1999 and Nucci *et al.*, 2002).

This study was conducted to isolate and identify *E. tarda* from the Nile tilapia fish, *Oreochromis niloticus (O. niloticus)*, which was suspected to be naturally diseased.

2. MATERIALS AND METHODS

2.1-Fish sampling

A random sample of 200 Nile tilapia fish, *O. niloticus*, (each of 50 ± 2 g) was collected alive from Barseek Fish Farm at Behera Governorate; fifty of which displayed the clinical signs of *Edwardsiella* infection. Collected fish were transported alive to the laboratory in large plastic bags filled with oxygen and water.

In the laboratory, they were kept in five identical glass aquaria measuring 90X60X50 cm each. In each aquarium, thirty healthy and ten symptom-showing fish were placed. The aquaria were supplied with chlorine-free tap water (Innes, 1966) to which filtered air was pumped (Sunny Inc., Japan). During holding the fish for investigation, they were fed on a commercial fish diet containing 25% crude protein. The feed was added daily at the level of 3% of body weight (Eurell *et al.*, 1978). Five recently dead fish, of

those exhibited disease symptoms, were used for examination and analysis.

2.2. Clinical examination, microbial isolation and identification

The fish were clinically examined according to Amlacher (1970) and Lucky (1977). For bacteriological examination, samples were individually taken from different internal tissues and organs to be inoculated into Tryptic Soya Broth (TSB) tubes, and then plated on Tryptic Soya Agar (TSA) medium. Plates were incubated for 24 – 48 h at 30°C and the morphologically suspected colonies were picked up to be cultured on TSA tubes for identification (Amlacher, 1970; Ling *et al.*, 2001 and Lim *et al.*, 2003).

The morphological features of the bacterial colonies and their growth characters on TSA medium, conventional biochemical reactions as well as API-20E system (Analytab Products, Plainview, New York) were used for identification of isolated bacteria according to Hawke *et al.* (1981) and Morrison *et al.* (1981). For confirmation, all isolated bacteria were subjected to sensitivity tests towards recommended concentrations of ten antibiotics by using disc diffusion technique (Lennette *et al.*, 1980 and Reger *et al.*, 1993).

2.3- *In vitro* **infection of healthy fish by** *E. tarda*

Since all seven strains that proved to be *E. tarda* gave the same results, one of them was used for artificial infection of healthy *O. niloticus* fish to confirm their pathogenicity. Eighty healthy fish (60 ± 5 g) were divided into 4 groups (each of 20 fish). Fish of the first three groups were intramuscularly injected with 0.2 ml of saline suspension containing the tested bacterium, at 5 X 10^3 CFU/ml, as recommended by Shehata *et al.* (1988). The fourth group was injected with 0.2 ml of sterile saline solution to serve as control. All four groups were separately kept under observation for two weeks to record clinical signs and mortality. The temperature was maintained at 25 – 27°C allover the experimental period.

3. RESULTS & DISCUSSION

3.1. Microbial isolation and identification

Clinical signs of natural Edwardsiellosis in *O. niloticus* fish were typified by loss of scales from areas of the skin, and excessive

mucous allover the body surface with petechial hemorrhages over the dorsal musculature. Large necrotic lesions extending down into the musculature in the posterior part of the body, especially in the caudal peduncle, with rotten fins were observed in some cases. These lesions were characterized by hyperemic borders and emitting foul odor. Skin showed superficial ulcers, especially at the caudal peduncle with extensive hemorrhagic raised areas on the dorsal musculature. Fish escape and defensive reflexes were weak or absent. The recorded clinical signs were identical to the records of several authors (Stoskopf, 1993; Eissa and Yassien, 1994; Baya *et al.*, 1997, Plumb, 1999, Miwa and Mano, 2000 and Pressley *et al.*, 2005). In Egypt, Soliman *et al.* (1991) reported that catfish injected with *E. ictaluri* showed hemorrhagic raised edematous area with liquefaction of underlying tissues, congestion of the fins and petechial hemorrhages allover the body surface.

Attempts to isolate *E. tarda* from different organs (*i.e.*, liver, kidneys, spleen, and intestine) and musculature of naturally infected *O. niloticus* resulted in seven isolates. The colonies appeared after 24 h at 30°C on TSA medium. They were small, circular, transparent, raised and grayish white punctuating colonies. Suspected cells proved to be Gram-negative motile short rods.

The physiological tests of the seven obtained isolates gave identical results (Table 1). Though the resulted morphological, biochemical, and API-20E system profiles of the isolated strains were very typical for *E. tarda*, they were all considered as presumptive identification.

The present work followed a biochemical scheme that is basically similar to those of Buxton and Fraser (1977), Hawke *et al.* (1981) and Morrison *et al.* (1981) which proved to be highly efficient. Results of the biochemical tests are in line with those reported by other investigators (Farmer & McWohrter, 1984; Waltman and Shotts, 1986; Waltman *et al.*, 1986; Roberts, 1989; Stoskopf, 1993 and Ling *et al.*, 2001). *E. tarda* (Ewing, *et al.*, 1965) was earlier isolated from channel catfish in the United States (Meyer and Bullock, 1973) and cultured fish from other parts of the world (Eissa and Yassien, 1994 and Muratori *et al.*, 2000).

Table (1): Biochemical profile of the isolated strains.

Test	Result	Test	Result
Catalase	+	Oxidase	-
Indole	+	Voges & Proskaur (VP)	-
Methyl red	+	Sodium pyruvate	-
Nitrate reduction	+	Fermentation of glucose	+
Citrate utilization	-	„ of arabinose	-
Malonate utilization	-	„ of sucrose	-
Gelatin	-	„ of xylose	-
Urease	-	„ of mannitol	-
H_2S	+	„ of sorbitol	-
Lysine decarboxylase	+	„ of rhamnose	-
Ornithine decarboxylase	+	„ of melibiose	-
Arginine dihydrolase	-	„ of inositol	-
Tryptophane deaminase Oxidase	-	„ of trehalose	-
		„ of adonitol	-

The confirmatory antibiotic susceptibility results indicated that the isolated strains were highly sensitive to Lincospectin (10μg), Neomycin (30μg), Oxonilic acid (30μg), Norfloxacin (10μg), Oxytetracycline (10μg) and Ampicillin (30μg), slightly sensitive to Doxycycline (30μg), Erythromycin (30μg), while it was resistant to Spectinomycin (30μg) and Chloramphenicol (30μg). These results clearly confirmed that all of the seven isolates were *E. tarda*. The distribution of these isolates in the naturally infected fish, presented in Table (2), is in harmony with the information provided by Plumb (1993), Reger *et al.* (1993) and Rashid *et al.* (1997).

Table (2): Distribution of *E. tarda* in various tissues and organs of *O. niloticus* fish killed by Edwardsiellosis.

Tissues and organs	*E. tarda*	
	No. of isolates	%
Liver	2	28.57
Kidneys	2	28.57
Spleen	2	28.57
Gills	0	0.00
Intestine	1	14.29
Deep layer of musculature	0	0.00
	7	100

Concerning the antibiotic susceptibility patterns of the isolated *Edwardsiella tarda* in this study, it was almost the same as those reported in the literature. Heo *et al.* (1996) found that *E. tarda* was susceptible to Ciprofloxacin in food at a dose of 100 mg/kg body weight or more for 3 days and were highly sensitive to

Norfloxacin at a dose of at least 100 mg/kg body weight daily for 3 days. Moreover, Eissa and Yassien (1994) found that *E. tarda* was highly sensitive to Oxytetracycline, Chloramphenicol and Kanamycin. The majority of the results of this work confirmed the comprehensive report of Stock and Wiedemann (2001).

3.2. Experimental infection

In all experimentally infected fish, disease symptoms started to appear after two days. The clinical signs on experimentally infected fish were similar to those on the naturally infected and very typical for Edwardsiellosis. Among the experimentally infected fish, death started from the third day of infection, and 57.5% of the treated fish died within the observation period (2 weeks). The control group of fish remained healthy up to the end of the experiment.

It was clear that *E. tarda* infection spread from lesions of visceral organs into the musculature and then to the skin, where large lesions develop in the musculature and dermis. These observations agreed with those reported by Egusa (1976), Plumb (1993), Stoskopf (1993), Eissa & Yassien (1994), Baya *et al.* (1997), Miwa & Mano (2000) and Pressley *et al.*, (2005). The post-mortem examination revealed congested liver, kidney and spleen with excessive mucus in gills. These could be attributed to septicemia induced by *E. tarda* exotoxins (Nowotny, 1979; Ullah & Arai, 1983; Hirono *et al.*, 1997 and Mathew *et al.*, 2001).

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عزل وتصنيف بكتريا *Edwardsiella tarda* من أسماك البلطي النيلي المصابة" *Oreochromis niloticus* "

وليد ضياء الدين صالح

قسم الميكروبيولوجيا – كلية الزراعة – جامعة القاهرة – الجيزة – مصر

ملخص

تعتبر بكتريا *Edwardsiella tarda* أحد مسببات أمراض أسماك المياه الدافئة التي تنجم عنها خسائر اقتصادية فادحة، و لقد وضعت خطة هذا البحث بغرض العزل و التصنيف (بطرق مختلفة) لبكتريا *E. tarda* الممرضة من أسماك البلطي النيلي *Oreochromis niloticus* المصابة إصابة طبيعية.

ظهرت أعراض المرض علي خمسين سمكة من بين مائتين جمعت عشوائيا من إحدى المزارع السمكية، و من تلك الاسماك المصابة (باستخدام مواصفات المستعمرات البكتيرية) تم الحصول علي سبع عزلات اشتبه في كونها من نوع *E. tarda* الممرض. وقد اعطت العزلات السبع ذات الصفات البيوكيماوية للميكروب المذكور، ثم جاءت نتائج تأثير عشر مضادات حيوية لتؤكد تطابق العزلات السبعة وأنها جميعا لبكتريا *E. tarda* الممرضة.

إحتوي كل من الكبد والكلى والطحال علي ٢٨,٥٧% من الميكروبات المعزولة بينما احتوت الامعاء علي ١٤,٢٩% منها، أما العضلات العميقة فكانت خالية من البكتريا الممرضة. و قد ظهرت أعراض المرض علي جميع الاسماك التي أجريت لها عدوى صناعية (بحقن البكتريا في العضلات)، و مات ٥٧,٥% منها خلال أسبوعين.

المجلة العلميـــة لكلية الزراعة – جامعة القاهرة – المجلد (٥٦) العدد الرابع (أكتوبر ٢٠٠٥): ٨٣٩-٨٤٨.