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Identification and Antibiotic Resistance *Edwardsiella tarda* from Clown Knifefish (*Chitala chitala*) in the Mekong Delta, Vietnam

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ABSTRACT

This investigation is intended to isolate, identify, and assess the pathogenicity of *Edwardsiella tarda*, which originated from diseased clown knifefish. A total of 43 isolates were obtained from infected fish samples in Hau Giang and Dong Thap provinces of the Mekong Delta, Vietnam. Two isolates of DT37 and HG41 were identified as *E. tarda* by morphological, biochemical, and 16S rRNA gene sequencing. Experimental challenge studies revealed that isolate DT37 leads to 83.33% at a 10^8 CFU/ml concentration after 60 hours. Meanwhile, in isolate HG41, mortality reached 100% within 48 hours post-injection at the highest concentration of 10^8 CFU/ml. The challenged clown knifefish exhibited gross signs of abnormal swimming, skin ulcerations, and petechial hemorrhages in the body. Internally, ascites with hemoperitoneum, light-colored nodules on the liver, hemorrhagic kidneys, and splenomegaly were also recorded. The LD_{50} of two isolates, DT37 and HG41, was 4.89×10^5 and 4.07×10^5 CFU/ml, respectively. The antibiogram result showed that most of the isolates were highly susceptible to ampicillin (65%), enrofloxacin (85%), florfenicol (100%), flumequine (90%), cefotaxime (80%), and trimethoprim and sulfamethoxazole (70%). However, the bacterial isolates were highly resistant to doxycycline (75%) and streptomycin (100%).

1. Introduction

Clown knifefish (*Chitala chitala*) is a freshwater fish with a large size that is easy to raise, has fast growth, and has good meat quality (Long *et al.* 2014). It should be widely farmed in some Asian countries such as India, Bangladesh, the Philippines, Myanmar, and Pakistan (Talwar and Jhingran 1991). In addition, this fish can be cultured at high densities, with low oxygen tolerance and adverse environmental conditions (Huong *et al.* 2020). In the Mekong Delta, the fish is mainly cultured in a few provinces such as Bac Lieu, Vinh Long, Tien Giang, Tra Vinh, and Dong Thap, of which the most fish are farmed in Hau Giang province (So and Tuan 2022). According to the statistics of the functional sector, the area and output of clown knifefish in Hau Giang province in 2017 were 50.8 ha and 2,775 thousand tons, respectively. However, by 2020, the area and fish production had increased to 86 ha and 6,880 thousand tons (So and Tuan 2022).

Presently, clown knifefish are grown commercially mainly by two models with high economic efficiency, namely in earthen ponds and fence nets (Viet 2015). However, the rapid development of farming areas and increasing density are some of the reasons for the increase in epidemics, especially bacterial infectious diseases, which have caused serious damage to aquaculture (Le and Cheong 2010). Many types of bacteria causing great losses in many freshwater fish species in the world and Vietnam have been recorded as *Aeromonas* spp., *Pseudomonas* spp., *Edwardsiella* spp., *Vibrio* spp., *Flavobacterium* spp., *Streptococcus* spp., and *Mycobacterium* spp. (Mohanty and Sahoo 2007; Abowei and Briyai 2011; Kumari 2020; Aziz and Abdullah 2021; Haenen *et al.* 2023). In addition, farmers often use fresh feed such as zooplankton, worms, pureed trash fish, or minced trash fish (Khanh 2006; Tien *et al.* 2012).

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Edwardsiella includes bacterial species of the Enterobacteriaceae family, first discovered in 1962 by Sakazaki in Japan (Inglis *et al.* 1993) and described by Ewing *et al.* (1965). Since recently, the genus has comprised five species, consisting of *E. ictaluri, E. tarda, E. hoshinae, E. piscicida*, and *E. anguillarum* (Kerie *et al.* 2019). Three species, including *E. ictaluri, E. tarda*, and *E. anguillarum,* have been implicated in the infection and mortality of numerous fish (Clavijo *et al.* 2002; Crumlish *et al.* 2002; Soto *et al.* 2012; Oh *et al.* 2020). *Edwardsiella tarda*, causing edwardsiellosis, putrefactive disease, or *Edwardsiella septicemia*, is a Gram-negative bacterium, motile, short, rod-shaped of the family Enterobacteriaceae (Janda *et al.* 1991). It is recognized as a disease of many fish, including catfish, eel, and tilapia (Janda *et al.* 1991; Clavijo *et al.* 2002; Diaz and Lopez 2015; Oh *et al.* 2020). This disease frequently causes septicemia in fish, leading to huge economic losses and large fatalities (up to 70%) in freshwater and marine fish farms in many nations (Alcaide *et al.* 2006; Park *et al.* 2012). In Vietnam, studies have shown that *E. ictaluri* bacteria cause disease in catfish (Crumlish *et al.* 2002; Dung *et al.* 2008). Many studies reveal that the *E. ictaluri* bacteria that causes disease in pangasius is resistant to many antibiotics (Dung *et al.* 2008; Thi *et al.* 2014). Until now, however, there have been no reports of isolation, identification, or antibiotic resistance in *E. tarda* causing disease in clown knifefish. Therefore, the study was carried out to provide information for the diagnosis, prevention, and treatment of bacterial diseases in clown knifefish in a reasonable way.

2. Materials and Methods

2.1. Collection of Fish Samples

During disease outbreaks, 112 fish samples were taken from different commercial farms and hatcheries in the provinces of Hau Giang and Dong Thap, Vietnam (Figure 1). Infected fish with gross signs of abnormal swimming, skin ulcerations, and petechial hemorrhages in the body were collected and bacteriologically examined (Figure 1). In cases where fish farms were far away from the laboratory, diseased fish were also analyzed on-farm to avoid the death and decomposition of samples during transportation.

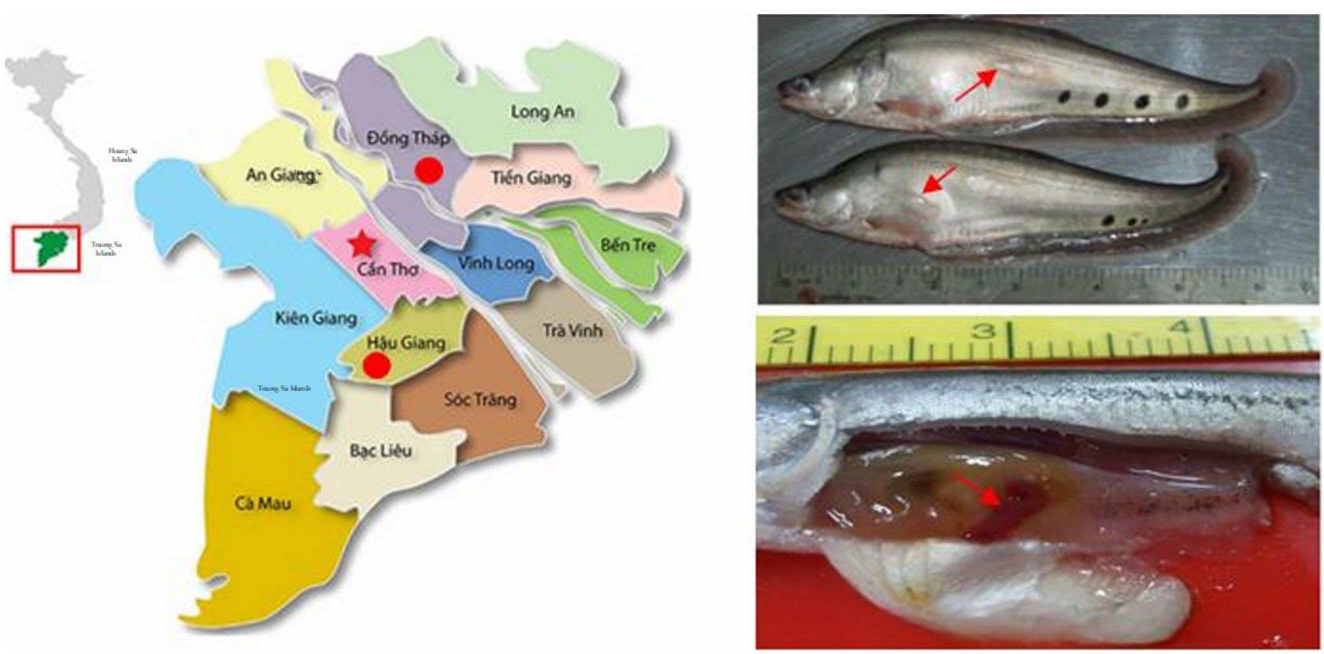

Figure 1. Location of diseased fish sample collection (red circle) and diseased fish (ulcerative lesion, congested spleen, and fluid in the abdominal cavity, red arrow) for *E. tarda* isolation

2.2. Isolation of Bacteria

E. tarda bacteria were isolated on tryptone soya agar medium (TSA, Meck, Germany) according to the manuals of Frerichs and Millar (1993). Briefly, the fish was cleaned of fish slime and aseptically dissected. Bacterial isolates were recovered from the kidney, liver, and spleen on TSA by streak plate method and incubated at 28°C for 24 hours. The presumptive and purified colonies were routinely subcultured on TSA for observation and identification after 24–48 hours of incubation. A few purified colonies were then enriched in brain heart infusion broth (BHIB, Meck, Germany) at 28°C for 24 hours and stored with 20% glycerol at -80°C.

2.3. Bacterial Identification

The primary tests of morphological and biochemical characterization, including Gram stain, motility, oxidase, catalase, oxidation/fermentation (O/F), and O/129 (150 µg), were performed according to Barrow and Feltham (2004). Using the API 20E test kit, the bacteria were identified to species level (Biomérieux, France), and the biochemical profiles of the test were recorded after 24 hours. In addition, bacteria were also identified by biomolecular methods and sequenced with the 16S rRNA gene with primers 27F: 5'-AGAGTTTGATCCTGGCTC-3' and 1492R: 5'-TACGGTTACCTTGTTACGACT-3' (Heuer *et al.* 1997). The genomic DNA of the bacteria was extracted according to Miller *et al.* (1988). Using a UV spectrophotometer, the amount and quality of the bacterial DNA were assessed at absorbances of 260 and 280 nm.

PCR reaction components consist of 1X PCR buffer; 1.5 mM $MgCl_2$; 150 µM dNTPs; 2U Taq DNA polymerase; 20 pmol of primer 27F and primer 1492R, and 20–40 ng of bacterial DNA. The thermal cycle performed the PCR reaction, consisting of initial denaturation stages at 94°C for 5 minutes, then 35 cycles including denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute, extension at 72°C for 2 minutes, and the final elongation at 72°C for 10 minutes. The amplified DNA product is 1,500 bp in size. Two isolates, DT37 and HG41 (used in the challenge experiment), were selected for sequencing at Macrogen, Korea (www.macrogen.com).

2.4. Challenge Experiment

2.4.1. Preparation of Bacterial Suspension

Two isolates DT37 and HG41, were used in the challenge experiment. The bacteria were enriched in BHIB medium at 28°C for 24 hours. The cultures were centrifuged at 4,000 rpm for 15 minutes and washed thrice in sterile normal saline (0.85% NaCl). The turbidity of bacterial density was compared with the 0.5 McFarland standard (bioMerieux, France), equivalent to a concentration of 10^8 CFU/ml. The bacterial concentration used in this study was diluted in sterile normal saline (0.85% NaCl) from 10^8 to 10^4 CFU/ml.

2.4.2. Fish

A total of 210 healthy clown knifefish fingerlings with an average weight of 15±4 g were collected from a hatchery in Dong Thap province and used for infection experiments. Before the injection challenge, fish were acclimated in a 500 L tank and maintained under standard experimental conditions with continuous aeration for two weeks. For experimental assurance, ten fish were randomly examined for external parasites and the presence of bacteria.

2.4.3. Study Design

There were six treatments and a control group in triplicate. Ten fish were randomly delivered in a 60 L tank for each treatment and control group. Each fish was inoculated intraperitoneally with 0.1 mL of bacterial suspension at the dose described in Table 1. Control groups received 0.1 ml volumes of sterile 0.9% NaCl solutions (w/v) as an inoculation. The water temperature was maintained at 28–30°C, and mortalities were observed during the 14 days of the challenge. All moribund and dead fish were sampled, reisolated, and identified for any clinical signs of disease. Finally, the Reed and Muench (1983) approach was used to calculate the lethal dose (LD_{50}) value.

2.5. Disc Diffusion Method

Antibiotic susceptibility was assessed using the Kirby-Bauer disc diffusion method (Bauer *et al.* 1966). Ten antibiotics (Oxoid, UK) were used to conduct the antibiogram: ampicillin

Table 1. Treatment groups, and bacterial concentrations used in this study

Treatment group	Number of fish	Isolates	Challenge dose (CFU/ml)
1	10	*E. tarda* DT37	10^4
2	10		10^6
3	10		10^8
4	10		10^4
5	10	*E. tarda* HG41	10^6
6	10		10^8
7	10	Control (0.9% NaCl solution)	No bacteria

CFU: colony-forming units

(AMP/10µg), cephalexin (CL/10µg), cefotaxime (CTX/10µg), cefazolin (KZ/10µg), doxycycline (DO/30µg), enrofloxacin (ENR/5µg), flumequine (UB/30µg), florfenicol (FFC/30µg), streptomycin (S/10µg), and sulfamethoxazole and trimethoprim (SXT/1,25/23,75µg) were used in this method.

In brief, single bacterial colonies after incubation of 24 hours were suspended in 0.85% saline solution, and the turbidity matched the 0.5 McFarland standard (bioMerieux, France). Then, the bacterial solution was spread on the surface of the Muller-Hinton agar (MHA, Merck, Germany). Finally, the antibiotic discs were placed on the agar. The inhibition zone diameter was measured in mm after 24–48 hours of incubation at 28°C. The determination of inhibitory zone diameters as susceptible (S), intermediate (I), and resistant (R) was based on a document from the CLSI (2020). The reference strain *Escherichia coli* ATCC 25922 (purchased from MicroBiologics, USA) was used as quality control.

2.6. Data Analysis

Descriptive statistics were used to determine antimicrobial resistance, and cumulative mortality. The BLASTn tool was used to compare the sequence similarity of bacterial strains with sequences in the NCBI database (National Center for Biotechnology Information).

3. Results

3.1. Bacterial Characterization

A total of 43 isolates from clown knifefish morphologically appeared circular, convex, and opaque. They were Gram-negative, short-rod-shaped, positive catalase, negative oxidase, and fermentative in anaerobic and aerobic conditions (Figure 2). They were motile and could grow at 37°C. Besides, the results also showed that all of the bacteria isolates could grow in the media with 0-3% NaCl (Table 2).

The API 20E test kit results showed that two isolates, DT37 and HG41 (two artificially infected strains), were positive for lysine, ornithine, citrate, H_2S production, indole, and glucose. On the contrary, they were negative for orthnitrophenyl galactosidase, arginine, urease, tryptophane deaminase, Voges–Proskauer reaction, gelatin, inositol, mannitol, saccharose, amygdalin sorbitol, rhamnose, melibiose, and arabinose. The phenotypic and biochemical properties of isolates originating from clown knifefish are presented in detail in Table 2.

3.2. PCR Identification

Approximately 1,500 bp amplicons were obtained by PCR based on the 16S rRNA gene (Figure 3). The result of sequencing showed that isolate DT37 had a 99.22% similarity to *E. tarda* strain Colony44 (CP070604.1). Meanwhile, the obtained sequences of isolate HG41 showed a 99.84% similarity to *E. tarda* ATCC 15947 = NBRC 105688 strain ATCC 15947 (CP084506.1).

3.3. Challenge Experiments

The first mortality occurred at 12 hours post-infection in all treatments in which fish were exposed to two isolates of *E. tarda*, DT37, and HG41. The clinical signs of experimentally infected fish were similar to those of naturally infected fish. In isolate DT37, the mortality reached 83.33% at a 10^8 CFU/ml concentration after 60 hours (Figure 3). Meanwhile, for *E. tarda* (HG41), mortality reached 100% within 48 hours post-injection at the highest concentration of 10^8 CFU/ml. At a 10^6 CFU/ml concentration, the average mortality in *E. tarda* DT37 and HG41 was 66.67% and 50%, respectively. However, no death fish in *E. tarda* DT37 or *E. tarda* HG41 were treated at 10^4 CFU/ml during the experimental challenge. In the control groups, there were no fatalities or obvious changes. Bacteria with the same characteristics as *E. tarda* were isolated from all dead clown knifefish. The LD_{50} values for *E. tarda* DT37 and HG41 isolates were 4.89×10^5 and 4.07×10^5 CFU/ml, respectively.

3.4. Antibiogram Results

The findings indicated that the bacteria were highly susceptible to ampicillin (65%), enrofloxacin (85%), florfenicol (100%), flumequine (90%),

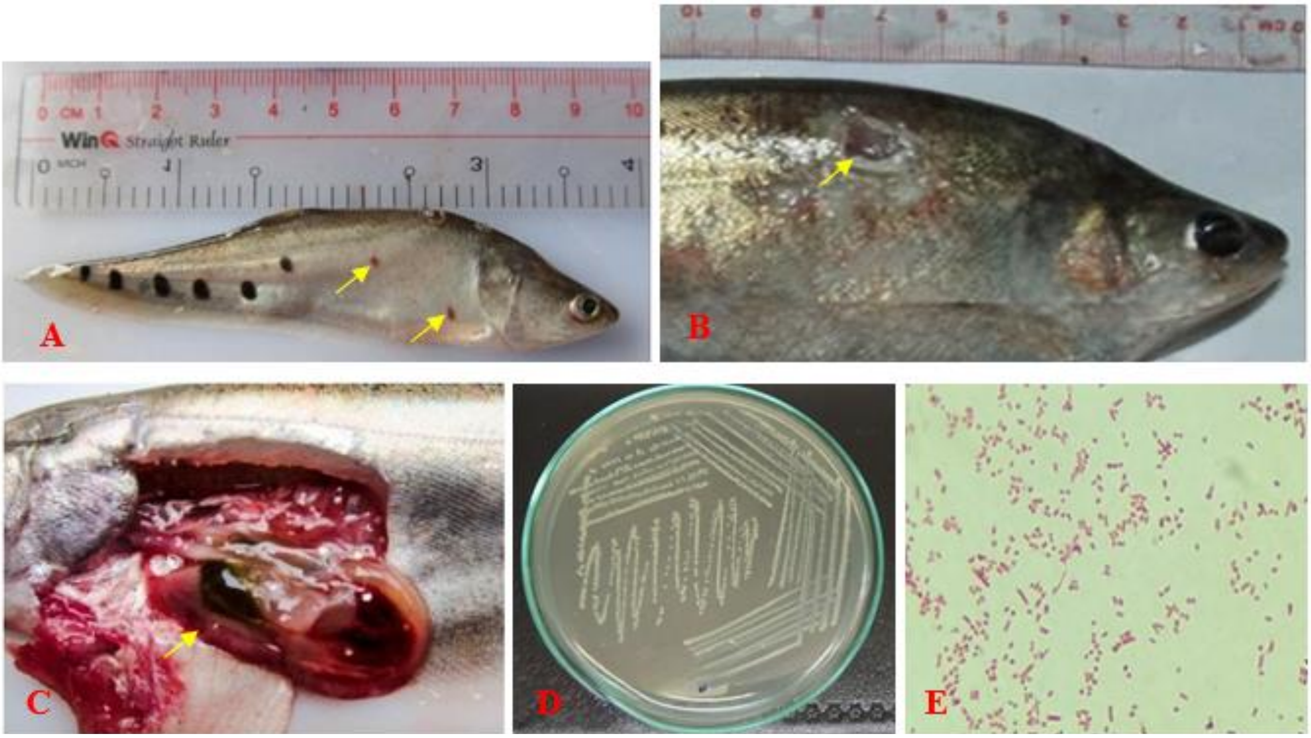

Figure 2. The external and internal clinical signs and characteristics of *E. tarda* isolate DT37 derived from diseased clown knifefish. (A) Petechial hemorrhages and hemorrhagic and exophthalmic eyes (yellow arrow), (B) ulcerative lesion and hemorrhages (yellow arrow), (C) fluid in the abnominal cavity (yellow arrow), (D) bacterial colonies grow on TSA medium, (E) gram staining (100X)

Table 2. Phenotypic and biochemical properties of isolates originated from clown knifefish

Phenotypic characteristics	Isolate DT37	Isolate HG41	*E. tarda**
Gram stain	Gram negative	Gram negative	Gram negative
Shape	Short-rod	Short-rod	Short-rod
Motility	+	+	+
Oxidase reaction	+	+	+
Catalase reaction	+	+	+
Oxidation/Fermentation	+/+	+/+	+/+
Growth in different sodium chloride:			
0% NaCl	+	+	+
1% NaCl	+	+	+
2% NaCl	+	+	+
3% NaCl	+	+	+
Orthnitrophenyl galactosidase	+	+	+
Arginine	-	-	-
Lysine	-	-	-
Ornithin	+	+	+
Citrate utilization	+	+	W
H2S production	+	+	+
Urease	+	+	+
Tryptophane deaminase	-	-	-
Indole production	-	-	-
Voges–Proskauer reaction	+	+	+
Gelatin	-	-	-
Glucose	-	-	-
Mannitol	+	+	+
Inositol	-	-	-
Sorbitol	-	-	-

+: positive, -: negative, *Buller (2014)

Table 2. Continued

Phenotypic characteristics	Isolate DT37	Isolate HG41	*E. tarda**
Rhamnose	-	-	-
Sucrose	-	-	-
Melibiose	-	-	-
Amygdalin	-	-	-
Arabinose	-	-	-

+: positive, -: negative, *Buller (2014)

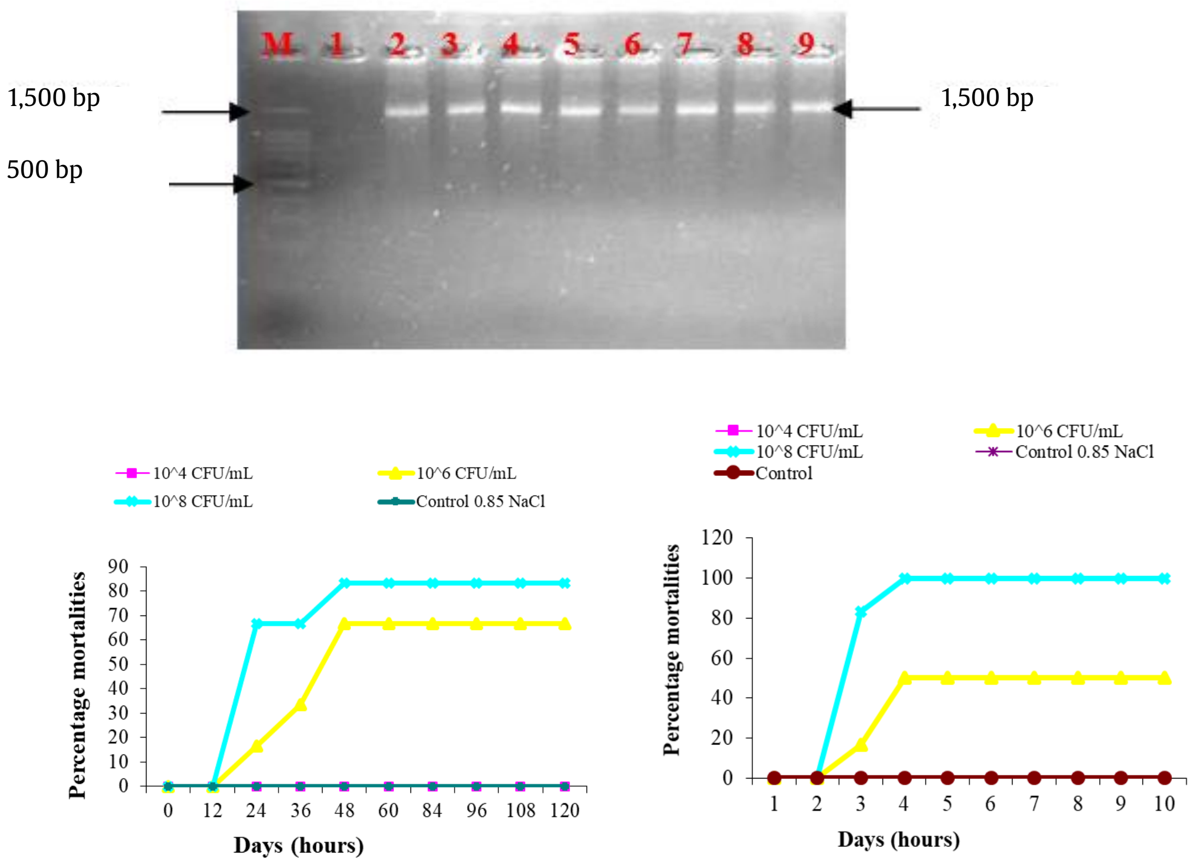

Figure 3. PCR-produced bacterial isolates from diseased clown knifefish were run on agarose gels. A. 100 bp DNA ladder; Lane 1. Negative control; Lane 2–9: isolates DT37, HG41, DT1, DT5. Cumulative mortality of clown knifefish infected with *E. tarda*

cefotaxime (80%), and sulfamethoxazole and trimethoprim (70%). However, the bacterial isolates were highly resistant to doxycycline (75%) and streptomycin (100%) (Figure 4).

4. Discussion

Numerous aquatic species worldwide have been documented to have *E. tarda*-caused Edwardsiellosis (Shetty *et al.* 2014; Dubey *et al.* 2019; Preena *et al.* 2022). In this study, 43 *E. tarda* isolates were obtained from diseased clown knifefish exhibiting hemorrhages, skin ulcerations, and petechiae on the body in Hau Giang and Dong Thap provinces of the Mekong Delta, Vietnam. The tested results showed that two bacterial strains, DT37 and HG41, had similar morphological, physiological, and biochemical characteristics to previous studies (Nagy *et al.* 2018; Nantongo *et al.* 2019). Specifically, two isolates in the current study are Gram-negative, motile, short-rod-shaped, positive catalase, negative oxidase, and fermentative in both anaerobic and aerobic conditions (Algammal *et al.* 2022; Rediet *et al.* 2022). Besides, the results showed that all bacteria isolates could grow in the media with 0-3% NaCl. The findings align with Abraham *et al.* (2015) and Ishihara and Kusuda

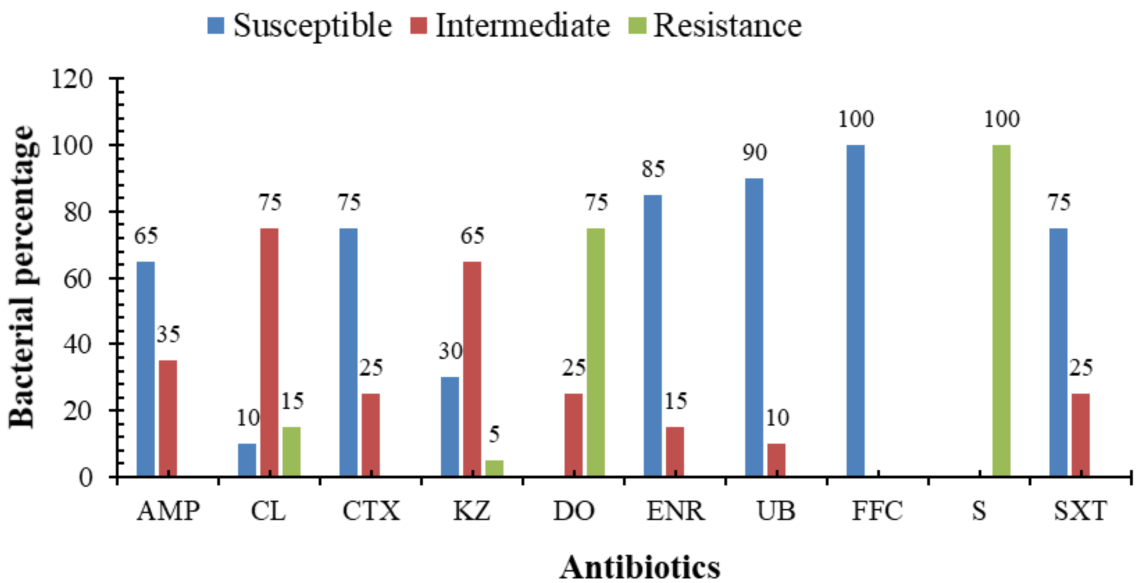

Figure 4. Antimicrobial susceptibility test of twenty *E. tarda* isolates ampicillin (AMP), cephalexin (CL), cefotaxime (CTX), cefazolin (KZ), doxycycline (DO), enrofloxacin (ENR), flumequine (UB), florfenicol (FFC), streptomycin (S), and trimethoprim-sulfamethoxazole (SXT)

(1982), who reported that *E. tarda* can grow in 0-4% sodium chloride. However, *E. tarda* (isolate EH-202) from infected turbots (*Scophthalmus maximus*) was able to survive in a medium supplemented with 5% NaCl in a study by Xiao *et al.* (2009), confirming its excellent halo-tolerating capabilities.

Biochemically, the results showed that two isolates, DT37 and HG41, were positive for lysine, ornithine, citrate, and glucose (Table 2). This result was in agreement with strains obtained from wild European eels (*Anguilla anguilla*) in Spain (Alcaide *et al.* 2006), wild Asian swamp eels (*Monopterus albu*) in Malaysia (Najiah and Lee 2006), and pacu (*Myleus micans*) in Canada (Lima *et al.* 2008). Interestingly, the bacteria were positive for indol and H_2S production, which were two important features that separate *E. tarda* from *E. ictaluri* (Inglis *et al.* 1993). Besides, two isolates, DT37 and HG41, were negative for orthnitrophenyl galactosidase, arginine, urease, tryptophane deaminase, Voges–Proskauer reaction, gelatin, inositol, mannitol, saccharose, amygdalin sorbitol, rhamnose, melibiose, and arabinose (Table 2). These biochemical characteristics of two isolates, DT37 and HG41, in this study are consistent with the previous report (Abraham *et al.* 2015; Nantongo *et al.* 2019; Wolde *et al.* 2022). Several phenotypic and biochemical characteristics variations have been found in *E. tarda* isolates (Kim *et al.* 2014). Hence, morphological and biochemical traits are crucial for distinguishing *E. tarda* from other *Edwardsiella* species. However, a PCR reaction and 16S rRNA gene fragment sequencing also identified two representative isolates (DT37 and HG41) as *E. tarda* (Figure 3).

The data produced from this study fulfilled Koch's postulates, thus confirming that *E. tarda* is the causative agent of hemorrhagic infections in clown knifefish. The clinical presentation of two bacterial strains, DT37 and HG41, supported field observations during natural disease outbreaks in clown knifefish. Diseased fish in this study showed gross signs of abnormal swimming, skin ulcerations, and petechial hemorrhages in their bodies (Figure 2). Internally, ascites with hemoperitoneum, hemorrhagic kidneys, light-colored nodules on the liver, and splenomegaly were also found (Figure 2). Generally, the symptoms of diseased clown knifefish in this study largely align with those of *E. tarda* infected aquatic animals (Butar-Butar *et al.* 2020; Algammal *et al.* 2022). In particular, the infected fish in the study emitted an unpleasant odor from swollen areas, similar to the smell of rotten eggs (Meyer and Bullock 1973; Noga 2010). However, previous studies have shown that *E. tarda* species will have different pathological signs in different fish species. Turgay (2020) reported that Edwardsiellosis in freshwater angelfish (*Pterophyllum scalare*) showed the most obvious external findings in the moribund fish: hemorrhage in the eyes, loss of scales, and skin depigmentation. Meanwhile, the pale liver, the enlarged spleen, and the thinned intestinal walls were internal signs. Murwantoko *et al.* (2019) revealed that *E. tarda* infected catfish (P*angasius pangasius*) showed clinical features in the form of loss of skin pigmentation due to lesions, abdominal swelling, hemorrhage in the fins, and necrosis in the fin area. According to Abraham *et al.* (2015), *E. tarda*-infected *Clarias gariepinus* displayed vertical hanging, frothing, excess mucus production, listing, a swollen abdomen, anorexia, fin and tail rot, and a reddish operculum.

The results demonstrated that the LD_{50} virulence value of *E. tarda* strain DT37 (4.89×10^5 CFU/ml) sampled in Dong Thap had a higher value than the *E. tarda* HG41 strain recovered from Hau Giang province (4.07×10^5 CFU/ml). Therefore, bacterial strains collected in Hau Giang were more virulent than those in Dong Thap. However, the difference in cumulative mortality between the treatments of these two strains was not statistically significant ($p>0.05$). The obtained LD_{50} values also gave much higher results than previous experiments. Typically, Xiao *et al.* (2009) revealed that the LD_{50} values for strains isolated from diseased turbots (*Scophthalmus maximus*) ranged between 3.8 × 103 and 3.8×10^5 CFU/g, with EH-202 exhibiting the lowest LD_{50} value among them. Infectious experiments on Nile tilapia also resulted in an equivalent LD_{50} in the range of 10^4 CFU/ml (Ibrahem *et al.* 2011). In addition, the LD_{50} value of the *C. garipinus* catfish experiment in Egypt was also recorded as 1.5×10^5 CFU/ml (Mahmoud and Abd El-Galil 2012). The experimental results and compared with the previous authors show that the LD_{50} value of the obtained bacterial strains has different virulence between geographical regions, among susceptible species. The pathogenicity of *E. tarda* species is influenced by many factors, and the pathogenic entry mechanisms are still under investigation and have not been determined specifically.

The findings revealed that 70% of isolates were highly susceptible to trimethoprim-sulfamethoxazole (Figure 4). In the present study, the percentage of bacteria sensitive to trimethoprim-sulfamethoxazole was higher than that reported by Charles *et al.* (2020), who showed that 41.7% of *E. tarda* from *Oreochromis niloticus* in Nigeria were resistant to trimethoprim-sulfamethoxazole. However, the study by Niu *et al.* (2019) indicated that *E. tard*a obtained from hybrid red tilapia (*Oreochromis* sp.) in the Ping River, Northern Thailand, was resistant to trimethoprim and sulfamethoxazole at a rate of 83.3%. Meanwhile, the study by Algammal *et al.* (2022) showed that 90.9% of *E. tarda* from Nile tilapia (*O. niloticus*) and African catfish (*Clarias gariepinus*) were resistant to trimethoprim-sulfamethoxazole. Since 1985, the *E. tarda* strains derived from diseased fish in the US and Taiwan have been recorded as having high sensitivity to antibiotics such as quinolones, beta-lactams, sulfamethoxazole, and trimethoprim. Also, the bacteria were found to have low resistance to ampicillin (25–34%), which is consistent with the results of this study (Sahoo and Mukherjee 1997; Nadirah *et al.* 2012).

Antibiotics belonging to the quinolone class are banned or restricted for use in aquaculture in Vietnam (MARD 2016, 2018). The study showed that *E. tarda* bacteria were at 85% and 90% sensitive to enrofloxacin and flumequine (Figure 4). In the current study, the percentage of bacteria sensitive to enrofloxacin was higher than that reported by Niu *et al.* (2019), who demonstrated that 66.7% of *E. tarda* isolated from *Oreochromis* sp. in Northern Thailand was sensitive to enrofloxacin. Meanwhile, Katharios *et al.* (2015) showed that 100% of *Edwardsiella* sp. was isolated from diseased cultured sharpsnout sea brew (Edwardsiellosis) sensitive to flumequine. Some recently published works also show similar sensitivity to tetracyclines, aminoglycosides, beta-lactams, quinolones, chloramphenicol, and gentamycin (Stock and Wiedemann 2001).

The study presented that 100% of *E. tarda* bacteria were sensitive to florfenicol (Figure 4). The results of this study are consistent with the report of Noor El Deen *et al.* (2017), which presented that *E. tarda* from cultured *O. niloticus* in Egypt is sensitive to florfenicol. This investigation aligns with a report by Gaunt *et al.* (2003), who revealed that florfenicol has become available and has rapidly become popular in several animal industries, including aquaculture. In a study, Dung *et al.* (2008) reported that *E. ictaluri* causing bacillary necrosis in striped catfish (*Pangasianodon hypophthalmus*) in Vietnam was 100% susceptible to florfenicol. Similarly, the study by Katharios *et al.* (2015) exhibited that *Edwardsiella* sp. was obtained from diseased cultured sharpsnout sea bream (Edwardsiellosis) sensitive to florfenicol. However, the results of the study by Niu *et al.* (2019) showed that *E. tarda* collected from *Oreochromis* sp. in Northern Thailand was resistant and intermediate to florfenicol, with rates of 16.7% and 63.3%, respectively.

Antibiogram analysis results (Figure 4) depicted that bacteria were highly sensitive to beta-lactam antibiotics, specifically ampicillin (65%) and cefotaxime (80%). According to Stock and Wiedemann (2001), *Edwardsiella* bacteria are naturally sensitive to β-lactam antibiotics due to their inability to secrete β-lactamase enzyme. Waltman and Shotts (1986) tested drug resistance on 118 strains of *E. ictaluri* isolated in the United States with 37 antibiotics, and the results indicated that the bacteria were susceptible to most of the tested drugs. The results of this study are similar to those of Nantongo *et al.* (2019), who reported that *E. tarda*, which originated from farmed Nile tilapia and African catfish in Uganda, was still susceptible to ampicillin and cefotaxime. However, the research by Algammal *et al.* (2022) revealed that *E. tarda* from *O. niloticus* and *C. gariepinus* were resistant to cefotaxime and ampicillin, with rates of 86.4% and 100%, respectively. Similarly, *E. tarda* collected from red tilapia in Thailand was 73.3% resistant to ampicillin (Niu *et al.* 2019). Research by Charles *et al.* (2020) indicated that 91.7% of *E. tarda* from *O. niloticus* in Nigeria were resistant to cefotaxime.

In this study, 100% of the isolates resisted streptomycin (Figure 4). This result is consistent with the research by Algammal *et al.* (2022), which indicated that 81.8% of *E. tarda* from *O. niloticus* and *C. gariepinus* were resistant to streptomycin. In the study of Niu *et al.* (2019), the prevalence of *E. tarda* resistance to streptomycin was 33.3%. In addition, the study of Clark *et al.* (1991) also noted that this species is sensitive to aminoglycosides, penicillin, and ciprofloxacin. In this present study, on the contrary, 75% of the isolates were highly resistant to doxycycline (Figure 4). The rate of bacteria resistant to doxycycline in this study was lower than that of Nidirah *et al.* (2012), who showed that 100% of *E. tadar* isolated from Asian seabass and Lates calcarifer were sensitive to doxycycline. Reger *et al.* (1993) also recorded strains of *E. tarda* that are highly sensitive to antibiotics of the quinolone, gentamycin, and doxycycline groups. According to Nagy *et al.* (2018), *E. tarda* recovered from *O. niloticus* in Egypt, was resistant to tetracycline and intermediately sensitive to doxycycline. Lo *et al.* (2014) revealed that the percentages of *E. tarda* strains isolated from diseased eels in Taiwan that were resistant to doxycycline and oxytetracycline were 21.3% (20/94 isolates).

In conclusion, this study obtained 43 isolates from diseased clown knifefish collected in Hau Giang and Dong Thap provinces of the Mekong Delta, Vietnam. Two isolates of DT37 and HG41 were identified as *E. tarda* by morphological, biochemical, and 16S rRNA gene sequencing. Experimental challenge studies revealed that isolated DT37 and HG41-infected knifefish showed clinical signs similar to those of naturally diseased fish. The LD_{50} of two isolates, DT37 and HG41, was 4.89×10^5 and 4.07×10^5 CFU/ml, respectively. The antibiogram result revealed that the bacterial isolates were highly susceptible to ampicillin, enrofloxacin, florfenicol, flumequine, cefotaxime, sulfamethoxazole, and trimethoprim. In contrast, the isolates showed high resistance to doxycycline and streptomycin. To our knowledge, this is the first report of *E. tarda* recovered from hemorrhagic clown knifefish in Vietnam.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

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