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An immunochromatographic test strip for rapid detection of fish pathogen *Edwardsiella tarda*

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Abstract

Background: *Edwardsiella tarda*, the etiologic agent of edwardsiellosis, is a devastating fish pathogen prevailing in worldwide aquaculture industries and accounting for severe economic losses. There is a raising concern about *E. tarda* being a significant zoonotic pathogen, and it is urgent to develop a rapid detection of this pathogen. This is the first study to develop a test strip for rapid detection of *E. tarda* in turbot.

Results: Mouse monoclonal antibodies (MAbs) and rabbit polyclonal antibody (PAb) against *E. tarda* were generated from immunization of mice and rabbits with a virulent isolate of *E. tarda* EIB202. Two MAbs specific to isolates of *E. tarda* were obtained, and one of them (25C1) was selected to conjugate with colloidal gold as the detector antibody. Rabbit PAb was used as the capture antibody. It was found the strip had no cross-reactivity with non-*E. tarda* bacterial microbes and the limit of detection (LOD) was 1×10^5 colony-forming units (CFU)/ml. The detection could be visually observed by the naked eye within 5 min. This test strip was verified with a similar detection limit and much less analysis time compared with a dot blot immunoassay (1×10^5 CFU/ml for LOD and 120 min for reaction time). When the samples were mixed with turbot tissue homogenates, strong immunoreactivity was observed over 10^5 CFU/ml, which suggested that the turbot tissue homogenates did not affect the detection of the strip. Pre-enrichment with homogenized turbot tissue for 12 h could increase the detection limit of the *E. tarda* present in the sample up to 1 to 10 CFU/ml. In practice, in detecting 20 turbot ascite samples infected by *E. tarda*, the immunochromatographic test strip showed a high accuracy (100% positive).

Conclusions: The immunochromatographic test strip offers great promise for a rapid, simple, and economical method of *E. tarda* on-site detection, and with different antibodies, it might be used to detect other aquatic pathogens.

Keywords: Detection; Dot blotting; *Edwardsiella tarda*; Immunochromatographic test strip; Pathogen diagnosis

Background

Edwardsiella tarda, the etiologic agent of edwardsiellosis, is a devastating fish pathogen prevailing in worldwide aquaculture industries and accounting for severe economic losses. *E. tarda* can cause systemic hemorrhagic septicemia and emphysematous putrefactive disease in more than 20 species of freshwater and marine fishes, including turbot, tilapia, eel, flounder, and catfish. There is a raising concern about *E. tarda* being a significant fish pathogen, and it is urgent to develop a rapid detection of this pathogen. The traditional diagnostic test available for identification of *E. tarda* in diseased fish is bacterial culture and identification using

biochemical tests [1]. During the past decade, a number of immunoassay methods, including enzyme-linked immunosorbent assay (ELISA) and dot blot immunoassay, have been used to detect *E. tarda* in infected organs like the liver, spleen, and kidney of the diseased fish [2,3]. Polymerase chain reaction (PCR) and modified PCR methods such as loop-mediated isothermal amplification (LAMP) were also used in the diagnoses of *E. tarda* [1,4,5], and the LAMP method was proved to be successful for *E. tarda* detection with a limit of detection (LOD) of 10 colony-forming units (CFU) [6]. Although the above methods are effective for *E. tarda* diagnosis, they are time-consuming (2 h to 7 days) and depend on expensive instruments and highly skilled personnel. Colloidal gold-based immunochromatographic assay (ICA) is a user-friendly format with few simple procedures and has been widely applied as a

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diagnostic tool especially for on-site identification of pathogens. The aim of our research was to develop a portable one-step method for the rapid detection of *E. tarda*. With a strip, the infecting pathogen of diseased fish could be determined on-site in several minutes without any instrument.

Methods

Strains, media, and chemicals

The strains used in this study are listed in Table 1. *E. tarda* strains were grown in tryptic soy broth (TSB) (Difco, Detroit, MI, USA) or tryptic soy agar (TSA) (Difco, Detroit, MI, USA) at 28°C, while *Escherichia coli* strains were cultured in Luria-Bertani (LB) broth at 37°C. *Edwardsiella ictaluri*, *Aeromonas hydrophila*, and *Chromobacterium violaceum* were cultured at 30°C for 20 h in brain-heart infusion (BHI) broth, LB medium with 1% NaCl, and LB medium with NaCl 2.5%, respectively. Then, the bacteria were collected by centrifugation at 10,000× *g* for 5 min at 4°C, followed by wash for three times with pre-chilled phosphate-buffered saline (PBS; 10 mmol/l, pH 7.4). The bacterial suspension in PBS was enumerated with a UV-visible spectrophotometer and inactivated with 1% (*v/v*) formalin for 1 h at 30°C. The formalin-killed bacteria were washed three times with pre-chilled PBS, resuspended, and stored at -80°C for further use.

Table 1 Strains used in this study

Bacteria	References or sources
<i>Edwardsiella tarda</i>	
EIB202	Xiao et al. [7]
WY37	Bai et al. [3]
ATCC15947	ATCC
ATCC23692	ATCC
DT	Guangdong, 2007
<i>Edwardsiella ictaluri</i>	
LH51	Liaoning, 2006
ATCC33202	ATCC
<i>Vibrio anguillarum</i>	
MVM425	Wu et al. [8]
VIB72	Wang et al. [9]
<i>Vibrio parahaemolyticus</i>	Ye et al. [10]
<i>Vibrio alginolyticus</i> EPGS	Rui et al. [11]
<i>Vibrio harveyi</i> VIB647	Pang et al. [12]
<i>Chromobacterium violaceum</i> CV026	Ravn et al. [13]
<i>Aeromonas hydrophila</i>	Wang et al. [14]
<i>Escherichia coli</i>	
CC118	Dennis and Zylstra [15]
DH5a	Winzer et al. [16]

The goat anti-mouse IgG was purchased from Tiangen Biotech (Beijing, China). Freund's complete adjuvant and Freund's incomplete adjuvant, H₂AuCl₄·3H₂O, sodium citrate, and bovine serum albumin (BSA) were from Sigma (St. Louis, MO, USA). Nitrocellulose membranes were purchased from Millipore (Billerica, MA, USA). PVC sheets, absorbent paper, and glass paper were purchased from Shanghai Goldbio Tech (Shanghai, China). Other reagents were of analytical purity, and Milli-Q (Millipore purification system)-purified water was used for all experiments.

Preparation of outer membrane proteins

The outer membrane proteins (OMPs) of *E. tarda* EIB202 were extracted as described by Darwish et al. [17] with minor modifications. Briefly, *E. tarda* EIB202, isolated from diseased turbot, was cultured in LB medium containing 1% glucose at 30°C for 20 h. The bacteria were collected by centrifugation at 10,000× *g* for 5 min at 4°C and then washed three times with pre-chilled PBS. The pelleted bacteria were suspended in 10 ml of HEPES buffer (10 mmol/l, pH 7.4) with 2 mmol/l phenylmethanesulfonyl fluoride and sonicated at 350 W for 120 times (4 s once with 6-s interval). Intact cells and large debris were removed by centrifugation at 10,000× *g* for 15 min at 4°C. Supernatants were pelleted and centrifuged at 100,000× *g* for 1 h at 4°C. The precipitate was resuspended with 5 ml HEPES buffer, and the same volume of HEPES buffer containing 2% sodium lauroyl sarcosine was added to solubilize inner membrane proteins. Thirty minutes later, the pellets were ultra-centrifuged at 100,000× *g* for 1 h at 4°C. The purified OMPs were suspended in 1 ml PBS and aliquoted in small portions and stored at -80°C.

Production of antibodies against *E. tarda*

Monoclonal antibody (MAb) specific to *E. tarda* was produced according to previously described methods [18]. *E. tarda* was cultured in TSB at 28°C for 16 h, and the bacteria were then collected and resuspended in sterile water for deoxycholate hydrogen sulfide lactose (DHL) agar plate counting. It was inactivated at 37°C for 48 h in 1% formaldehyde and then used as the immunogen after washing three times with PBS to remove the formaldehyde. Five BALB/c mice were immunized by intraperitoneal injection of the 200 μl suspension containing 10⁸ inactivated bacteria per ml or OMPs mixed with an equal volume of Freund's adjuvant (complete Freund's adjuvant for the first immunization and incomplete for the second and third immunization) for three times at 2-week intervals. One week after the third immunization, serum antibody titers of immunized mice were determined by indirect ELISA on inactivated *E. tarda* or OMP-coated plates. Mice producing the highest ELISA titer were given

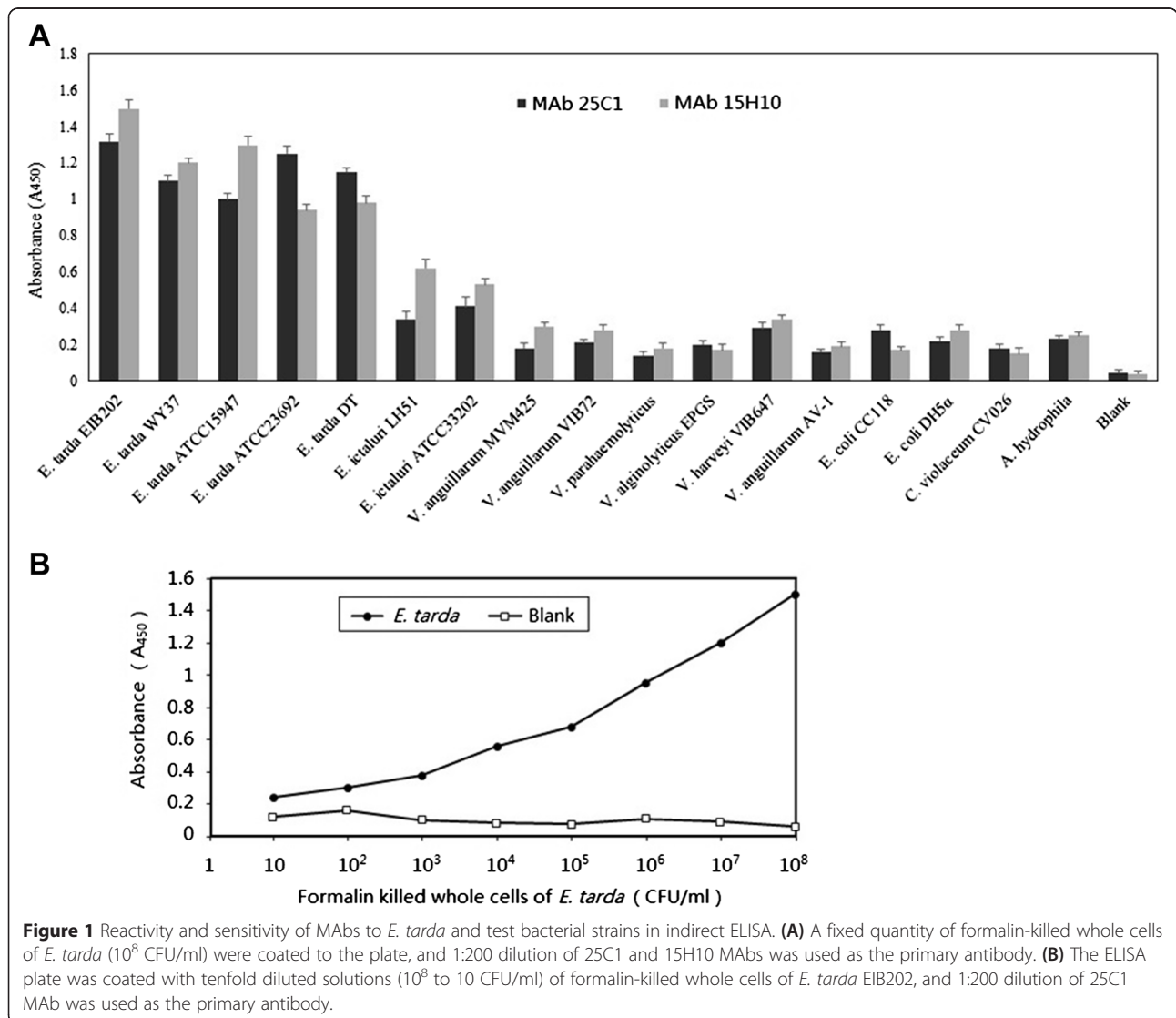
an intravenous boost (OMP, 50 µg) without adjuvant. One week after the intravenous boost, two BALB/c mice were sacrificed for the fusion with SP2/0-Ag14 myeloma cells. The dispersed spleen lymphocytes were prepared and fused with myeloma cells (SP2/0-Ag-14) to produce hybridomas. For the screen of MABs against the somatic antigen of *E. tarda*, indirect ELISA was processed using supernatants of hybridomas with formalin-killed whole cells of *E. tarda* coating on plates. Two of the strongly reacting MABs (designated as 25C1 and 15H10) were used for further characterizations and applications in immunoassays. Positive hybridoma cells selected by indirect ELISA and cloned by limiting dilution were cultured and injected intraperitoneally into the BALB/c mice with 5×10^6 cells each mouse for ascite production. After 7 to 14 days, ascite was collected. MAb in ascite fluid was precipitated with 50% ammonium sulfate, centrifuged, and dialyzed

against PBS before being purified using a protein G agarose column.

New Zealand white rabbits were subcutaneously and intramuscularly injected with a total of 20 ml suspension containing 10^8 inactivated bacteria per ml mixed with an equal volume of Freund's adjuvant (complete Freund's adjuvant for the first immunization and incomplete for the second and third immunization) for four times at 3-week intervals, and then the rabbit antisera were collected by ear bleeding. The sensitivity and specificity of the antisera were determined by dot blotting and Western blotting. High-titer and highly specific antisera were pooled and purified using a protein A agarose column.

Reactivity and characterization of MABs

Indirect ELISA tests were performed to assess the reactivity of MABs to the formalin whole cells of *E. tarda* isolates. ELISA plates were coated with 50 µl per well of



formalin-killed whole bacterial cells (10^8 CFU/ml) in carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. All tests were performed in duplicate. The indirect ELISA tests were performed as Kumar et al. described [18]. The isotyping of MAbs was carried out with a mouse monoclonal antibody isotyping kit (Sigma, St. Louis, MO, USA). Indirect ELISA was also used to test the sensitivity of the MAbs to detect formalin-killed whole cells of *E. tarda*. Briefly, ELISA plates were coated and dried overnight with 50 µl of tenfold diluted solutions of whole bacterial cells (10^8 to 10 CFU/ml). The subsequent steps were similar as mentioned above.

Preparation of test strip

The MAb specific to *E. tarda* was conjugated to colloidal gold particles (diameter = 40 nm) as the method described by Frens [19] and dispersed onto a glass fiber membrane as the conjugate pad. The rabbit polyclonal antibody (2 mg/ml) and goat anti-mouse IgG (1 mg/ml) were micro-sprayed with BioDot XYZ3050 (BioDot, Irvine, CA, USA) onto a nitrocellulose membrane separately at the position that would become the test line (T) at the upper position and control line (C) around the bottom of the membrane as the analytical membrane. A glass fiber membrane and absorbent paper were used as the sample pad and absorbent paper, respectively. The sample pad, conjugate pad, analytical membrane, and absorbent pad were assembled with 2 mm overlapping between each component on a plastic adhesive backing. The assembled master card was then cut into strips of 5 mm wide with a numerical controlled slitter (Shanghai Goldbio Tech, Shanghai, China) and stored in a desiccated plastic bag.

Specificity and sensitivity of test strip

In order to determine the cross-reactivity of the test strip, five strains of *E. tarda*, two strains of *E. ictaluri*, and ten other bacterial strains (Table 1) were diluted in the same volume of test to a concentration of 10^6 CFU/ml with PBS and applied to the test strips.

The sensitivity of the strip was determined qualitatively. The serially diluted *E. tarda* of several concentrations (1×10^6 , 5×10^5 , 1×10^5 , and 5×10^4 CFU/ml) in sample buffer was used in sensitivity detection. The strip sensitivity results were compared with the results from dot blotting. All of the detections were performed in triplicate.

Dot blotting

The bacterial suspension used to determine the specificity of the test strips and *E. tarda* EIB202 of serial dilutions were spotted onto a nitrocellulose membrane (2 µl/spot), dried at 37°C, and quenched in 5% Blotto (5% nonfat dry milk, 0.05% Tween-20 in PBS) for 2 h.

After being incubated with MAb at 37°C for 1 h, the membrane was extensively washed three times with PBST (0.05% Tween-20 in PBS) and incubated in goat anti-mouse IgG antibody horseradish peroxidase conjugate (GAM-HRP) at 37°C for 1 h. Then, the membrane was washed three times and incubated in TMB membrane substrate for 5 min. After revealing dark gray spots, the reaction was terminated by incubation of the membrane in water.

Detection of *E. tarda* in artificially contaminated fish tissue

Spleen tissues from fresh turbot were dissected and homogenized in TSB supplemented with 2% NaCl at a ratio of 1:9 (*w/v*). The turbot homogenate (10 ml) was spiked with *E. tarda* EIB202 to various original concentrations from 1 to 10^7 CFU/ml by tenfold serial dilutions. One milliliter from each tube was collected at 0, 6, 12, and 24 h and immediately heat killed at 60°C for 30 min and stored at -20°C before testing with the test strip. For the strip test, 400 µl from each sample was mixed with PBS at a ratio of 1:1, and 100 µl of the mixture was applied to the test strip.

On-site detection with test strip

Twenty ascite samples of diseased turbot from an aquaculture farm (Shandong Province, China) was prepared and applied directly to the test strip. Simultaneously, 10 µl ascite sample was spread on a DHL plate. The colonies with a black center (indicating H₂S production) were counted and identified to be *E. tarda* with the commercial API 20E kit (bioMérieux, Marcy-l'Étoile, France) and 16S rRNA sequencing.

Results and discussion

Preparation and characteristics of antibodies against *E. tarda*

In order to obtain MAb, BALB/c mice were immunized with the formalin-killed bacteria and purified OMPs of *E. tarda*, respectively. Compared with the antibody titer of anti-serum against OMP, the antibody titer of anti-serum against the formalin-killed bacteria was lower even after the fifth immunization and not suitable for hybridization. It might be attributed to the characteristics of *E. tarda* as an intracellular pathogen. MAbs against the OMP of *E. tarda* have been reported in detection of *E. tarda* from different geographic locations [18]. Therefore, OMP was used as the immunogen in this study. Out of the 1,536 wells of culture supernatants from two fusions, two hybridomas generated a strongly positive signal in indirect ELISA. Stable hybridoma cell lines

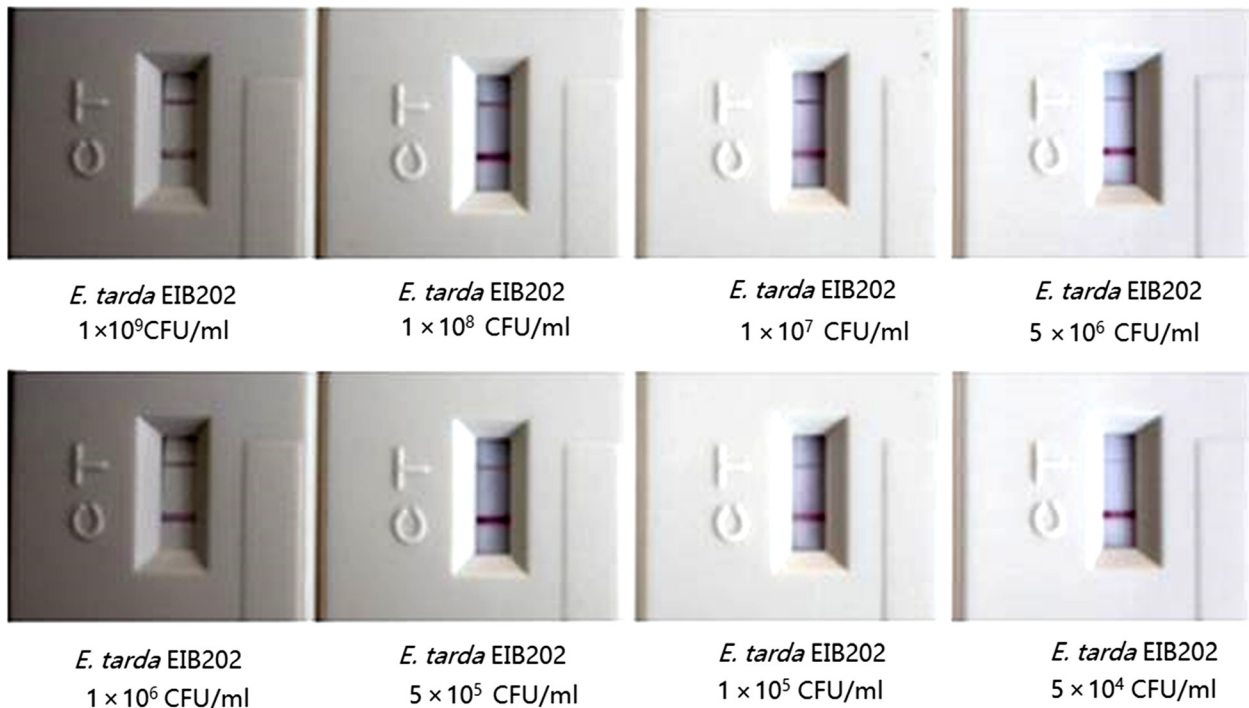


Figure 2 Sensitivity evaluation of the test strip prepared with monoclonal antibody 25C1 against *E. tarda*. The standard bacterial suspension of *E. tarda* EIB202 was enumerated and serially diluted to 1 × 10⁹, 1 × 10⁸, 1 × 10⁷, 1 × 10⁶, 5 × 10⁶, 5 × 10⁵, 1 × 10⁵, and 5 × 10⁴ CFU/ml and directly applied to the test strips. The detection limit of the test strip was determined by the diminishing of red color at the test line.

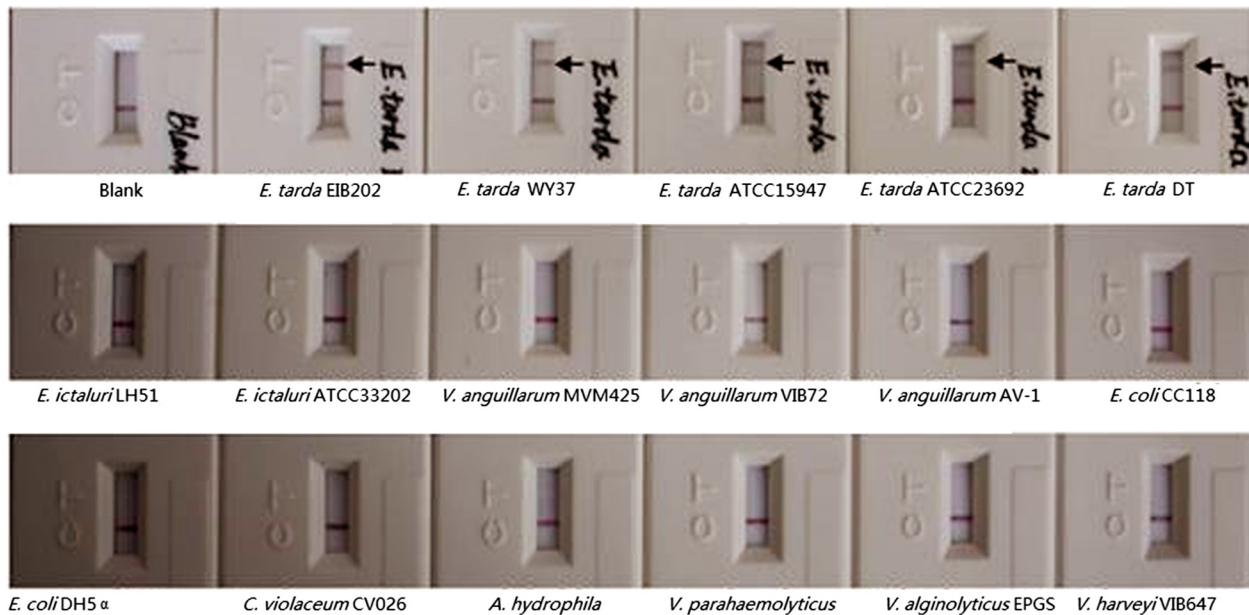


Figure 3 Specificity evaluation of the test strip prepared with monoclonal antibody 25C1 against *E. tarda*. Bacteria were diluted to 1 × 10⁶ CFU/ml with PBS and applied to the sample pad of the test strip.

25C1 and 15H10 were obtained following three rounds of limiting dilution and the cloning procedure.

The isotyping results classified the two anti-*E. tarda* MAbs as subclass IgG2 α . The reactivity of MAbs with different strains of *E. tarda* and with other bacteria was investigated using indirect ELISA. The two MAbs, 25C1 and 15H10, reacted strongly with formalin-killed whole bacterial cells from all isolates of *E. tarda* but not with the whole bacterial cells of *E. coli*, *E. ictaluri*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Chromobacterium violaceum*, and *Aeromonas hydrophila* (Figure 1A). The results showed that MAb 15H10 reacted with *E. ictaluri* more than MAb 25C1 (Figure 1A). The sensitivity of MAb 25C1 to detect whole cells of *E. tarda* was up to a level of 1×10^4 CFU/ml (Figure 1B). According to the results above, the cell line 25C1 produced an IgG2 α subtype of MAb with high specificity to *E. tarda* which was selected to prepare MAb used in subsequent experiments. Rabbit anti-*E. tarda* EIB202 anti-sera were purified through a protein A column which demonstrated specificity to all of the tested *E. tarda* isolates and could be used as the capture antibody in test strip production.

Preparation of test strip

The test strip was assembled in a sandwich format. MAb 25C1 was used for conjugation with colloidal gold and served as the detection antibody. The rabbit anti-*E. tarda* polyclonal antibody (PAb) was used as the capture antibody. Transmission electron microscope (TEM)

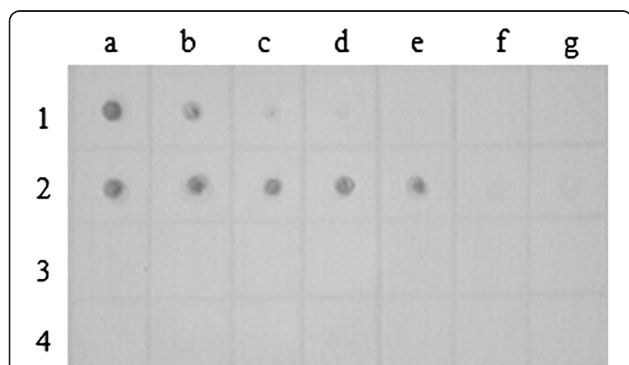


Figure 4 Dot blotting detection with monoclonal antibody 25C1 against *E. tarda*. Bacterial strains used for the cross-reactivity test at a concentration of 1×10^6 CFU/ml and *E. tarda* EIB202 of serial dilutions were spotted onto the nitrocellulose membranes (2 μ l/spot) and processed dot blotting using MAb 25C1. Row 1: *E. tarda* EIB202 (a) 1×10^6 CFU/ml, (b) 5×10^5 CFU/ml, (c) 1×10^5 CFU/ml, (d) 5×10^4 CFU/ml, (e) 1×10^4 CFU/ml, (f) 5×10^3 CFU/ml, (g) 1×10^3 CFU/ml. Row 2: (a) *E. tarda* EIB202, (b) *E. tarda* WY37, (c) *E. tarda* ATCC15947, (d) *E. tarda* ATCC23692, (e) *E. tarda* DT, (f) *E. ictaluri* LH-51, (g) *E. ictaluri* 33202. Row 3: (a) *V. anguillarum* MVM425, (b) *V. anguillarum* VIB72, (c) *V. anguillarum* AV-1, (d) *E. coli* CC118, (e) *E. coli* DH5 α , (f) *C. violaceum* CV026, (g) *A. hydrophila*. Row 4: (a) *V. parahaemolyticus*, (b) *V. alginolyticus* EPGS, (c) *V. harveyi* VIB647, (d) blank.

images revealed that the optimal diameter of colloidal gold particles ranged from 36 to 42 nm, with a mean diameter of 40 nm, to produce a distinguishable red color. The optimal concentrations of antibodies used to form the positive line and test line were determined by signal visualization. A clear bright-red display emerged with 6.0 μ g/ml of gold-labeled MAb 25C1 and 1.0 μ g/ml of rabbit anti-*E. tarda* PAb (data not shown).

Specificity and sensitivity of test strip

According to the recently described method, the detections of all the *E. tarda* strains used in this study produced positive results when 100 μ l bacterial suspension at a concentration of 1×10^6 CFU/ml was added on the strip. There were not any cross-reactions of the strip with *E. ictaluri*, *V. anguillarum*, *E. coli*, *C. violaceum*, *A. hydrophila*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. harveyi* (Figure 2). The test strip showed a high specificity to *E. tarda* and supposed to detect different isolates of *E. tarda*.

To determine the sensitivity of the ICA test strip, different concentrations of bacterial suspension of *E. tarda* EIB202 were applied to the ICA strip and tested in triplicate. The sensitivity of the ICA test strip showed a positive correlation with bacterial concentration when the bacterial concentration was between 5×10^4 and 1×10^9 CFU/ml. The color intensity of the test line increased with *E. tarda* concentration. When the suspension with a concentration of 1×10^5 CFU/ml was added on the strip, the intensity of the test line was significantly weaker (within 5 min) than that of the control line (Figure 3), and the test line at 5×10^4 CFU/ml bacteria was visible just at about 60 min. Therefore, 1×10^5 CFU/ml of *E. tarda* could be considered as the low limit of the ICA test strip for visual detection (within 5 min).

Comparison to dot blot hybridization

As an easy technique, dot blot hybridization is currently used for pathogen detection. However, the dot blotting procedure is laboratory-dependent and time-consuming which needs long result turn-around time (approximately 6 h) [20,21]. In this study, the dot blotting using MAb 25C1 described earlier as a binding antibody was performed to determine the specificity and sensitivity of this method. The results showed that MAb 25C1 could recognize all isolates of *E. tarda* used in the study and had no cross-reaction with all the bacterial strains except for *E. tarda*, which displayed the perfect specificity of MAb 25C1. The LOD of dot blotting was 1×10^5 CFU/ml, which is similar to that of the ICA method (Figure 4). Compared with that of the dot blotting, the detection process of the ICA method was simple and time-saving (5 min vs 6 h). Although the dot blotting produced a similar LOD to that of the ICA strip, it was reported

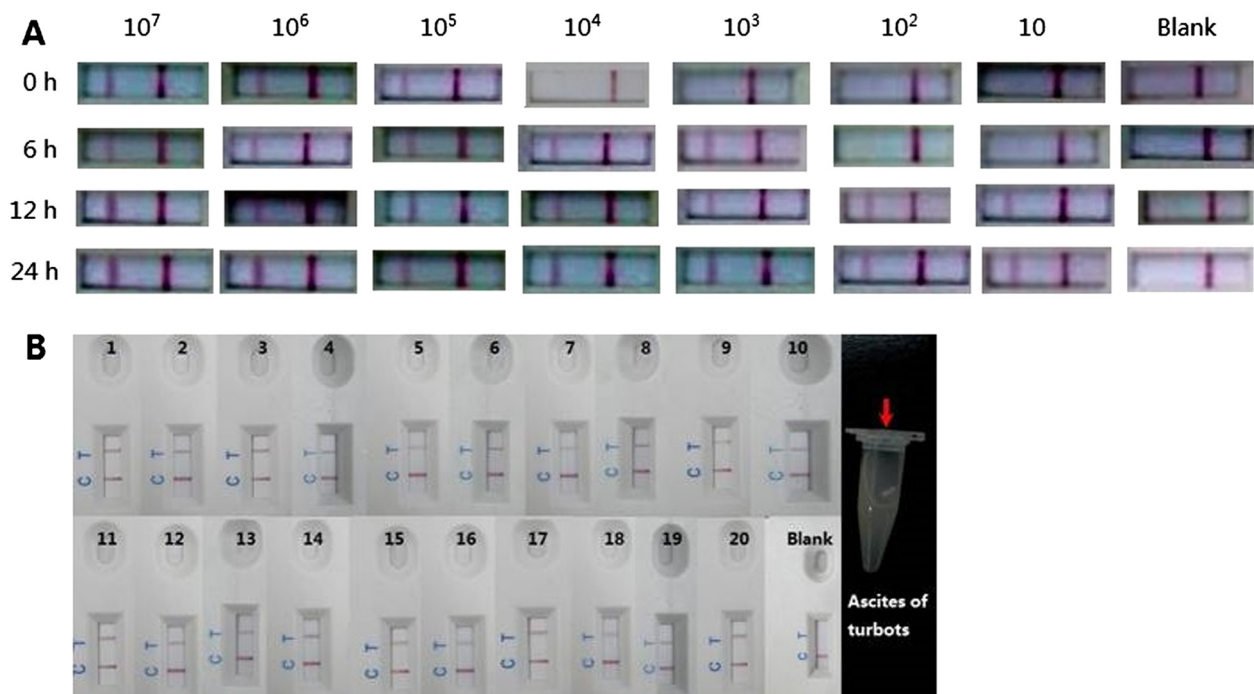


Figure 5 Strip test of *E. tarda* EIB202 with turbot tissue homogenates and on-site detection of turbot ascite. **(A)** *E. tarda* EIB202 at various concentrations (1 to 10⁷ CFU/ml) were pre-enriched with turbot tissue homogenates for 0 to 24 h before being applied to the strip. PBS was used as the blank sample. **(B)** Ascites of diseased turbot collected from an aquaculture farm (Shandong Province, China) numbered 1 to 20 were prepared and applied directly to the test strip. The concentrations of *E. tarda* in ascites ranged from 5.1 × 10⁵ to 7.8 × 10⁷ CFU/ml by DHL agar counting. PBS was used as the blank sample.

that the limited capacity of the nitrocellulose membrane might reduce the detection sensitivity of this method because the high protein in the sample could compete with the bacterial antigen [22]. The test strip offered similar detection sensitivity to that of the dot blotting and would be a convenient tool for direct identification of fish pathogen *E. tarda*.

Detection of *E. tarda* in artificially contaminated fish tissue

The application of test strips to detect the targeted microorganisms in the fish tissue samples was evaluated. When mixed with homogenized turbot tissue, a strong immunoreactivity was observed in the samples inoculated with *E. tarda* EIB202 over 10⁵ CFU/ml, which is similar to that of the sample in TSB. The results suggested that the existence of turbot tissue homogenates did not affect the detection of the strip. After pre-enrichment with the tissue of turbot for 6 h, a moderate immunoreactivity was observed in the turbot homogenate samples inoculated with *E. tarda* over 10³ CFU/ml (Figure 5A). After pre-enrichment for 12 and 24 h, a moderate positive observation could be detected at the inoculate of 10¹ CFU/ml in TSB. Therefore, pre-enrichment of the sample for 12 h could increase the

detection limit of the *E. tarda* present in the sample up to 1 to 10 CFU/ml.

On-site detection of *E. tarda* infection with test strip

E. tarda is an important fish pathogen and has caused huge losses to the aquaculture industry throughout the world. Outbreaks of edwardsiellosis appear first in the form of infections affecting only a few fish, and these diseased fish are able to survive only for a few days. When early detection and proper measurement are not undertaken and the diseased fish not treated as soon as possible, the infection spreads to the surrounding areas to create an edwardsiellosis epidemic [6]. Since early detection is critical for controlling this disease, rapid and accurate methods for edwardsiellosis diagnosis would be very valuable. To evaluate the method developed in this work, the immunochromatographic test strip was practically utilized to detect *E. tarda* in an industrial turbot farm (Yantai, Shandong Province, China). Diseased turbot with typical symptoms of edwardsiellosis such as eye tumefaction, inflammation, hemorrhages, and ascites were sampled. Each of 20 ascite samples of diseased turbot was collected and applied directly to the test strip for *E. tarda* on-site detection, and PBS was used as blank control due to the absence of ascite in healthy turbot. Among the 20 ascite samples, 20 samples (100%) were

positively detected, and the test lines were clearly indicated (Figure 5B). The concentrations of *E. tarda* in ascites ranged from 5.1×10^5 to 7.8×10^7 CFU/ml by DHL agar counting. The major colonies in the 20 ascite samples were further measured using API 20E and 16S rDNA sequencing to confirm that the diseased turbot were infected with *E. tarda* rather than other possible pathogens. Fortunately, the complete genome of *E. tarda* is available [23]. The results of 16S rRNA sequencing displayed that all the colonies isolated from the ascite samples belong to the same *E. tarda* strain with 99% identity to *E. tarda* FL6-60 [GenBank:CP002154.1] and EIB202 [GenBank:CP001135.1]. It was suggested that the diseased turbot from the aquaculture farm might be infected by the same *E. tarda* strain. In the conventional bacterial culture test, the procedure for isolation and identification of *E. tarda* from diseased fish samples usually requires 2 to 3 days, including bacterial enrichment, plating onto DHL agars, sequential subculture, and biochemical characteristic test, which is time-consuming and tedious. Therefore, the test strip is effective for the on-site detection of *E. tarda* in ascite samples.

Conclusions

The results in this work indicated that the detection limit, simplicity, and specificity of the test strips are sufficient for on-site use. In conclusion, the one-step immunodiagnostic test presented here could prove to be a useful tool for the inexpensive, rapid, and convenient on-site detection of *E. tarda* in clinical samples.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HL carried out the preparation of the monoclonal antibody targeting *E. tarda* and development of the test strip. YW carried out the on-site detection of the test strip and helped in the manuscript preparation. JX, QW, QL, and YZ have made substantive intellectual contributions to this study and substantial contributions to the design of the study. All authors read and approved the final manuscript and helped design the whole study.

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