**Table 1.** Detailed information of *Edwardsiella ictaluri* disease outbreaks in tilapia farms in this study.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Farm no. | Farm code§ | Fish species | Fish size (g) | Source of stocking fish | Water temperature (ºC) | Cumulative mortality (%)\* | No. of fish used for bacterial isolation | No. and % of fish with dominant pinpoint colonies |
| 1 | HB-01.19 | NT | 83-106 | imported | 24.6 | 40-45 | 15 | 13 (86.67) |
| 2 | HB-04.20 | NT | 118-341 | imported | 27.2 | 45-46 | 12 | 10 (83.34) |
| 3 | HB-05.20 | NT | 23-42 | imported | 25.3 | 60-65 | 12 | 8 (66.67) |
| 4 | HB-06.21 | NT | 146-256 | imported | 28.1 | 45-50 | 15 | 13 (86.67) |
| 5 | HB-09.21 | RT | 62-81 | domestic | 27.5 | 50-55 | 15 | 9 (60.00) |
| 6 | YB-02.20 | NT | 25-42 | imported | 26.1 | 40-45 | 12 | 10 (83.34) |
| 7 | YB-03.21 | RT | 187-215 | imported | 27.8 | 50-55 | 10 | 9 (90.00) |
| 8 | YB-05.21 | RT | 78-103 | imported | 24.2 | 50-55 | 14 | 13 (92.86) |
| 9 | TQ-01.20 | NT | 55-71 | imported | 28.3 | 40-45 | 15 | 11 (73.34) |
| 10 | TQ-03.21 | RT | 73-98 | domestic | 28.5 | 30-35 | 15 | 9 (60.00) |
| 11 | HD-02.19 | NT | 27-43 | domestic | 26.8 | 35-37 | 12 | 9 (75.00) |
| 12 | HD-04.20 | NT | 248-319 | imported | 25.5 | 45-50 | 12 | 10 (83.34) |
| 13 | HD-05.20 | NT | 36-43 | imported | 25.8 | 35-38 | 12 | 11 (91.67) |
| 14 | HD-06.21 | RT | 45-61 | imported | 23.3 | 40-42 | 14 | 12 (85.72) |
| 15 | TB-01.20 | NT | 67-81 | domestic | 24.4 | 45-50 | 15 | 10 (66.67) |
| 16 | TB-03.20 | NT | 168-192 | imported | 26.0 | 50-55 | 15 | 11 (73.34) |
| 17 | TB-04.21 | RT | 51-65 | imported | 27.6 | 45-50 | 15 | 14 (93.34) |
| 18 | TB-05.21 | RT | 32-43 | imported | 29.1 | 35-40 | 11 | 8 (72.73) |
| 19 | BN-02.20 | NT | 42-54 | domestic | 26.5 | 30-35 | 11 | 9 (81.82) |
| 20 | BN-03.21 | NT | 27-36 | domestic | 27.3 | 30-35 | 13 | 11 (84.62) |
| 21 | HY-01.20 | NT | 43-54 | imported | 25.4 | 45-50 | 13 | 12 (92.31) |
| 22 | HY-03.21 | NT | 17-21 | imported | 26.2 | 40-45 | 13 | 9 (69.24) |
| 23 | SL-02.21 | NT | 45-56 | imported | 27.4 | 45-50 | 15 | 13 (86.67) |
| 24 | SL-03.21 | RT | 47-64 | imported | 26.7 | 40-45 | 15 | 12 (80.00) |
| 25 | HNa-01.20 | NT | 32-48 | domestic | 26.4 | 30-32 | 10 | 8 (80.00) |
| 26 | HNa-03.21 | NT | 29-43 | imported | 26.6 | 30-35 | 10 | 7 (70.00) |

HB, Hoa Binh; TQ, Tuyen Quang; HD, Hai Duong; TB, Thai Binh; YB, Yen Bai; BN, Bac Ninh; HY, Hung Yen; SL, Son La; HNa, Ha Nam

§Each code indicates geographical location, sample code, and year of collection. Underlined codes indicate earthen-pond farms.

NT, Nile tilapia (*O. niloticus*); RT, hybrid red tilapia (*Oreochromis* sp.)

\*Estimated by farmers

**Table 2**. Primers for species identification and virulence gene detection.

|  |  |  |
| --- | --- | --- |
| **Primer sequence (5′→3′)** | **Target**  **Gene (size)** | **References** |
| ***Sequencing*** | | |
| Uni-Bact-F/AGAGTTTGATCMTGGCTCAG | *16S rRNA*  (~1500 bp) | Weisburg et al. (1991) |
| Uni-Bact-R/ACGGHTACCTTGTTACGACTT |
| GyrB-1245F/ATCRTCYTTCATGGTCCARA | *gyrB*  (1860 bp) | Griffin et al. (2014) |
| GyrB-1949R/GGAGAGCATCTTGTCGAAGC |
| ***Specific PCR assay*** | | |
| *Edwardsiella-*F/ACAGCCTGGAAGAGTCCTAC | Fimbrial genes  (848 bp)  (470 bp) | Sakai et al. (2009) |
| *Edwardsiella-*R/TTGAGAGTCGCTGCTTAC |
| Ed-ictaluri-F/GTAGCAGGGAGAAAGCTTGC |
| Ed-ictaluri-R/GAACGCTATTAACGCTCACACC |
| ***Edwardsiella ictaluri pathogenicity islands*** | | |
| *esrC-F/*CGTTCATGGCTGCCACAG | T3SS  (3214 bp) | Rogge et al. (2013) |
| *esrC-R/*AAACAGGAGGGTACAGGC |
| *evpC-F/*ATGCCAAGTGGAATTCGCTG | T6SS  (2369 bp) |
| *evpC-R/*CACCGCTTTGGCCATATTGA |
| *virD4-F/*GTTGGCGGGTGTGTTTATCGTT | T4SS  (3373 bp) |
| *virD4-R/*TCAGATTACGGGTCAGCTCGTT |
| *ureA-F/*CACCTGTAGATTTCAGCG | Urease enzyme  (2116 bp) |
| *ureC-R/*GACAGAGCATGATAAGCC |
| *eseI-F/*ATGTTACCTATCAACCGCATCA | T3SS effector  (1019 bp) |
| *eseI-R/*TGGGATGAAGACTCGCCGTACAGTGGAGGC |
| *escDeseI-F/*GTACCAACGCCGACTAATCCCTAACGCCTCCCAC | T3SS effector and chaperone  (1748 bp) |
| *esdDeseI-R/*TGGGATGAAGACTCGCCGTACAGTGGAGGC |

T3SS, type III secretion system; T4SS, type IV secretion system; T6SS, type VI secretion system

**Table 3**. Morphological and biochemical characteristics of *Edwardsiella ictaluri* isolates from diseased tilapia.

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **Tilapia isolates in this study**  **(n = 26)** | **Reference isolates** | |
| ***E. ictaluri***  **Tilapia isolate**  **(Dong *et al*., 2019)** | ***E. ictaluri***  **Catfish isolate**  **(Crumlish *et al*., 2002)** |
| Gram | Negative | Negative | Negative |
| Bacterial morphology | Rod | Rod | Rod |
| Oxidase | - | - | - |
| Catalase | + | + | + |
| ONPG | - | - | - |
| Arginine dihydrolase | - | - | - |
| Lysine decarboxylase | + | + | + |
| Ornithine decarboxylase | - | - | - |
| Citrate utilization | V | V | V |
| H2S production | - | - | - |
| Urease | - | - | - |
| TDA | - | - | - |
| Indole production | - | - | - |
| Voges-Proskauer | V | + | - |
| Gelatin | - | - | - |
| Acid production |  |  |  |
| D-glucose | + | + | + |
| D-mannitol | - | - | - |
| Inositol | - | - | - |
| D-sorbitol | - | - | - |
| L-rhamnose | - | - | - |
| D-sucrose | - | - | - |
| D-melibiose | - | - | - |
| Amygdalin | - | - | - |
| L-arabinose | - | - | - |

V, variable

**Table 4**. List of 26 *Edwardsiella ictaluri* isolates collected from tilapia farms in northern Vietnam.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Farm no.** | **Strains** | **Organ isolated** | **Farm code** | **Genus/species specific PCR** | **PCR results for presence of virulence genes** | | | | | | **Antimicrobial resistant phenotype** |
| ***esrC*** | ***evpC*** | ***virD4*** | ***ureA-C*** | ***eseI*** | ***escD*** |
| 1 | Ed.HB-02\*ɤ | Kidney | HB-01.19 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ne+Ox+ST+Va |
| 2 | Ed.HB-03 | Spleen | HB-04.20 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Na+Ox+ST+Va |
| 3 | Ed.HB-05 | Kidney | HB-05.20 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST+Va |
| 4 | Ed.HB-07 | Liver | HB-06.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST+Va |
| 5 | Ed.HB.08 | Kidney | HB-09.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST |
| 6 | Ed.YB-03 | Spleen | YB-02.20 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Na+Ox+ST+Va |
| 7 | Ed.YB-04 | Liver | YB-03.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Na+Ox+ST+Va |
| 8 | Ed.YB-08\*ɤ | Kidney | YB-05.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST |
| 9 | Ed.TQ-02 | Liver | TQ-01.20 | +/+ | + | + | - | + | - | - | Ax+Er+FL+Ox+ST |
| 10 | Ed.TQ-06ɤ | Spleen | TQ-03.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST+Va |
| 11 | Ed.HD-03 | Spleen | HD-02.19 | +/+ | + | + | - | + | - | - | Ax+Ct+Er+Fl+Of+Ox+ST+Va |
| 12 | Ed.HD-07 | Liver | HD-04.20 | +/+ | + | + | - | + | - | - | Ax+Ac+Er+Fl+Na+Ox+ST+Va |
| 13 | Ed.HD-09\*ɤ | Spleen | HD-05.20 | +/+ | + | + | - | + | - | - | Ax+Ac+Er+Fl+Na+Ox+ST+Va |
| 14 | Ed.HD-11 | Kidney | HD-06.21 | +/+ | + | + | - | + | - | - | Ax+Ct+Er+Fl+Ox+ST+Va |
| 15 | Ed.TB-01 | Kidney | TB-01.20 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Of+Ox+ST+Va |
| 16 | Ed.TB-07\*ɤ | Kidney | TB-03.20 | +/+ | + | + | - | + | - | - | Fl+Ne+Ox+ST |
| 17 | Ed.TB-08 | Spleen | TB-04.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST+Va |
| 18 | Ed.TB-12 | Kidney | TB-05.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Na+Ox+ST+Va |
| 19 | Ed.BN-04ɤ | Spleen | BN-02.20 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Ox+ST+Va |
| 20 | Ed.BN-06 | Spleen | BN-03.21 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Of+Ox+ST+Va |
| 21 | Ed.HY-05 | Kidney | HY-01.20 | +/+ | + | + | - | + | - | - | Ax+Er+Fl+Na+Ox+ST+Va |
| 22 | Ed.HY-06ɤ | Kidney | HY-03.21 | +/+ | + | + | - | + | - | - | Er+Fl+Ox+ST+Va |
| 23 | Ed.SL-01 | Kidney | SL-02.21 | +/+ | + | + | - | + | - | - | Fl+Ne+Ox+ST+Va |
| 24 | Ed.SL-07ɤ | Liver | SL-03.21 | +/+ | + | + | - | + | - | - | Fl+Ne+Ox+ST |
| 25 | Ed.HNa-02ɤ | Spleen | HNa-01.20 | +/+ | + | + | - | + | - | - | Er+Fl+Ox+ST+Va |
| 26 | Ed.HNa-05 | Spleen | HNa-03.21 | +/+ | + | + | - | + | - | - | Ax+Fl+Ne+Ox+ST+Va |

ɤ: isolates subjected to *16S rRNA* and *gyrB* sequencing

\* Isolates chosen for the LD50 test

Underlined codes indicate earthen-pond farms

See section 2.6 for antimicrobial codes

**Table 5**. Antimicrobial susceptibility of *Edwardsiella ictaluri* isolates. Percent frequency out of n = 26 is shown in brackets.

|  |  |  |  |
| --- | --- | --- | --- |
| **Antimicrobial** | **Susceptible**  **(S)** | **Intermediate**  **(I)** | **Resistant**  **(R)** |
| **Penicillins (PNs)** | | | |
| Amoxicillin (Ax) | 4 (15.3) | 1 (3.9) | 21 (80.8) |
| Oxacillin (Ox) | 0 (0) | 0 (0) | 26 (100) |
| **Β-Lactam/β-Lactamase inhibitor combination (BL/BLI)** | | | |
| Amoxicillin /Clavulanate (Ac) | 19 (73.1) | 5 (19.3) | 2 (7.6) |
| **Cephems (CPs)** | | | |
| Cefotaxime (Ct) | 22 (84.7) | 2 (7.6) | 2 (7.7) |
| Ceftriaxone (Cx) | 26 (100) | 0 (0) | 0 (0) |
| Cefuroxime (Cu) | 26 (100) | 0 (0) | 0 (0) |
| **Macrolides (MCs** | | | |
| Erythromycin (Er) | 3 (11.4) | 1 (3.9) | 22 (84.7) |
| **Tetracyclines (TCs)** | | | |
| Doxycycline (Dx) | 26 (100) | 0 (0) | 0 (0) |
| Oxytetracycline (OTC) | 26 (100) | 0 (0) | 0 (0) |
| **Sulfonamides (SULs) (Folate pathway inhibitors)** | | | |
| Sulfamethoxazole/Trimethoprim (ST) | 0 (0) | 0 (0) | 26 (100) |
| **Amphenicols (AMPs)** | | | |
| Florfenicol (Fl) | 0 (0) | 0 (0) | 26 (100) |
| **Fluoroquinolones (FQNs)** | | | |
| Ofloxacin (Of) | 21 (80.8) | 2 (7.6) | 3 (11.6) |
| Norfloxacin (No) | 26 (100) | 0 (0) | 0 (0) |
| **Quinolones (QLs)** | | | |
| Nalidixic acid (Na) | 17 (65.4) | 2 (7.6) | 7 (27.0) |
| **Aminoglycosides (AMGs)** | | | |
| Neomycin (Ne) | 5 (19.2) | 16 (61.6) | 5 (19.2) |
| **Glycopeptide (GLs)** | | | |
| Vancomycin (Va) | 3 (11.6) | 2 (7.6) | 21 (80.8) |