Review

Edwardsiellosis in fish: a brief review

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Edwardsiellosis is one of the most important bacterial diseases in fish. Scientific work on this disease started more than forty years ago and numerous workers around the world are continually adding to the knowledge of the disease. In spite of this, not a single article that reviews the enormous scientific data thus generated is available in the English language. This article briefly discusses some of the recent research on edwardsiellosis, describing the pathogen's interaction with the host and environment, its pathogenesis and pathology as well as diagnostic, preventive and control measures.

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1. Introduction

The occurrence of disease is a significant setback for successful aquafarming. Bacteria are the most common pathogens of cultured warm water fish, and cause major losses to the freshwater aquaculture industry in India and elsewhere. They are also the most prevalent cause of morbidity and mortality among wild populations of fish. Out of the most annihilating bacteria, motile Aeromonads and *Edwardsiella* sp. are the most significant. *Edwardsiella tarda* (Ewing *et al* 1965) is a commonly found pathogen causing edwardsiellosis/emphysematous putrefactive disease leading to mass mortality in various populations and age groups of fish. Fish species commonly affected by edwardsiellosis include carp, tilapia, eel, catfish, mullet, salmon, trout and flounder.

Epizootics of edwardsiellosis, which had a disastrous effect on fish culture, occurred in northern and southern Europe in turbot in recent years. Four outbreaks occurred in both the regions in 2005 and three peaks of mortality at an interval of one month were recorded in northern Europe in 2006 (Castro *et al* 2006). The enormous loss caused by this pathogen is being felt in the USA, Japan, Europe and Asian countries; however, no exact figures are available to quantify the loss. Prevalence of the disease in ponds is seldom above 5%; however, it can reach 50% when fish are confined to tanks (Meyer and Bullock 1973). The quantum of loss due to this pathogen in India is yet to be determined. The authors have come across a few cases of mass mortality in stocks, particularly of Indian major carps and Asian catfish, during hatchery rearing operations. This pathogen is also important from a public health point of view, as it is known to produce disease in reptiles, birds, humans and other mammals. In spite of these facts, there is no established successful commercial treatment/control/preventive measure available. In this article, we present a brief review of edwardsiellosis, exploring the pathogen, its pathogenesis and pathology, and diagnostic and control measures.

2. Edwardsiellosis

2.1 *The bacterium*

Edwardsiellosis is caused by *Edwardsiella tarda*, a Gram-negative, motile, short, rod-shaped bacterium (1 μm in

Keywords. Bacterial disease; *Edwardsiella tarda*; edwardsiellosis; fish

Abbreviations used: ECP, extracellular protein; EPDC, emphysematous putrefactive disease of catfish; FISH, fluorescence *in situ* hybridization; GFP, green fluorescent protein; HPCE, high performance capillary electrophoresis; LAMP, loop-mediated isothermal amplification; OMP, outer membrane protein; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; T3SS, type III secretion system

diameter and 2–3 μm long), which affects a wide range of hosts including both freshwater and marine fish (Thune *et al* 1993). The genus *Edwardsiella*, named after the American bacteriologist P R Edwards (1901–1966), was proposed in 1965 (Ewing *et al* 1965) for a group of 37 organisms differing biochemically from other members of Enterobacteriaceae. These 37 cultures were principally isolated from faeces, blood, wounds and urine of humans. All of them have been placed into a single species, *E. tarda* (Ewing *et al* 1965; Meyer and Bullock 1973). McWhorter *et al* (1967) developed a provisional scheme for the O and H antigenic characteristics of *Edwardsiella*. Another species under this genus, *E. ictaluri,* responsible for causing enteric septicaemia, was isolated in 1979 in channel catfish (Hawke 1979). *E. tarda* is facultatively anaerobic and mesophyllous (Holt *et al* 1994), and characterized as cytochrome oxidase-negative, indole-positive and strong H_2S producer in nature; thus, the disease is often named emphysematous putrefactive disease. It forms typical green colonies with black centres on Rimler–Shott agar medium (Acharya *et al* 2007). Table 1 shows the biochemical characteristics of *E. tarda* (Wyatt *et al* 1979; Grimont *et al* 1980; Fang *et al* 2006).

Analyses of the serological relationship among *E. tarda* strains isolated from eel and flounder in Japan by Park *et al* (1983) and Rashid *et al* (1994), respectively, revealed that there are four different serotypes (A, B, C and D), out of which only serotype A is highly virulent and predominant. A number of serovar groups (>60) have been identified in *E. tarda* based on the somatic (O) and flagellar (H) antigens (Sakazaki 1984; Tamura *et al* 1988). Following the discovery of two proteins, a flagellin and an SseB (a protein belonging to the type III secretion system), a nominal distinction between virulent and avirulent *E. tarda* has been made possible by comparative proteomic analysis (Tan *et al* 2002).

Table 1. Biochemical characteristics of *Edwardsiella tarda*

Characteristics	Response[1]	Characteristics	Response
Motility		Acid from	
25 °C	+	Glucose	+
35 °C	+	Lactose	–
Indole production	+	Mannitol	–
Methyl red	+	Salicin	–
Citrate		Inositol	–
Simmons	–	Arabinose	–
Christensen	+	Rhamnose	+
H_2S production		Trehalose	+
Triple sugar iron agar	+	Erythritol	–
Peptone iron agar	+	Mannose	–
Tolerance to NaCl		Maltose	–
1.5%	+	Sucrose	–
3%	+	Dulcitol	–
Mol % G+C of DNA	55–58	Adonitol	–
Voges–Proskauer	–	Sorbitol	–
Phenylalanine deaminase	–	Raffinose	–
Lysine decarboxylase	+	Xylose	–
Ornithine decarboxylase	+	Cellobiose	–
Malonate utilization	–	Esculin	–
Urease	–	KCN	–
Salicin	–	Catalase	+
Acetate utilization	–	Arginine dihydrolase	–
Deoxyribonuclease	–	Gelatin hydrolysis	–
Lipase	–	Nitrate reductase	+
Pectate hydrolysis	–	β-Galactosidase	–
Cytochrome oxidase	–	Triple sugar iron agar	K/AG[2]

[1]'+' indicates over 90% of isolates are positive for the characteristic and '–' indicates over 90% are negative.

[2]K represents alkaline or no reaction, A represents acid production and G represents gas production.

Random amplified polymorphic DNA profiles of *E. tarda* isolated from fish and humans from diverse geographical locations have also shown recognizable differences among isolates (Nucci *et al* 2002). Also, polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) of its 16S rDNA has been found to be specific for detecting habitat-specific isolates (Acharya *et al* 2007). Thus, *E. tarda* appears to be more plastic, phenotypically more polymorphic and capable of adapting to survival within a broad range of hosts, in contrast to *E. ictaluri* (Panangala *et al* 2006).

In fish, *E. tarda* was first reported as *Paracolobactrum anguiillimortiferum* associated with red disease of Japanese eel, *Anguilla japonica* (Hoshina 1962). Thereafter, it has been reported in a number of cultured fish such as eel (*Anguilla japonica*), channel catfish (*Ictalurus punctatus*), mullet, brook trout (*Salvelinus fontinalis*), red sea bream (*Pagrus major*), Japanese flounder (*Paralichthys olivaceus*), Chinook salmon (*Oncorhynchus tshawytscha*) (Meyer and Bullock 1973; Egusa 1976; Kusuda *et al* 1976; Amandi *et al* 1982; Uhland *et al* 2000; Sano *et al* 2001), turbot (*Scophthalmus maximus* L.) (Nougayrede *et al* 1994), Asian catfish (*Clarias batrachus*) (Sahoo *et al* 1998), climbing perch (*Anabas testudineus*) (Sahoo *et al* 2000), tilapia (Clavijo *et al* 2002) and oyster toadfish (*Opsanus tau*) (Horenstein *et al* 2004) (table 2). Matsuyama *et al* (2005) found differences in the pathogenicity of motile and atypical (non-motile) *E. tarda* examined in yellowtail, Japanese flounder and red sea bream.

E. tarda has long been considered as an unusual human pathogen. It is primarily associated with gastroenteritis, wound infections such as cellulitis or gas gangrene and generalized infections in humans with impaired immune systems (Thune *et al* 1993; Plumb 1999; Srinivasa Rao *et al* 2001; Nucci *et al* 2002). The extraintestinal manifestations of infection include biliary tract infection, bacteraemia, skin and soft tissue infection, liver abscess, peritonitis, intra-abdominal abscess, and tubo-ovarian abscess (Wang *et al* 2005). It has also been reported to cause myonecrosis, puerperal intrauterine infection, septic arthritis and empyema (Ashford *et al* 1998; Slaven *et al* 2001; Mikamo *et al* 2003; Mizunoe *et al* 2006). Many systemic diseases such as septicaemia, meningitis, cholecystitis, endocarditis, liver abscess and osteomyelitis have also been reported (Janda and Abbott 1993). Such observations have raised a concern about *E. tarda* being a significant zoonotic bacterium.

2.2 *The environment*

Environmental stresses such as high temperature, poor water quality and high organic content primarily contribute to the onset and severity of *E. tarda* infections in fish (Plumb 1999; Uhland *et al* 2000; Zheng 2004). Juveniles of channel catfish exposed to high carbon dioxide and ammonia, and low dissolved oxygen showed higher rates of *E. tarda* infection than fish that were not environmentally stressed

Table 2. *Edwardsiella tarda* infection reported in various fish species

Sl. No.	Fish	Reference	
		Year	Author(s)
1	Channel catfish (*Ictalurus punctatus*)	1973	Meyer and Bullock
2	Largemouth bass (*Micropterus salmoides*)	1973	White *et al*
3	Japanese eel (*Anguilla japonica*)	1976	Egusa
4	Mullet (*Mugil cephalus*)	1976	Kusuda *et al*
5	Crimson sea bream (*Evynnis japonicus*)	1977	Kusuda *et al*
6	Freshwater catfish	1979	Wyatt *et al*
7	Tilapia (*Tilapia nilotica*)	1980	Van Damme and Vandepitte
8	Chinook salmon (*Oncorhynchus tshawytscha*)	1982	Amandi *et al*
9	Red sea bream (*Chrysophrys major*)	1982	Yasunaga *et al*
10	Yellowtail (*Seriola gaingu eradiata*)	1982	Yasunaga *et al*
11	Japanese flounder (*Paralichthys olivaceus*)	1983	Nakatsugawa
12	Common carp (*Cyprinus carpio*)	1984	Sae-Oui *et al*
13	Stripped bass (*Morone saxatilis*)	1986	Herman and Bullock
14	Turbot (*Psetta maxima*)	1994	Nougayrede *et al*
15	Asian catfish (*Clarias batrachus*)	1998	Sahoo *et al*
16	Koi (*Anabas testudineus*)	2000	Sahoo *et al*
17	European eel (*Anguilla anguilla*)	2006	Alcaide *et al*

(Walters and Plumb 1980). Muratori (2000) found *E. tarda* infection of the external surface in 17.2%, muscle samples in 14.3% and intestine samples in 11.2% of the five hundred and forty tilapia examined from an integrated fish farm using pig excrements as food, which may have accounted for the high organic load. Fish with abraded skin become more susceptible to *E. tarda* (Pressley 2005); thus, the risk of infection is higher from water contaminated with the bacteria. Wiedenmayer *et al* (2006) reported that channel catfish at high stocking density (20 fish/3 l water) are susceptible to *E. tarda* infection following immersion exposure even without prior mechanical skin injury. Thus, outbreaks of acute *E. tarda* infection are mostly found in channel catfish culture systems when the temperature rises, there is overcrowding and the organic load is high, although mortality often remains low and the infection becomes chronic (Meyer and Bullock 1973; Noga 2000). This may be true for other freshwater fish culture systems also. Matsuoka (2004) found that fish challenged with *E. tarda* shed bacterial cells into the water before and after death, which may play an important role in the spread of edwardsiellosis among cultured fish.

3. Pathogenesis

The bacterium has the ability to live outside the host in pond water and mud. The presence of these bacteria increases with a rise in water temperature, and increased organic load caused by increased feeding rates and amphibian populations. The organism remains in carrier condition in mammals, birds, reptiles, amphibians and aquatic invertebrates. Du *et al* (2007) noted that under unfavourable conditions when the bacteria become non-culturable but still remain viable, they gradually decrease in size and change in shape from short rods to coccoid forms. When such cells are resuscitated in experimental chick embryos, not only is their morphology restored but they also become capable of causing infection and mortality in trout.

The pathogenesis of *E. tarda* is multifactorial. The mechanisms are poorly understood, and the site of attachment and penetration not known, although the intestine and abraded skin are the most likely sites for penetration of the bacteria. The ability of *E. tarda* to adhere to and penetrate epithelial cells has been reported by several workers (Janda *et al* 1991; Strauss *et al* 1997; Ling *et al* 2000). Electron microscopy revealed *E. tarda*-induced formation of extensive projections on the plasma membrane of HEp-2 cells, which resemble membrane ruffles and may be involved in pathogenesis (Phillips *et al* 1998).

To understand the mode of pathogenicity of a pathogen, identification and characterization of virulence factors are strongly needed. Some potential virulence factors of *E. tarda* such as siderophores, cell adhesion factors and cell invasion activity, and two types of haemolysin have been described by several workers (Watson and White 1979; Kokubo *et al* 1990; Janda *et al* 1991; Janda and Abbott 1993; Chen *et al* 1996). One type of haemolysin that is cell associated and iron regulating is secreted into the culture supernatant by iron-regulated culture conditions (Janda and Abbott 1993; Watson and White 1979) while the other type is extracellular hole-forming, and is not regulated by iron (Chen *et al* 1996).

The bacteria are resistant to the bactericidal/static activity of serum and phagocyte-mediated killing (Ainsworth and Chen 1990). The capability of a pathogen to infect its host is partly due to its ability to detoxify various toxic forms of oxygen ($O^{\cdot}_2$, O_2^-, H_2O_2 and $OH^{\cdot}$) present in the host body by producing enzymes such as catalase, peroxidase and superoxide dismutase (SOD). This is undoubtedly true for *E. tarda* also. Yamada and Wakabayashi (1999) identified the SOD gene (*sodB*) of *E. tarda*, which was found to be different in non-virulent and virulent strains (Han *et al* 2006). Srinivasa Rao *et al* (2003) found that a major catalase enzyme, KatB, provides resistance to $H_2O_2^-$ and phagocyte-mediated killing by the host defence system.

Although the genome of *E. tarda* has not been completely sequenced, many important genes are well characterized (table 3). Srinivasa Rao *et al* (2003) have reported 14 genes (derived from 15 Tn*phoA* mutants) that are important in the pathogenesis of *E. tarda*, which encode for virulence factors homologous to phosphate transporters, secretion system regulatory proteins, enzymes such as catalase and glutamate decarboxylase, fimbrial proteins and two novel proteins. The *fimA* gene is responsible for adherence of the bacteria to the host for invasion. Inside the host, the bacteria fight against serum- and phagocyte-mediated killing, which is does with the *gadB, isor, katB, ompS2* and *ssrB* genes. The *pst* and *astA* genes help in acquiring nutrients such as phosphate and iron within the host, which the bacteria need for their proliferation. Some regulatory elements such as *phoU* and *ssrB* probably enable the bacteria to switch on the necessary virulence genes for greater infectivity. Shen and Chen (2005) also observed the expression of several virulent genes (*hlyA*, *citC*, *fimA*, *gadB*, *katB* and *mukF*) that correlated with the mortality of *E. tarda*-infected fish. Fimbrial proteins were found to be induced more significantly under higher sodium chloride concentrations; this may play an important role in the virulence of *E. tarda* in marine environments (Yasunobu *et al* 2006).

As noted earlier, Han *et al* (2006) reported that virulent strains possessed the type I *sodB* (superoxide dismutase) and *katB* (catalase) genes whereas non-virulent strains have type II *sodB*, but not *katB*. They also suggested that the ability of *E. tarda* to resist complement activity and phagocytosis is due to the presence of superoxide dismutase and catalase. Resistance to complement activity might be also achieved by speeding up destruction of the C3 convertase enzyme (Tambourgi *et al* 1993) and by binding host complement regulators onto their own cell surface

Table 3. A few important genes identified and characterized in *E. tarda*

Sl. No.	Gene name (Gene Bank ID)	Description	Reference
1.	*hlyA* (ETAHLYA)	Haemolysin gene	Chen *et al* (1996)
2.	*ethA, ethB* (D89876)	Iron-regulated haemolysin gene	Hirono *et al* (1997)
3.	*determinant* (E03239)	Determinant gene	Aoki and Kitao (1991)
4.	*sodB* (AB009850)	Iron-containing superoxide dismutase gene	Yamada and Wakabayashi (1999)
5.	*flagellin* (AF487406)	Flagellin gene	Tan *et al* (2002)
6.	*gadB* (AY078505)	Glutamate decarboxylase gene	Srinivasa Rao *et al* (2003)
7.	*waaX & isoR* (AY078508)	Beta 1,4-galactosyltransferase and Fe-S oxidoreductase genes	Srinivasa Rao *et al* (2003)
8.	*ompS2* (AY078509)	Outer membrane protein gene	Srinivasa Rao *et al* (2003)
9.	*citrate lyase ligase* (AF491963)	Citrate lyase ligase gene	Srinivasa Rao *et al* (2003)
10.	*fimbrial operon* (AF491964)	Fimbrial operon gene	Srinivasa Rao *et al* (2003)
11.	*pstS* (AF491965)	Phosphate binding protein gene	Srinivasa Rao *et al* (2003)
12.	*katB & ankB* (AY078506)	Catalase precursor and ankyrin genes	Srinivasa Rao *et al* (2003)
13.	*gyrB* (AY370834)	Gyrase B gene	Delmas *et al* (2006)
14.	*gap* (AB198939)	Glyceraldehyde-3-phosphate dehydrogenase gene	Liu *et al* (2005)
15.	*fimA* (DQ914634)	Major fimbrial subunit protein	Guo *et al* (2006)

(Horstmann *et al* 1988). All virulent strains showed esterase and naphthol AS-BI phosphohydrolase activity, and also stronger alkaline phosphatase activity in contrast to their non-virulent counterparts. Apart from these, cell surface structures such as lipopolysaccharide and peptidoglycan also help the bacteria to overcome the hostile environment inside the host (Matthew *et al* 2001). Like other members of the Enterobacteriaceae family, *E. tarda* also possess a siderophore-mediated iron acquisition system, which is necessary for its survival in the host (Kokubo *et al* 1990; Dhaenens *et al* 1999). *E. tarda* is also capable of surviving and being transported as free cells in the blood.

It has recently been found by several workers that *E. tarda* can inject toxic protein into the host cell by means of a type III secretion system (Srinivasa Rao *et al* 2004; Tan *et al* 2005; Zheng *et al* 2005). Zheng *et al* (2007) showed that EseB, EseC and EseD, which form a protein complex after secretion, are secreted by the T3SS and constitute one of the major components of the extracellular proteins (ECPs). Furthermore, it has been noted that the fish isolate of this bacterium produces more virulence factors at 25 °C and lowers the secretion of proteins belonging to the *TTSS* and *evp* gene cluster, thus virulence is reduced when grown at 37 °C (Srinivasa Rao *et al* 2004). This suggests that the bacteria have better adaptability for survival at host internal temperature ranges.

The haemolysin and dermotoxins produced by most of the strains of this bacterium are responsible for major damage (Ullah and Arai 1983a, b; Suprapto *et al* 1996; Hirono *et al* 1997). Aranishi and Mano (2000) reported that during infection the usual level of skin cathepsins, which indicate antibacterial properties, is raised. Thus, the organism can cause septicaemia and is pyogenic in nature.

The recent introduction of the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* as an endogenous fluorescent tag provides a means of both rendering the bacteria visible and tracing their activity in living host cells (Prasher *et al* 1992; Chalfie *et al* 1994; Valdivia *et al* 1996). Using GFP, Ling (2000) found that both avirulent and virulent *E. tarda* strains adhere to, enter and replicate in fish cells using internalization mechanisms involving host microfilaments and protein tyrosine kinase. Histological and infection kinetics studies using GFP revealed that the gastrointestinal tract, gills and body surface of fish are the sites of entry of virulent *E. tarda* (Ling *et al* 2001).

Some workers have recently revealed the relationship between the kinetic change of apoptosis in lymphoid organs and the inflammatory process. The extensive apoptosis in the thymus and spleen suggests that *Edwardsiella* septicaemia generates systemic immunosuppression via lymphocyte apoptosis (Pirarat *et al* 2007).

4. Pathology and diagnosis

4.1 *Gross pathology*

Fish affected by edwardsiellosis shows signs of spiralling movement and die with the mouth agape and opercula flared,

which may be due to the development of anaemia leading to oxygen insufficiency. The fish reveal gross lesions on the skin, pale gills, tumefaction of the eye, excessive mucus secretion, scale erosion and ulcers in a few cases. Swelling and bleeding of the anus leading to reddening is often noticed. In peracute cases congestion of the ventral parts of the body is mostly seen (Meyer and Bullock 1973; Padros *et al* 2006). However, the symptoms or gross signs are not of much help in arriving at a diagnosis as many of these symptoms and signs match those of infection with other bacteria.

In mild infections, the only manifestation of the disease is small cutaneous lesions (3–5 mm in diameter) located on the posterolateral parts of the body. As the disease progresses, abscesses develop within the muscles of the flanks or caudal peduncle. These abscesses rapidly increase in size and develop into large cavities filled with gas and, in the acute stage, are visible as convex, swollen areas. Loss of pigmentation over the lesions is common (Sahoo *et al* 1998). If the lesion is incised, a foul odour is emitted. Necrotic tissue remnants may fill up to one third of the cavity. As the infection progresses, affected fish lose control over the posterior half of the body. The name 'emphysematous putrefactive disease of catfish' (EPDC) has been aptly chosen since it describes the gross appearance of infected fish (Meyer and Bullock 1973).

4.2 *Histopathology*

On histopathological examination, suppurative interstitial nephritis is found most often followed by suppurative hepatitis (figure 1). *E. tarda* infection causes hypertrophy of the liver cells and enlargement of their nuclei (Miwa and Mano 2000). Bacteria-laden phagocytes are found in the sinusoids of the anterior kidney (figures 2 and 3), liver and spleen. Liquefaction and gaseous necrosis are seen in the kidney (figure 4), liver (figure 1), spleen and body musculature leading to ulcer formation (Sahoo *et al* 2000; Jin *et al* 2000). Increased melanomacrophage reaction is frequently observed in the kidney (figure 5). The gills show hyperplastic changes. Due to massive haemolysis of the red blood cells, the spleen accumulates haemosiderin pigments along with the presence of hyperaemia and necrotic changes (Herman and Bullock 1986; Darwish *et al* 2000). The authors have witnessed major damage to the primary ovarian follicles along with oophoritis in Asian catfish due to *E. tarda* infection (Sahoo *et al* 1998). Thus, the pathogen may hamper the breeding process and lead to vertical transfer of infection. Ultrastructural observations indicate that macrophages are the main cell type implicated in the inflammatory response. Most of the bacteria observed within the phagocyte cytoplasm show no degenerative changes and some are seen to be dividing. Degenerative changes observed in the macrophages indicate their failure to prevent the infection (Padros *et al* 2006). However, diagnosis of *E. tarda* infection is made usually on the basis of isolation of the organism and biochemical characterization.

4.3 *Haematology and serology*

Analyses of blood parameters of *E. tarda*-infected tilapia showed reduction in haematocrit values, haemoglobin, erythrocytes, total plasma proteins, Mg^{2+}, K^{+}, Na^{+}, Cl^{-}, and increase in the total leukocyte count and level of plasma glucose (Benli and Yildiz 2004). Caruso *et al* (2002) observed a considerable increase in plasma lysozyme level in *E. tarda*-challenged sheatfish (*Silurus glanis*). A positive correlation between superoxide production during respiratory burst activity and survival against challenge by *E. tarda* was noticed in coho salmon (Balfry *et al* 1994) and rohu (*Labeo rohita*) (Mohanty *et al* 2007), which could serve as a immunological marker for resistance. Serum samples can be analysed using dot, indirect or competitive ELISA methods and antigens can be detected by agglutination tests (Swain *et al* 2001, Swain and Nayak 2003).

4.4 *Expression of immune-related genes*

Expression of c-type lysozyme was found to increase in the anterior kidney, spleen and ovary of Japanese flounder (*Paralichthys olivaceus*) following *E. tarda* infection (Hikima *et al* 1997). Matsuyama *et al* (2007) found that immune-related genes such as MMP-9, MMP-13, CXC chemokine, CD20 receptor and hepcidin are upregulated during edwardsiellosis in Japanese flounder, whereas genes encoding for Mx protein, MHC class II-associated invariant chain, MHC class II alpha and MHC class II beta, immunoglobulin light chain precursor, immunoglobulin light chain and IgM are downregulated.

4.5 *Molecular diagnosis*

PCR-based diagnosis of *E. tarda* infection in blood samples of oyster toadfish was reported by Horenstein *et al* (2004). They had also tried more sensitive real-time PCR methods. TnphoA transposon tagging can be effectively used to identify the genes responsible for virulence in this pathogen. The differences in interactions of virulent and avirulent *E. tarda* organisms with fish phagocytes and PhoA1 fusion mutants could be used to identify virulence genes as suggested by Srinivasa Rao *et al* (2001).

A rapid and sensitive method using loop-mediated isothermal amplification (LAMP) for the diagnosis of edwardsiellosis was reported by Savan *et al* (2005). LAMP is an autocyling strand displacement DNA synthesis

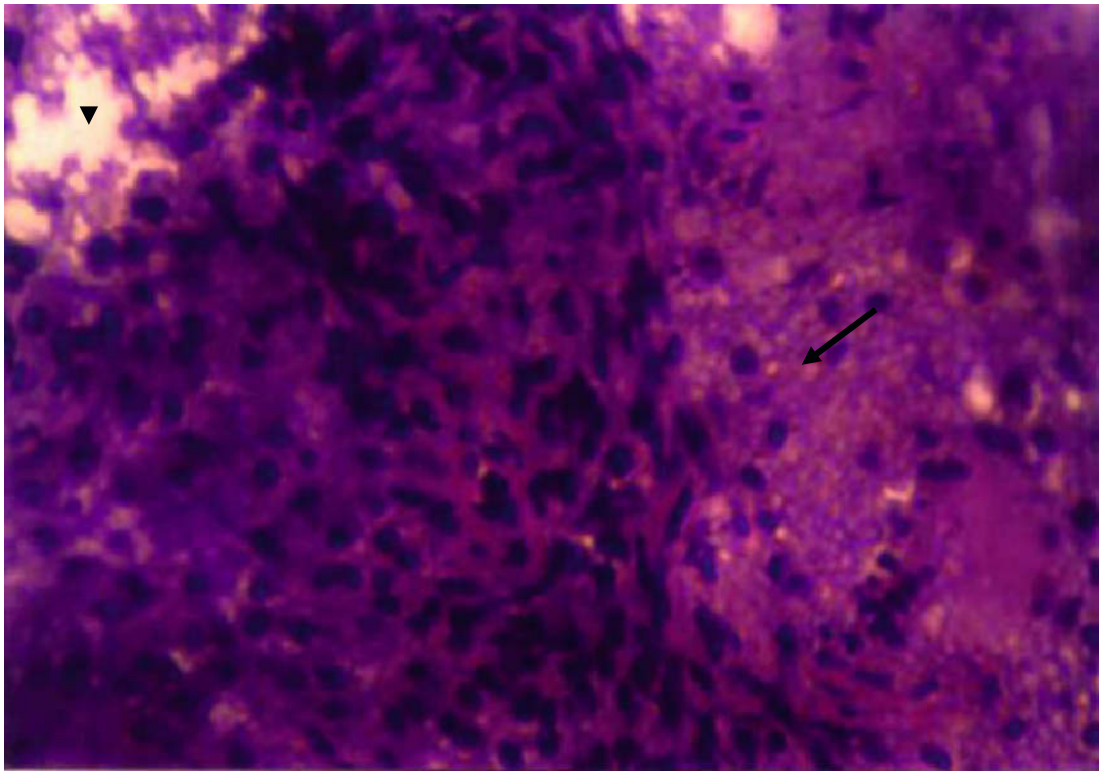

Figure 1. Microabscess formation in the liver (arrow) and massive necrosis of hepatocytes (arrow head) (H&E × 400).

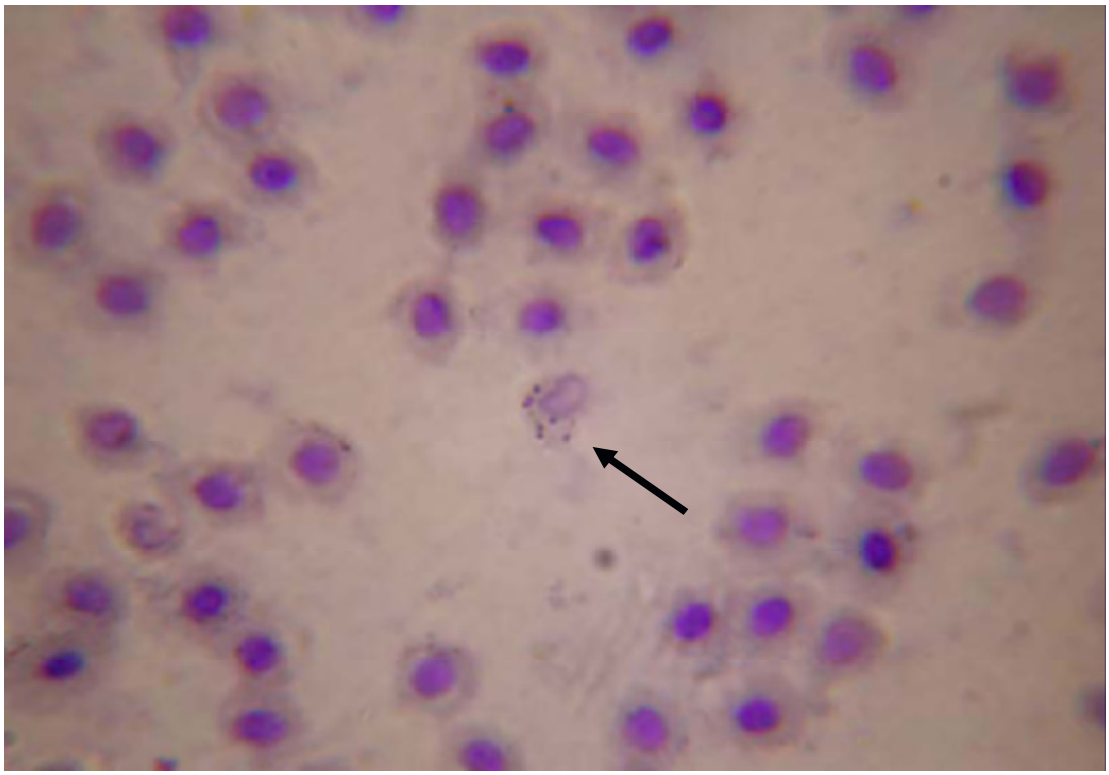

Figure 2. Impression smear from kidney of experimentally *E. tarda*-infected rohu showing bacteria-laden macrophage (arrow) (Giemsa × 1000).

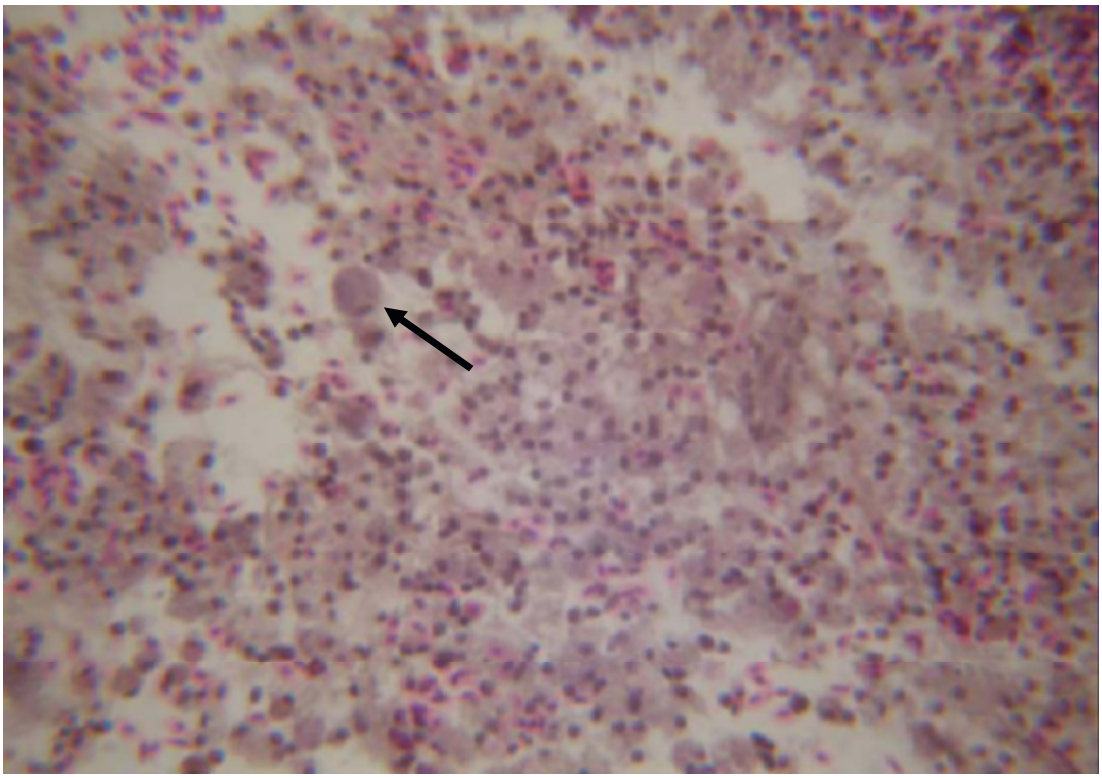

Figure 3. Kidney section from experimentally *E. tarda*-infected rohu showing bacteria-laden macrophage (arrow) (H&E × 400)

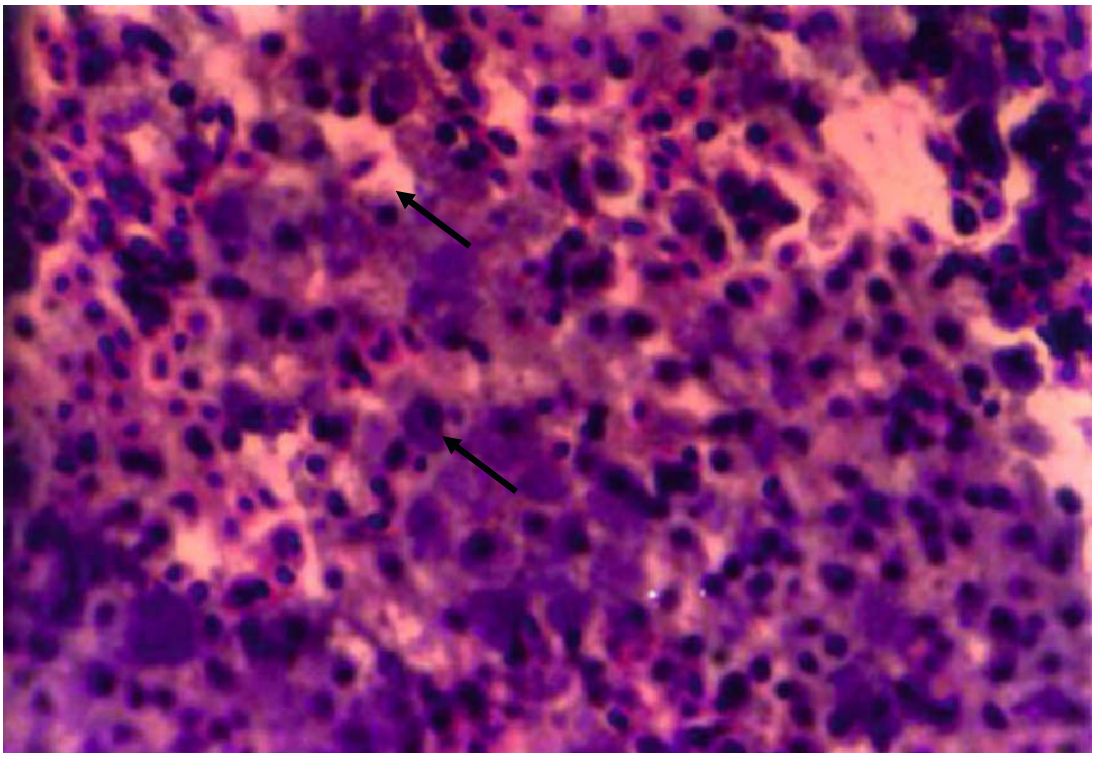

Figure 4. Massive necrosis of haematopoietic tissue and increased macrophage reaction (arrow) in anterior kidney of rohu (× 400).

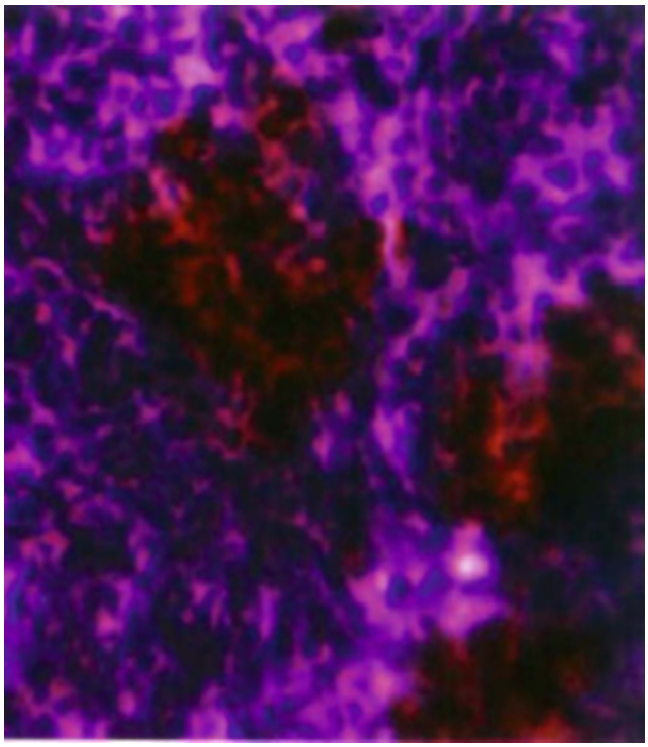

Figure 5. Increased melanomacrophage reaction in anterior kidney tissue of rohu (H&E × 400)

performed using a DNA polymerase with a high level of strand displacement activity and a set of specially designed inner and outer primers (Notomi *et al* 2000).

Various new techniques for more senstitive detection of *E. tarda* are now available for rapid diagnosis. Ootsubo *et al* (2002) devised a fluorescence *in situ* hybridization (FISH) technique using a 24-mer oligonucleotide probe (probe D) that allows the detection of bacterial cells belonging to Enterobacteriaceae, including *E. tarda*, without giving any false-positive reaction. Another technique, which identifies, separates and quantifies intact bacteria using high-performance capillary electrophoresis (HPCE), has been reported by Yu *et al* (2004). They identified *E. tarda* in fish and traced the bacteria (<10 min) after direct injection into fish fluid using CE blue light-emitting diode-induced fluorescence and a cell-permeable green nucleic acid stain (SYTO 13).

5. Prevention and control

As the bacteria are prevalent in many farms, the best way to prevent the disease is to maintain proper environmental conditions in ponds. The physicochemical parameters of the environment should be at the optimum to prevent any infection from flaring up. Hatcheries should maintain proper hygiene and sanitation. The best way to control edwardsiellosis is to constantly monitor for its presence and keep pathogen-free stocks.

Antibiotics and chemotherapy have been used to prevent disease outbreaks and control proliferation of pathogens for a long time, causing the emergence of drug-resistant bacteria. Moreover, four tetracycline-resistant determinant genes, namely *tet-A*, *tet-D*, *tet-B* and *tet-G* have been reported in *E. tarda,* of which the first two types are present on the mobile plasmid (Jin Jun *et al* 2004). Earlier studies indicated that *E. tarda* is susceptible to various antibiotics such as the aminoglycosides, cephalosporins, penicillins, imipenem, aztreonam, ciprofloxacin, sulphamethoxazole/trimethoprim, nitrofurantoin, the quinolones and antibiotic betalactamase-inhibitor agents (Waltman and Shotts 1986; Clark *et al* 1991). Presently, although a good number of antibiotics such as norfloxacin, ciprofloxacin, oxytetracycline, gentamicin, chloramphenicol (Sahoo and Mukherjee 1997), cefazolin (Zhang *et al* 2005) and aztreonam (Zhu *et al* 2006), etc. for tetracycline-resistant strains have been proven to be successful in controlling the infection, these have their own disadvantages such as the development of disease-resistant strains, carry over, high cost and dose problems, as well as indiscriminate use by aquafarmers. Thus, the overuse of antibiotics and other chemicals needs to be checked and the use of alternative methods should be stressed (Taoka *et al* 2006).

Stress factors such as overcrowding, malnutrition, sudden change of water temperature, pH and fluctuations in dissolved oxygen can be avoided. Antistress substances such as probiotics, ascorbic acid, lipopolysaccharides may be added to the feed. Probiotics are microorganisms that impart positive effects on a host animal by improving the microflora of its gastrointestinal tract (Fuller 1989). Probiotics include the metabolites of live or dead bacterial cells, which modify the enzymatic activity or microflora in the gastrointestinal tract. Pirarat *et al* (2006) suggested that in *E. tarda*-infected fish, *Lactobacillus rhamnosus* enhances the alternative complement system of the fish, enables phagocytic cell aggregation, increases phagocytic activity and subsequently protects the fish from acute septicaemic death. Prevention of thymic necrosis by the probiotic supplement seems to initiate an immune response against edwardsiellosis. Taoka *et al* (2006) have also reported that administration of commercial probiotics containing *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum* and *Saccharomyces cerevisiae* enhanced the non-specific immune system, slightly improved the survival of tilapia to salinity stress and reduced mortality due to *E. tarda* infection.

Due to variation in the serotypes of *E. tarda*, a practical effective vaccine was not available for a long time. Nevertheless, some workers reported on a few trials for developing a suitable vaccine. Swain *et al* (2002) found

that 15 min exposure of rohu and catla juveniles to *E. tarda* bacterin suspension improved their resistance to challenge by *E. tarda*. A 37 kDa outer membrane protein (OMP) was detected in several *E. tarda* serotype strains and was designated as an effective vaccine candidate against experimental *E. tarda* infection in Japanese flounder (Kawai *et al* 2004). However, such vaccines require high cost and labour and are thus not practicable. Liu *et al* (2005) prepared a recombinant glyceraldehyde 3-phosphate dyhydrogenase (GAPDH) of *E. tarda,* which could serve as an effective and practical vaccine antigen against *E. tarda* infection in Japanese flounder. It is interesting to note that the GAPDH of *E. tarda* also effectively protect Japanese flounder from *Vibrio anguillarum* infection because the GAPDH of the two bacterial pathogens are highly homologous (91%) (Liu *et al* 2007). Verjan *et al* (2005) detected seven antigenic proteins of *E. tarda* using rabbit polyclonal antiserum and their amino acid sequences had identity with lipoproteins, periplasmic proteins, and exported and secreted proteins with roles in the transport of metabolites across the cell membrane, stress response and motility. The detected genes and their products could be useful for developing DNA or recombinant subunit vaccines.

Recently, *E. tarda* ghosts produced by gene E-mediated lysis were found to be new candidates for developing a vaccine. These ghosts showed higher bactericidal activity and protection in tilapia than those injected with formalin-killed *E. tarda* (Kwon *et al* 2006). In another recent attempt to produce an attenuated vaccine, Lan *et al* (2007) have developed mutants for the *esrB* gene (encoding for a regulator protein of the type III secretion system) of *E. tarda*, which elicited significant protection against edwardsiellosis in turbot. Evans *et al* (2006) have discovered a novel rifampicin-resistant strain of *E. tarda*, which serves as a potent live vaccine against *E. tarda* infection in tilapia, channel catfish and other fish. Moreover, this vaccine provides long-lasting acquired immunity that is superior to experimentally killed *E. tarda* vaccines. It is cost-effective, as it can be administered by bath immersion as well as by injection, and can provide years instead of only months of protection.

Apart from vaccines, farmers have another important, newer and successful method of control/treatment/prevention of the disease, i.e. by the use of immunostimulants. We have screened a good number of immunostimulants against *E. tarda* infection in Indian major carps. A few of them, such as *β*-1,3 glucan from the cell wall of *Saccharomyces cerevisiae* in a dose of 0.1 mg/kg feed for seven days or levamisole in a dose of 5 mg/kg feed on 5 occasions at 3-day intervals were found to be most effective in treating the disease and reducing mortality in Indian major carp (*Labeo rohita*) in acute cases (Sahoo and Mukherjee 2002). These substances also give protection against other bacterial, viral, parasitic and fungal pathogens without creating any environmental hazards. Some other immunostimulants, although found to be less successful, such as megadoses of ascorbic acid and vitamin E, can be also tried. Kim *et al* (2003) found no synergistic effects following dietary supplementation with ascorbic acid, selenium and alpha-tocopheryl acetate in Nile tilapia to *E. tarda* challenge. However, Wang *et al* (2006) showed that high intake of dietary vitamin E with 213 mg *α*-tocopherol/kg feed significantly decreased cumulative mortality and delayed the days to first mortality after a 7-day *E. tarda* challenge in Japanese flounder. They also reported that, under experimental conditions, dietary vitamin E and n-3 HUFA have a synergistic effect on non-specific immune responses and disease resistance. Recently, tuftsin, when injected in three doses of 10 mg/kg body weight was found to enhance the immune response in *Labeo rohita* fingerlings (Misra *et al* 2006).

Genetic selection for disease resistance provides an apparently permanent and cost-effective method of preventing a disease. A wide variation in disease resistance (with per cent survival ranging from 0 to 100) and immune responses against edwardsiellosis was observed recently in *Labeo rohita* by Mohanty *et al* (2007), which indicates the enormous scope of inducing disease resistance in rohu through selection.

6. Conclusion and future directions

Since 1962, when *E. tarda* was first established as a fish pathogen, it has been reported as a consistent hindrance to aquafarming. People around the world are meticulously trying to lighten this burden. As with any other disease, the environment plays an important role in occurrence of the disease. Better management practices of fish culture systems with intermittent examination of water and organic material for the presence of pathogens helps in reducing edwardsiellosis. To bring about a permanent solution, in-depth knowledge of the host–pathogen interaction and its mechanism of action is needed. Recent advances in vaccine development against *E. tarda* are no doubt promising. A deeper understanding of the pathogenicity will help in producing an appropriate and effective vaccine for all fish.

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References

Acharya M, Maiti N K, Mohanty S, Mishra P and Samanta M 2007 Genotyping of *Edwardsiella tarda* isolated from freshwater fish culture system; *Comp. Immunol. Microbiol. Infect. Dis.* **30** 33–40

Ainsworth A J and Chen D X 1990 Differences in the phagocytosis of four bacteria by channel catfish neutrophils; *Dev. Comp. Immunol.* **14** 201–209

Alcaide E, Herraiz S and Esteve C 2006 Occurrence of *Edwardsiella tarda* in wild European eels *Anguilla anguilla* from Mediterranean Spain; *Dis. Aquat. Org.* **73** 77–81

Amandi A, Hiu S F, Rohovec J S and Fryer J L 1982 Isolation and characterization of *Edwardsiella tarda* from fall Chinook salmon (*Oncorhynchus tshawytscha*); *Appl. Environ. Microbiol*. **43** 1380–1384

Aoki C and Kitao T 1991 Determinant gene DNA of fish pathogenic strain and its use; *Patent: JP 1991280882-A 3 11-DEC-1991, Fujisawa Pharmaceut. Co. Ltd.*

Aranishi F and Mano N 2000 Antibacterial cathepsins in different types of ambicoloured Japanese flounder skin; *Fish Shellfish Immunol.* **10** 87–89

Ashford R U, Sargeant P D and Lum G D 1998 Septic arthritis of the knee caused by *Edwardsiella tarda* after a catfish puncture wound; *Med. J Aust.* **168** 443–444

Balfry S K, Iwama G K and Evelyn T P T 1994 Components of the non-specific immune system in coho salmon associated with strain differences in innate disease resistance; *Dev. Comp. Immunol.* **18** S82

Benli A C K and Yildiz H Y 2004 Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*; *Aqua. Res.* **35** 1388–1390

Caruso D, Schlumberger O, Dahm C and Proteau J 2002 Plasma lysozyme levels in sheatfish *Silurus glanis* (L.) subjected to stress and experimental infection with *Edwardsiella tarda*; *Aqua. Res.* **33** 999–1008

Castro N, Toranzo A E, Barja J L, Nunez S and Magarinos B 2006 Characterization of *Edwardsiella tarda* strains isolated from turbot, *Psetta maxima* (L.); *J. Fish Dis.* **29** 541–547

Chalfie M, Tu Y, Euskirchen G, Ward W W and Prasher D C 1994 Green fluorescent protein as a marker for gene expression; *Science* **263** 802–805

Chen J D, Lai S Y and Huang S L 1996 Molecular cloning, characterization and sequencing of the hemolysin gene from *Edwardsiella tarda*; *Arch. Microbiol.* **165** 9–17

Clark R B, Lister P D and Janda J M 1991 In vitro susceptibilities of *Edwardsiella tarda* to 22 antibiotics and antibiotic-beta-lactamase-inhibitor agents; *Diagn. Microbiol. Infect. Dis.* **14** 173–175

Clavijo A M, Conray G, Santander J and Aponte F 2002 First report of *E. tarda* from tilapias in Venezuela; *Bull. Eur. Assoc. Fish Pathol.* **22** 280–282

Darwish A, Plumb J A and Newton J C 2000 Histopathology and pathogenesis of experimental infection with *Edwardsiella tarda* in channel catfish; *J. Aquat. Anim. Health* **12** 255–266

Delmas J, Breysse F, Devulder G, Flandrois J P and Chomarat M 2006 Rapid identification of Enterobacteriaceae by sequencing DNA gyrase subunit B encoding gene; *Diagn. Microbiol. Infect. Dis.* **55** 263–268

Dhaenens L, Szczebara F, Van Nieuwenhuyse S and Husson M O 1999 Comparison of iron uptake in different *Helicobacter* species; *Res. Microbiol.* **150** 475–481

Du M, Chen J, Zhang X, Li A, Li Y and Wang Y 2007 Retention of virulence in a viable but nonculturable *Edwardsiella tarda* isolate; *Appl. Environ. Microbiol.* **73** 1349–1354

Egusa S 1976 Some bacterial diseases of freshwater fishes in Japan; *Fish Pathol.* **10** 103–114

Evans J J, Klesius, P H and Shoemaker C A 2006 Modified live *Edwardsiella tarda* vaccine for aquatic animals; *United States Patent 7067122*

Ewing W H, McWhorter A C, Escobar M R and Lubin A H 1965 *Edwardsiella*, a new genus of Enterobacteriaceae based on a new species; *Int. Bull. Bacteriol. Nomencl. Taxon*. **15** 33–38

Fang H, Zhang X, Chen C, Jin X and Wang X 2006 Studies on the edwardsiellosis and characterization of pathogenic bacteria from diseased flounder (*Paralichthys olivaceus* L.) and turbot (*Scophthalmus maximus* L.); *Acta Oceanol. Sin.* **25** 138–147

Fuller R 1989 Probiotics in man and animals; *J. Appl. Bacteriol.* **66** 365–378

Grimont P A D, Grimont F, Richard C and Sakazaki R 1980 *Edwardsiella hoshinae*, a new species of Enterobacteriaceae; *Curr. Microbiol.* **4** 347–351

Guo L-X, Li S-S, Jiang S-X, Chen W-B, Shao B-Y and Yang J 2006 Cloning and sequence analysis of fimbrial subunit gene (*fimA*) of *Edwardsiella tarda*; *Shipin Kexue* (*Beijing*) **27** 45–48

Han H-J, Kim D-H, Lee D-C, Kim S-M and Park S-I 2006 Pathogenicity of *Edwardsiella tarda* to olive flounder, *Paralichthys olivaceus* (Temminck and Schlegel); *J. Fish Dis.* **29** 601–609

Hawke J P 1979 A bacterium associated with disease of pond cultured channel catfish; *J. Fish. Res. Board Can.* **36** 1508–1512

Herman R L and Bullock G L 1986 Pathology caused by the bacterium *Edwardsiella tarda* in striped bass; *Trans. Amer. Fish. Soc.* **115** 232–235

Hikima J, Hirono I and Aoki T 1997 Characterization and expression of c-type lysozyme cDNA from Japanese flounder (*Paralichthys olivaceus*); *Mol. Mar. Biol. Biotechnol.* **6** 339–344

Hirono I, Tange N and Aoki T 1997 Iron-regulated haemolysin gene from *Edwardsiella tarda*; *Mol. Microbiol.* **24** 851–856

Holt J G, Krieg N R, Sneath P H A, Staley J T and Williams S T 1994 Family Enterobacteriaceae; in *Bergey's manual of determinative bacteriology* (ed.) J G Holt (Baltimore: Williams & Wilkins) pp 175–194

Horenstein S, Smolowitz R, Uhlinger K and Roberts S 2004 Diagnosis of *Edwardsiella tarda* infection in oyster toadfish (*Opsanus tau*) held at the Marine Resources Center; *Biol. Bull. Woods Hole Mass*. **207** 171

Horstmann R D, Sievertsen H J, Knobloch J and Fischetti V A 1988 Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H; *Proc. Natl. Acad. Sci. USA* **85** 1657–1661

Hoshina T 1962 On a new bacterium *Paracolobactrum anguillimortiferum* n. sp.; *Bull. Jap. Soc. Scient. Fish.* **28** 162–164

Janda J M and Abbott S L 1993 Expression of an iron-regulated hemolysin by *Edwardsiella tarda*; *FEMS Microbiol. Lett.* **111** 275–280

Janda J M, Abbott S L, Kroske-Bystrom S, Cheung W K, Powers C, Kokka R P and Tamura K 1991 Pathogenic properties of *Edwardsiella* species; *J. Clin. Microbiol.* **29** 1997–2001

Janda JM and Abbott SL 1993 Infections associated with the genus *Edwardsiella*: the role of *Edwardsiella tarda* in human disease; *Clin. Infect. Dis.* **17** 742–748

Jin Jun L, Bum Jeong J, Huh M-D, Chung J-K, Choi D-I, Lee C-H and Jeong H D 2004 Detection of tetracycline-resistance determinants by multiplex polymerase chain reaction in *Edwardsiella tarda* isolated from fish farms in Korea; *Aquaculture* **240** 89–100

Jin X, Huang W, Xia Y, Li Y and Li B 2000 Application of monoclonal antibody against *Edwardsiella tarda*; *J Fish. China* **24** 554–558

Kawai K, Liu Y, Ohnishi K and Oshima S 2004 A conserved 37 kDa outer membrane protein of *Edwardsiella tarda* is an effective vaccine candidate; *Vaccine* **22** 3411–3418

Kim K, Wang X, Choi S, Park G, Koo J and Bai S C 2003 No synergistic effects by the dietary supplementation of ascorbic acid, alpha-tocopheryl acetate and selenium on the growth performance and challenge test of *Edwardsiella tarda* in fingerling Nile tilapia, *Oreochromis niloticus* L; *Aqua. Res.* **34** 1053–1058

Kokubo T, Iida T and Wakabayashi H 1990 Production of siderophore by *Edwardsiella tarda*; *Fish Pathol.* **17** 243–256

Kusuda R, Itami T, Mumekiyo M and Nakajima H 1977 Characteristics of an *Edwardsiella* sp. from an epizootic of cultured crimson sea breams; *Bull. Jpn. Soc. Scient. Fish.* **43** 129–134

Kusuda R, Toyoshima T, Iwamura Y and Sako H 1976 *Edwardsiella tarda* from an epizootic of mullets (*Mugil cephalus*) in Okitsu Bay; *Bull. Jpn. Soc. Scient. Fish.* **42** 271–275

Kwon S R, Nam Y K, Kim S K and Kim K H 2006 Protection of tilapia (*Oreochromis mosambicus*) from edwardsiellosis by vaccination with *Edwardsiella tarda* ghosts; *Fish Shellfish Immunol.* **20** 621–626

Lan M Z, Peng X, Xiang M Y, Xia Z Y, Bo W, Jie L, Li X Y and Jun Z P 2007 Construction and characterization of a live, attenuated *esrB* mutant of *Edwardsiella tarda* and its potential as a vaccine against the haemorrhagic septicemia in turbot, *Scophthamus maximus* (L.); *Fish Shellfish Immunol.* (in press)

Ling S H M, Wang X H, Lim T M and Leung K Y 2001 Green fluorescent protein-tagged *Edwardsiella tarda* reveals portal of entry in fish; *FEMS Microbiol. Lett.* **194** 239–243

Ling S H M, Wang X H, Xie L, Lim T M and Leung K Y 2000 Use of green fluorescent protein (GFP) to track the invasive pathways of *Edwardsiella tarda* in the *in vivo* and *in vitro* fish models; *Microbiology* **146** 7–19

Liu Y, Oshima S-I and Kawai K 2007 Glyceraldehyde-3-phosphate dehydrogenase of *Edwardsiella tarda* has protective antigenicity against *Vibrio anguillarum* in Japanese flounder; *Dis. Aquat. Org.* **75** 217–220

Liu Y, Oshima S-I, Kurohara K, Ohnishi K and Kawai K 2005 Vaccine efficacy of recombinant GAPDH of *Edwardsiella tarda* against edwardsiellosis; *Microbiol. Immunol.* **49** 605–612

Mathew J A, Tan Y P, Srinivasa Rao P S, Lim T M and Leung K Y 2001 *Edwardsiella tarda* mutants defective in siderophore production, motility, serum resistance and catalase activity; *Microbiology* **147** 449–457

Matsuoka S 2004 Discharge of *E. tarda* cells from experimentally infected Japanese flounder; *Fish Pathol.* **39** 9–14

Matsuyama T, Fujiwara A, Nakayasu C, Kamaishi T, Oseko N, Hirono I and Aoki T 2007 Gene expression of leucocytes in vaccinated Japanese flounder (*Paralichthys olivaceus*) during the course of experimental infection with *Edwardsiella tarda*; *Fish Shellfish Immunol.* **22** 598–607

Matsuyama T, Kamaishi T, Ooseko N, Kurohara K and Iida T 2005 Pathogenicity of motile and non-motile *Edwardsiella tarda* to some marine fish; *Fish Pathol.* **40** 133–136

McWhorter A C, Ewing W H and Sakazaki R 1967 *Bacteriol. Proc*. p. 89

Meyer F P and Bullock G L 1973 *Edwardsiella tarda*, a new pathogen of channel catfish (*Ictalurus punctatus*); *Appl. Microbiol.* **25** 155–156

Mikamo H, Ninomiya M, Sawamura H and Tamaya T 2003 Puerperal intrauterine infection caused by *Edwardsiella tarda*; *J Infect. Chemother.* **9** 341–343

Misra C K, Das B K, Mukherjee S C and Meher P K 2006 The immunomodulatory effects of tuftsin on the non-specific immune system of Indian major carp, *Labeo rohita*; *Fish Shellfish Immunol.* **20** 728–738

Miwa S and Mano N 2000 Infection with *Edwardsiella tarda* causes hypertrophy of liver cells in Japanese flounder *Paralichthys olivaceus*; *Dis. Aquat. Org.* **42** 227–231

Mizunoe S, Yamasaki T, Tokimatsu I, Matsunaga N, Kushima H, Hashinaga K and Kadota J 2006 A case of empyema caused by *Edwardsiella tarda*; *J. Infect.* **53** 255–258

Mohanty B R, Sahoo P K, Mahapatra K D and Saha J N 2007 Innate immune responses in families of Indian major carp, *Labeo rohita*, differing in their resistance to *Edwardsiella tarda* infection; *Curr. Sci.* **92** 1270–1274

Muratori M C S, de Oliveira A L, Ribeiro L P, Leite R C, Costa A P R and da Silva M C C 2000 *Edwardsiella tarda* isolated in integrated fish farming; *Aqua. Res.* **31** 481–483

Nakatsugawa T 1983 *Edwardsiella tarda* isolated from cultured young flounders; *Fish Pathol.* **18** 99–101

Noga E J 2000 *Fish disease diagnosis and treatment* (Iowa: Iowa State University Press)

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N and Hase T 2000 Loop-mediated isothermal amplification of DNA; *Nucleic Acids Res.* **28** E63

Nougayrede P H, Vuillaume A, Vigneulle M, Faivre B, Luengo S and Delprat J 1994 First isolation of *Edwardsiella tarda* from diseased turbot (*Scophthalmus maximus*) reared in a sea farm in the Bay of Biscay; *Bull. Eur. Assoc. Fish Pathol.* **14** 128–129

Nucci C, Silveria W D, Correa S S, Nakazato G, Bando S Y, Ribeiro M A and Castro A F P 2002 Microbiological comparative study of isolates of *Edwardsiella tarda* isolated in different countries from fish and humans; *Vet. Microbiol.* **89** 29–39

Ootsubo M, Shimizu T, Tanaka R, Sawabe T, Tajima K, Yoshimizu M, Ezura Y, Ezaki T and Oyaizu H 2002 Oligonucleotide probe for detecting Enterobacteriaceae by *in situ* hybridization; *J. Appl. Microbiol.* **93** 60–68

Padros F, Zarza C, Dopazo L, Cuadrado M and Crespo S 2006 Pathology of *Edwardsiella tarda* infection in turbot, *Scophthalmus maximus* (L.); *J. Fish Dis.* **29** 87–94

Panangala V S, Shoemaker C A, McNulty S T, Arias C R and Klesius P H 2006 Intra- and interspecific phenotypic characteristics of fish-pathogenic *Edwardsiella ictaluri* and *E. tarda*; *Aqua. Res.* **37** 49–60

Park S, Wakabayashi H and Watanabe Y 1983 Serotype and virulence of *Edwardsiella tarda* isolated from eel and their environment; *Fish Pathol.* **18** 85–89

Phillips A D, Trabulsi L R, Dougan G and Frankel G 1998 *Edwardsiella tarda* induces plasma membrane ruffles on infection of HEp-2 cells; *FEMS Microbiol. Lett.* **161** 317–323

Pirarat N, Kobayashi T, Katagiri T, Maita M and Endo M 2006 Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*); *Vet. Immunol. Immunopathol.* **113** 339–347

Pirarat N, Maita M, Endo M and Katagiri T 2007 Lymphoid apoptosis in *Edwardsiella tarda* septicemia in tilapia, *Oreochromis niloticus*; *Fish Shellfish Immunol.* **22** 608–616

Plumb J A 1999 *Health maintenance and principal microbial diseases of cultured fish* (Ames: Iowa State University Press)

Prasher D C, Eckenrode V K, Ward W W, Prendgast F G and Cormier M J 1992 Primary structure of the *Aequorea victoria* green-fluorescent protein; *Gene* **111** 229–233

Pressley M E, Phelan P E III, Witten P E, Mellon M T and Kim C H 2005 Pathogenesis and inflammatory response to *Edwardsiella tarda* infection in the zebrafish; *Dev. Comp. Immunol.* **29** 501–513

Rashid M M, Honda K, Nakai T and Muroga K 1994 An ecological study on *Edwardsiella tarda* in flounder farms; *Fish. Pathol.* **29** 221–227

Sae-Oui D, Muroga K and Nakai T 1984 A case of *Edwardsiella tarda* infection in cultured colored carp *Cyprinus carpio*; *Fish Pathol.* **19** 197–199

Sahoo P K and Mukherjee S C 1997 *In-vitro* susceptibility of three bacterial pathogens of catfish to 23 antimicrobial agents; *Indian J. Fish.* **44** 393–397

Sahoo P K and Mukherjee S C 2002 The effect of dietary immunomodulation upon *Edwardsiella tarda* vaccination in healthy and immunocompromised Indian major carp (*Labeo rohita*) *Fish Shellfish Immunol.* **12** 1–16

Sahoo P K, Mukherjee S C and Sahoo S K 1998 *Aeromonas hydrophila* versus *Edwarsiella tarda*: A pathoanatomical study in *Clarias batrachus*; *J. Aqua.* **6** 57–66

Sahoo P K, Swain P, Sahoo S K, Mukherjee S C and Sahu A K 2000 Pathology caused by the bacterium *Edwarsiella tarda* in *Anabas testudineus (Bloch)*; *Asian Fish. Sci.* **13** 357–362

Sakazaki R 1984 Serological typing of *Edwardsiella tarda*; in *Methods in microbiology Vol 15* (ed) P H T Bergan (London: Academic Press) pp 213–225

Sano M, Minagowa M, Sugiyama A and Nakajima K 2001 Diseases of cultured marine fish in subtropical areas in Japan; *Bull. Nat. Res. Inst. Aquacult. Suppl.* **5** 15–18

Savan R, Kono T, Itami T and Sakai M 2005 Loop-mediated isothermal amplification: an emerging technology for detection of fish and shellfish pathogens; *J. Fish Dis.* **28** 573–581

Shen Y-R and Chen J-D 2005 Expression of virulent genes of *Edwardsiella tarda* correlated with mortality of diseased-fish infection; *J. Fish. Soc. Taiwan* **32** 80–81

Slaven E M, Lopez F A, Hart S M and Sanders C V 2001 Myonecrosis caused by *Edwardsiella tarda*: a case report and case series of extraintestinal *E. tarda* infections; *Clin. Infect. Dis.* **32** 1430–1433

Srinivasa Rao P S, Lim T M and Leung K Y 2001 Opsonized virulent *Edwardsiella tarda* strains are able to adhere to and survive and replicate within fish phagocytes but fail to stimulate reactive oxygen intermediates; *Infect. Immun.* **69** 5689–5697

Srinivasa Rao P S, Lim T M and Leung K Y 2003 Functional genomics approach to the identification of virulent genes involved in *Edwardsiella tarda* pathogenesis; *Infect. Immun.* **71** 1343–1351

Srinivasa Rao P S, Yamada Y and Leung K Y 2003 A major catalase (KatB) that is required for resistance to H_2O_2 and phagocyte mediated killing in *Edwardsiella tarda*; *Microbiology* **149** 2635–2644

Srinivasa Rao P S, Yamada Y, Tan Y P and Leung K Y 2004 Use of proteomics to identify novel virulence determinants that are required for *Edwardsiella tarda* pathogenesis; *Mol. Microbiol.* **53** 573–586

Strauss E J, Ghori N and Falkow S 1997 An *Edwardsiella tarda* strain containing a mutation in a gene with homology to sglB and hpmB is defective for entry into epithelial cells in culture; *Infect. Immun.* **65** 3924–3932

Suprapto H, Hara T, Nakai T and Muroga K 1996 Purification of a lethal toxin of *Edwardsiella tarda*; *Fish Pathol*. **31** 203–207

Swain P and Nayak S K 2003 Comparative sensitivity of different serological tests for seromonitoring and surveillance of *Edwardsiella tarda* infection of Indian major carps; *Fish Shellfish Immunol.* **15** 333–340

Swain P, Mukherjee S C, Sahoo P K, Das B K, Pattnaik P, Murjani G and Nayak S K 2001 Dot-enzyme-linked immunosorbent assay (Dot-ELISA) for the diagnosis of *Edwardsiella tarda* infection in fish; *Asian Fish. Sci.* **14** 89–93

Swain P, Nayak S K, Sahu A, Mohapatra B C and Meher P K 2002 Bath immunization of spawn, fry and fingerlings of Indian major carps using a particulate bacterial antigen; *Fish Shellfish Immunol.* **13** 133–140

Tambourgi D A, Kipnis T L, da Silva W D, Joiner K A, Sher A, Heath S, Hall B F and Ogden G B 1993 A partial cDNA clone of trypomastigote decay-accelerating factor (T-DAF), a developmentally regulated complement inhibitor of *Trypanosoma cruzi*, has genetic and functional similarities to the human complement inhibitor DAF; *Infect. Immun.* **61** 3656–3663

Tamura K, Sakazaki R, McWhorter A C and Kosako Y 1988 *Edwardsiella tarda* serotyping scheme for international use; *J. Clin. Microbiol.* **26** 2343–2346

Tan Y P, Lin Q, Wang X H, Joshi S, Hew C L and Leung K Y 2002 Comparative proteomic analysis of extracellular proteins of *Edwardsiella tarda*; *Infect. Immun.* **70** 6475–6480

Tan Y P, Zheng J, Tung S L, Rosenshine I and Leung K Y 2005 Role of type III secretion in *Edwardsiella tarda* virulence; *Microbiology* **151** 2301–2313

Taoka Y, Maeda H, Jo J-Y, Kim S-M, Park S-I, Yoshikawa and Sakata T 2006 Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*; *Fish. Sci.* **72** 755–766

Thune R L, Stanley L A and Cooper R K 1993 Pathogenesis of Gram-negative bacterial infections in warm water fish; *Annu. Rev. Fish Dis.* **3** 37–68

Uhland F C, Helie P, Higgins R, 2000 Infections of *Edwardsiella tarda* among brook trout in Quebec; *J. Aquat. Anim. Health* **12** 74–77

Ullah M A and Arai T 1983a Pathological activities of the naturally occurring strains of *Edwardsiella tarda*; *Fish Pathol.* **18** 65–70

Ullah M A and Arai T 1983b Exotoxic substances produced by *Edwardsiella tarda*; *Fish Pathol.* **18** 71–75

Valdivia R H, Hromockyj A E, Monack D, Ramakrishnan L and Falkow S 1996 Applications for green fluorescent protein (GFP) in the study of host–pathogen interactions; *Gene* **173** 47–52

Van Damme L R and Vandepitte J 1980 Frequent isolation of *Edwardsiella tarda* and *Plesiomonas shigelloides* from healthy Zairese freshwater fish: possible source of sporadic diarrhea in the tropics; *Appl. Environ. Microbiol.* **39** 475–479

Verjan N, Hirono I and Aoki T 2005 Genetic loci of major antigenic protein genes of *Edwardsiella tarda*; *Appl. Environ. Microbiol.* **71** 5654–5658

Walters G R and Plumb J A 1980 Environmental stress and bacterial infection in channel catfish, *Ictalurus puctatus* Rafinesque; *J. Fish Biol.* **17** 177–185

Waltman W D and Shotts E B 1986 Antimicrobial susceptibility of *Edwardsiella tarda* from the United States and Taiwan; *Vet. Microbiol.* **12** 277–282

Wang I K, Kuo H L, Chen Y M, Lin C L, Chang H Y, Chuang F R and Lee M H 2005 Extraintestinal manifestations of *Edwardsiella tarda* infection; *Int. J. Clin. Pract.* **59** 917–921

Wang Z, Mai K, Kangsen M, Liufu Z, Ma H, Xu W, Ai Q, Zhang W, Tan B and Wang X 2006 Effect of high dietary intakes of vitamin E and n-3 HUFA on immune responses and resistance to *Edwardsiella tarda* challenge in Japanese flounder (*Paralichthys olivaceus*, Temminck and Schlegel); *Aqua. Res.* **37** 681–692

Watson J J and White F H 1979 Hemolysins of *Edwardsiella tarda*; *Can. J. Comp. Med.* **43** 78–83

White F H, Simpson C F and Williams L E Jr 1973 Isolation of *Edwardsiella tarda* from aquatic animal species and surface waters in Florida; *J. Wildlife Dis.* **9** 204–208

Wiedenmayer A A, Evans J J and Klesius P H 2006 Experimental *Edwarsiella tarda* infection in nonabraded channel catfish *Ictalurus punctatus* by immersion; *Fish. Sci.* **72** 1124–1126

Wyatt L E, Nickelson II R and Vanderzant C 1979 *Edwardsiella tarda* in freshwater catfish and their environment; *Appl. Environ. Microbiol.* **38** 710–714

Yamada Y and Wakabayashi H 1999 Identification of fish pathogenic strains belonging to genus *Edwardsiella* by sequence analysis of sodB; *Fish Pathol.* **34** 145–150

Yasunaga N, Ogawa S and Hatai K 1982 Characteristics of the fish pathogen *Edwardsiella* isolated from several species of cultured marine fishes; *Bull. Nagasaki Prefecture Inst. Fish.* **8** 57–65

Yasunobu H, Arikawa Y, Fumtsuka-Uozumi K, Domo M, Iida T, Mahmoud M M, Okuda J and Nakai T 2006 Induction of hemagglutination activity of *Edwardsiella tarda* by sodium chloride; *Fish Pathol.* **41** 29–34

Yu L, Yuan L, Feng H and Li S F 2004 Determination of the bacterial pathogen *Edwardsiella tarda* in fish species by capillary electrophoresis with blue light-emitting diode-induced fluorescence; *Electrophoresis* **25** 3139–3144

Zhang X-J, Fang H, Chen C-Z, Ge M-X and Wang X-Y 2005 Sensitivity of pathogenic *Edwardsiella tarda* isolated from flounder (*Paralichthys olivaceus*) to some antimicrobial agents; *Fish. Sci.* **24** 15–18

Zheng D, Mai K, Liu S, Limin C, Liufu Z, Xu W, Tan B and Zhang W 2004 Effect of temperature and salinity on virulence of *Edwardsiella tarda* to Japanese flounder, *Paralichthys olivaceus* (Temminck et Schlegel); *Aqua. Res.* **35** 494–500

Zheng J, Li N, Tan Y P, Sivaraman J, Mok Y K, Mo Z L and Leung K Y 2007 EscC is a chaperone for the *Edwardsiella tarda* type III secretion system putative translocon components EseB and EseD; *Microbiology* **153** 1953–1962

Zheng J, Tung S L and Leung K Y 2005 Regulation of a type III and a putative secretion system in *Edwardsiella tarda* by EsrC is under the control of a two-component system, EsrA–EsrB; *Infect. Immun.* **73** 4127–4137

Zhu Z-C, Shi X-G, Zhang S-J, Jiang G-J, Xing Z-B, Zhao Y-L, Li Z-J and Wu P 2006 The pathogenic bacteria of the ascites in Japanese flounder (*Paralichthys olivaceus*); *Fish. Sci.* **7** 325–329

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