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# A comprehensive review on vaccine trials against *Edwardsiella tarda*: A potential pathogen in aquaculture

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**Abstract**
*Edwardsiella tarda* is a well-recognised bacterial pathogen that inhabits the aquatic environment. *E. tarda* can infect many different fish species cultured worldwide and cause danger to aquaculture production. Various types of vaccines have been developed against *E. tarda* infection in fish species. Published findings on disease outbreaks, pathogenesis and virulence factors of *E. tarda* and vaccine trials against edwardsiellosis in various fish species are collated in the present study.

**Keywords:** *Edwardsiella tarda, Aquaculture vaccine,* pathogenesis

## 1. Introduction
*Edwardsiella tarda* is a Gram-negative, rod-shaped, facultatively anaerobic, non-spore-forming bacteria, peritrichously flagellated, motile bacterium of the family 'Enterobacteriaceae'. *E. tarda* was first reported in Japan by Sakazaki and Murata [89]. In fishes, *E. tarda* was first reported as *Paracolobactrum anguiillimortiferum* associated with red disease of Japanese eel, *Anguilla japonica* [30]. Ewing, McWhorter, Escobar, and Lubin examined 37 isolates submitted to the Communicable Disease Center, Atlanta, Georgia, by various laboratories [21]. In 1965, they suggested the term 'Edwardsiella' as the generic name and 'tarda' as the specific name for this organism which they incorporated within the family Enterobacteriaceae [90]. The genus *Edwardsiella* was named after the American Bacteriologist P R Edwards (1901–1966), proposed in 1965 [21]. Wakabayashi confirmed that *P. anguiillimortiferum* was the same as *E. tarda* [113]. Since 1965, *E. tarda* has been isolated from different fish and non-fish species such as reptiles, birds, and mammals, including humans.

## 2. Disease outbreaks in various fish species
Edwardsiellosis, caused by the bacterium *E. tarda*, is a severe systemic bacterial disease, which affects a variety of fish taxa and has a worldwide distribution in fresh and marine waters [4]. *E. tarda* causes fish gangrene, emphysematous putrefactive disease of catfish or red disease of eels, and edwardsiella septicemia [83]. Clinical signs of *E. tarda* vary after onset. The infected fish usually show loss of pigmentation, swelling of the abdominal surface, watery and bloody ascites in the abdominal space, swollen internal organs, haemorrhage, congested liver, spleen, and kidney [73, 74]. Edwardsiella septicemia is a serious systemic bacterial infection of cultured channel catfish (*Ictalurus punctatus*), which causes gas-filled, malodorous lesions in the muscle tissue of channel catfish, and the bacteria was isolated from the lesions or kidneys of diseased catfish in the United States [73]. *E. tarda* was isolated from largemouth bass (*Micropterus salmoides*) and various other aquatic animal species in Florida, including six species of wild birds and five alligators (*Alligator mississippiensis*) [119], and it was found to be associated with haemorrhagic enteritis in some of the species. In Japan and Taiwan, it causes a severe infection, called red disease, of cultured Japanese eels (*A. japonica*) [19], and the disease was characterized by macroscopic putrefactive lesions in the kidney or liver frequently combined with high mortalities, mainly in the summer months. Outbreaks in Japanese flounder (*Paralichthys olivaceus*) and *Tilapia nilotica* result in abdominal inflation and accumulation of ascites [78, 46], and in sea bream (*Evynnis japonica*), the infection was characterized by haemorrhagic ulcers on head and body surface, and numerous bacterial colonies as greyish-white spots in spleen and kidney [49]. The first description of an epizootic of edwardsiellosis affecting wild adult striped bass population was observed in the Chesapeake Bay [6].
*E. tarda* was isolated and identified from freshwater catfish and their environment [120] and

from several species of cultured marine fish such as red seabream and yellow tail [131]. Edwardsiellosis was reported among cultured coloured carps, *Cyprinus carpio*, in Mihara city, Hiroshima Prefecture [87]. The bacterium can affect a variety of other fishes, including flounders and tropical fishes. These outbreaks are typically associated with warm summer months, elevated water temperatures, poor water quality, and increased dissolved organic material [6, 73]. Repeated outbreaks of edwardsiellosis in turbot culture have occurred in different geographical areas of Europe [9]. *E. tarda* was isolated from moribund *Scophthalmus maximus* in an outbreak of edwardsiellosis in a mariculture farm in Yantai, a northern coastal city of China [124]. The first outbreak of edwardsiellosis in a wild European eel population was reported in a mediterranean freshwater coastal lagoon, Albufera Lake, Valencia, Spain [2]. African sharptooth catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) are two main reared species in polyculture systems in Egypt that were highly affected by recurrent *E. tarda* outbreaks [32]. The disease occurrence in *A. marmorata* caused by *E. tarda* was observed in a freshwater farm in south China [75]. The incidence of edwardsiellosis in cultured sharpsnout seabream (*Diplodus puntazzo*) was reported in the Mediterranean Sea [42]. Multiple edwardsiellosis outbreak and concomitant mortality associated with *E. tarda* and *E. piscicida* were reported in farmed barramundi (*Lates calcarifer*) in US [66].

Although *E. tarda* is generally considered as a problem in warm water fishes, the bacterium was responsible for mortalities of economically important coldwater fishes, such as chinook salmon (*Oncorhynchus tshawytscha*) in the USA [3], in turbot (*S. maximus*) in Spain [79], in farmed rainbow trout (*O. mykiss*) [86], and in brook trout (*Salvelinus fontinalis*) [110]. The infections are associated with stress-related immunosuppression due to an increase in summer water temperatures and poor environmental conditions. In India, *E. tarda*-specific infection has been reported in many cultured fish species by several authors [41, 47, 88, 102].

## 3. Pathogenesis and virulence factors

*E. tarda* is an intracellular pathogen and is capable of infecting various types of cells [35, 60]. Pathogenesis of *E. tarda* is multifactorial, and many potential virulent factors have been suggested, including antiphagocytic killing [1], production of siderophore [44], hemolysins [118], the ability to invade epithelial cells and fish tissues [34, 59, 60], and production of toxins such as dermatotoxins and hemolysins [29, 111, 118]. Two types of hemolysins such as cell-associated and iron-regulated hemolysin [33,118], and extracellular hole-forming hemolysin [11] were reported. The gastrointestinal tract, the body surface, and gills were observed to be the sites of entry of virulent *E. tarda* [59]. Virulent strains of *E. tarda* could enter fish in large numbers via mucus, gills, and the gastrointestinal tract and multiply inside various internal organs, causing death [85]. Type III secretion system (T3SS) and type VI secretion system (T4SS) of *E. tarda* play significant roles in adherence, penetration, survival, and replication in epithelial cells and phagocytes of the host [81].

Using a genome-wide analysis of functional genomics such as transposon tagging [71, 95] and proteomics [94, 105], nearly 20 crucial virulence genes were identified to be involved in pathogenesis by *E. tarda*. The two most important virulence factors in *E. tarda* based on $LD_{50}$ studies are the T3SS and partial EVP (*E. tarda* virulence protein) gene clusters [94, 106]. The T3SS proteins include the *E. tarda* secretion system apparatus (EsaB and EsaN), effectors (EseB, EseC, and EseD), chaperones (EscA, EscB, and EscC), and regulators (EsrA, EsrB, and EsrC) [106, 114, 135].

### 3.1 Bacterial surface components as virulence factors

Flagellar filament structural protein, fliC, is essential for the growth and virulence of *E. tarda* [28]. Flagellar genes such as fliC12, fliA, and flhDC of *E. tarda* play crucial roles in filament structure of flagella, bacteria motility, biofilm formation, adherence, internalization, and pathogenicity [126]. Invasin Inv of *E. tarda* plays essential roles in the hemolytic activity, biofilm formation, adherence, internalization, and pathogenicity of the bacteria [15]. Invasin Inv1 is a surface-localized virulence factor that is involved in host infection [55]. Mutation of tryptophanase gene *tnaA* in *E. tarda* reduces antibiotic resistance, lipopolysaccharide (LPS) production, and virulence indicating that *TnaA* is involved in enhancing the pathogenicity of *E. tarda* [27]. The genes of *E. tarda* two-component system *qseB* and *qseC* were found to control flagellar motility, fimbrial hemagglutination, and intracellular virulence [117]. Membrane-bound lytic murein transglycosylase A (MltA) plays essential roles in b-lactam antibiotics and environmental stress resistance, autolysis, LPS biosynthesis, and pathogenicity of *E. tarda* [63]. Bacterial sialidases are a group of glycohydrolases that are known to play an essential role in the invasion of host cells and tissues. *NanA*, a sialidase, from *E. tarda* plays a vital role in pathogenesis [40].

### 3.2 DNA-binding proteins, lysozyme inhibitors and other proteins of virulence

DNA-binding protein from starved cells (Dps) is a member of ferritin-like proteins that exhibit properties of non-specific DNA binding, iron oxidation and storage. The two Dps, Dps1 and Dps2 are functional analogs that possess ferroxidase activity and DNA binding capacity and are required for optimum oxidative stress resistance and full bacterial virulence [136]. Alternative sigma factor 54 (rpoN) is an essential regulator of virulence and stress resistance [116]. Cpx (cpxP, cpxR, cpxA), 'Conjugative plasmid expression' is found to be involved in *E. tarda* virulence [80]. *E. tarda* possesses the genes of two lysozyme inhibitors, ivy, and mliC (ivyEt and mliCEt). IvyEt confers protection on *E. tarda* against lysozyme lysis in the presence of serum and is required for optimal infection of the host [115]. MliCEt is a lysozyme inhibitor implicated in various aspects of bacterial virulence and required for host infection. MliCEt as a virulence factor probably works in a parallel, non-redundant manner with IvyEt [57]. Eta1 (adhesin) is an *in vivo*-induced antigen that mediates pathogen-host interaction and, as a result, is required for optimal bacterial infection [101]. Hsp90 is a molecular chaperone involved in diverse cellular processes, including protein folding/repairing and signal transduction in eukaryotic cells. HtpG is the prokaryotic homolog of Hsp90 which is a biologically active protein required by *E. tarda* for coping with various stress conditions [13]. Members of the DnaJ/Hsp 40 family play an important role in protein homeostasis by regulating the activity of DnaK/Hsp70. *E. tarda* DnaJ is a virulence-associated molecular chaperone with immuno protective potential [14].

### 3.3 Pathogenesis by host-immune evasion

Catalases such as KatB and KatG, and a Two-component system (TCS), QseE, QseG, and QseF in *E. tarda* serve for the physiological fitness and pathogenesis of *E. tarda* [112].

EsrB and PhoP (TCS) are also involved in the pathogenicity of *E. tarda* [67]. The PhoP-PhoQ system of *E. tarda* detects changes in environmental temperature and $Mg2^{+}$ concentration as well as regulates the T3SS and T4SS through direct activation of esrB [10]. Thirteen proteins in *E. tarda* were found to require the presence of PhoP for full expression [68]. *E. tarda* circumvents serum attack by preventing, to a large extent, complement activation via the alternative pathway, and those heat-labile surface structures likely to play an essential role in the complement evasion [56]. *E. tarda* inhibits apoptosis by regulating the genes involved in the apoptotic process. *Fech, Prx3, Brms1a,* and *Ivns1a* are involved in apoptosis in teleost. Prevention of apoptosis is a virulence strategy of *E. tarda* that enables the pathogen to survive and replicate inside the host cells [138]. Sip1 [139] and Sip 2 [54], serum-regulated proteins, are essential for serum resistance and pathogenicity of *E. tarda*. *E. tarda* strain having more than one virulence gene (esrB, mukF, and gadB) results in more severe lesions than strains having one or even no virulence genes [77].

**Fig 1:** SS agar plate and BHI plate with *E. tarda* colonies.

**Fig 2a** **Fig 2b**

**Fig 2:** *Labeo rohita* experimentally infected with *E. tarda* via intraperitoneal injection ($3.5 \times 10^{8}$ cfu/ml). 2a) scale erosion and fin erosion. 2b) redness and cutaneous lesions in the musculature.

## 4. Vaccines trials against *Edwardsiella tarda* in various fish species

With increased aquaculture production globally, vaccine development has become more important [18]. A variety of antigen preparation methods have been used to develop effective vaccines against *E. tarda* infections, including formalin-killed cells (FKC), ECP (extracellular protein), LPS, avirulent *E. tarda*, live attenuated *E. tarda*, ghost cells, OMP (outer membrane protein), OMV (outer membrane vesicle), recombinant proteins, recombinant protein-expressing cells, and DNA vaccines [81]. Variations in the serotypes of *E. tarda* had resulted in a long time to develop vaccine and to report on vaccination trials [76]. Immunization of Japanese eel with LPS preparation of *E. tarda* showed good protection [93]. Immunization with *E. tarda* crude LPS and crude polysaccharide (PS) was an effective immunogen producing high antibody titers and higher protection than whole-cell preparation in eel when experimentally challenged with live *E. tarda* [92]. The lipid of *E. tarda* acts as an immuno suppressor in the eel [91]. Citrus limon peel essential oil as an organic waste showed enhancement on immune response in tilapia and increased disease resistance against *E. tarda* [5]. Glucose enhanced the immunity of tilapia (*O. niloticus*) against *E. tarda* infection through metabolome reprogramming. *E. tarda* infection would suppress the glucose level in the liver of fish, and so exogenous glucose to the fish would greatly enhance their survival ability. The exogenous glucose was preferably converted into fatty acid, which could replace glucose as an important source to help fish fight against pathogens [132].

### 4.1 FKC, ECP, and ICC

When the fish immunized with FKC, ECP, or ICC (intracellular components) and control fish were challenged by injection and immersion with live cells, death was delayed in most of the immunized groups, but clear protection was not observed in any of the groups [72]. When Japanese flounder were immunized with formalin inactivated monovalent *E. tarda* TX1, *Vibrio anguillarum* C312, *Streptococcus iniae* SF1, and *Vibrio harveyi* T4D, or with different combinations of these strains, the combinations of strains M4 (TX1, C312, SF1, and T4D mix), M3 (TX1, C312, and SF1 mix) and M2 (TX1 and C312 mix) all induced significantly higher levels of protection against *E. tarda*, and two of them (M2 and M4) also effected much higher RPS rates against *V. anguillarum* [98]. Progress in vaccine preparation using diverse antigens has led to highly effective vaccines. Several vaccine trials coupled with adjuvants have shown 100% relative survival, and most evaluated trials were shown to produce significant protective effects [81]. When Tilapia fish were intraperitoneally immunized with formalin-killed *E. ictaluri* whole cells and rGAPDH from *E. ictaluri*, and both of which were emulsified in ISA 763A adjuvant, the RPS values were found to exceed 71.4% in the fish after challenging with the *E. tarda* [8].

Crucian carp vaccinated with live cells of *E. tarda* showed high survival rates, high IFN-g and T-bet gene expression levels, and increased cytotoxic T lymphocytes (CTLs) after challenging whereas FKC-vaccinated fish had increased IL-4/13A and IL-10 expression levels and increased antibody titres, with suppressed Th1-like responses [127]. When Flounder were vaccinated by immersion of formalin-inactivated *E. tarda* following hyperosmotic treatments and challenged with *E. tarda*, the RPS of flounder treated with hyperosmotic immersion (HI) at 50, 60, and 70‰ salinities were 79, 71, and 57% respectively. HI (50‰ salinity) could efficiently enhance the immune response of flounder and show higher RPS [23]. Immune response of flounder was associated with the concentration and immersion time of formalin-inactivated *E. tarda* [16]. Flagellin was found to enhance the immunoprotection of formalin-inactivated *E. tarda* vaccine in Turbot, and it could be used as a potential adjuvant in the fish vaccine [64].

### 4.2 OMP vaccines

Exposure of rohu and catla juveniles to *E. tarda* bacterin suspension for 15 min showed significant resistance against challenge with virulent *E. tarda* [103]. A 37 kDa OMP was detected in several serotypes of *E. tarda* strains and was designated as an effective vaccine candidate against experimental *E. tarda* infection in Japanese flounder [43]. Liu *et al*. prepared a recombinant glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of *E. tarda*, which could serve as an effective and practical vaccine antigen against *E. tarda* infection in Japanese flounder [65]. Verjan *et al*. 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 transporting 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 [112].

Japanese flounder (*P. olivaceus*) given intraperitoneal injection with OMP of *E. tarda* in FIA and challenged at day 30 showed a higher relative percentage survival (RPS) of 71. The OMP of *E. tarda* could elicit strong and persistent immune responses in Japanese flounder protecting from *E. tarda* infection [109]. Olive flounder given OMVs (Outer Membrane Vesicles) were effectively protected, and OMVs may be useful in the development of an effective vaccine against edwardsiellosis [82]. *E. tarda* OmpA encapsulated in chitosan nanoparticles was protective when administered orally in Fringed-Lipped Penisula carp (*Labeo fimbriatus*), and the protection was superior to inactivated whole-cell vaccine without adjuvants [17]. Olive flounder fed *L. lactis* BFE920 expressing a fusion antigen composed of *E. tarda* OmpA (Outer membrane protein A) and FlgD (flagellar hook protein D) showed a strong protective effect against edwardsiellosis [7].

### 4.3 Ghost cells, live avirulent vaccines

*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* [50]. *E. tarda* ghost vaccine at different concentrations could induce immune responses against a homologous challenge and protect *Sparus macrocephalus* with no significant difference in SR and RPS [128]. A modified live vaccine was developed against *E. tarda* through the induction of rifampicin resistance in a native *E. tarda* isolate. Acquired immunity was stimulated against virulent *E. tarda* infection by single immersion treatment or injection of fish [20]. Mutants for the esrB gene (encoding for a regulator protein of T3SS) of *E. tarda* elicited significant protection against edwardsiellosis in turbot (*Scophthamus maximus*) [51]. Vaccination with natural *E. tarda* ATCC 15947 strain could also induce strong protective immunity against the infection of pathogenic *E. tarda* in Japanese flounder [12]. The zebra fish vaccinated with DaroC, DaroCDesrC, DaroCDslyA, and DaroC DeseBCD DesaC via intramuscular injection showed ideal protection, resulting in relative percent survival of 68.3, 71.3, 80.1, and 81% against subsequent challenge with the wild-type *E. tarda* EIB202. DaroC DeseBCD DesaC showed a low virulence and the highest RPS on zebra fish model. The mutant DaroC DeseBCD DesaC might serve as an effective live attenuated vaccine for edwardsiellosis [123].

Vaccination with live avirulent *E. tarda* (serotype A) activated specific antibody production against virulent *E. tarda* and increased the expression of cytokine genes, including interleukin-1b (IL-1b), type 1 interferon (IFN), and IFN-g in head-kidney of the Japanese flounder [104]. N163 is the immunodominant region of *E. tarda* FliC which may induce immune responses in Japanese flounder [38]. Using attenuated *V. anguillarum* named MVAV6203 to express protective antigen GAPDH of *E. tarda*, it was found that the multivalent bacterium presented protective efficacy against infection by both *V. anguillarum* and *E. tarda* (RPS = 70%) [137]. Using allelic exchange strategy, an *E. tarda* live attenuated vaccine candidate WED was developed, which is a mutant of *E. tarda* EIB202 with deletions in the T3SS genes eseB, eseC, eseD, and escA, along with the aroC gene. WED showed 5700-fold higher 50% lethal dose (LD50) than that of the wild-type *E. tarda* EIB202. Vaccination with WED by intraperitoneal or immersion injection routes elicited significant protection against the challenge of the wild-type *E. tarda* in turbot (Scophthamus maximus) [121]. A combination vaccine of live attenuated *E. tarda* WED, and *V. anguillarum* MVAV6203 was found to be effective. When the vaccinated Zebra fish were challenged with *E. tarda* and *V. anguillarum* at 30 days post-vaccination, the fish exhibited the relative protective survival of 70% and 90%, respectively [22]. EsrB variants generated by error-prone PCR mutagenesis were incapable of activating T3SS and T6SS expressions but efficiently enhanced the yields of hemolytic activity in ΔesrB mutant. The isolated strain was YWZ47 with attenuated virulence, increased host invasion ability and showed high protection efficiency for the challenge of wild-type *E. tarda* when inoculated in Turbot by both immersion and intraperitoneal injection routes [130]. Live vaccines activate biosynthesis of unsaturated fatty acids, the TCA cycle and reduce aminoacyl-tRNA biosynthesis, and oleate induces effective protection against *E. tarda*, and thus Live *E. tarda* vaccine enhances innate immunity by metabolic modulation in zebrafish [24].

### 4.4 Recombinant vaccines

A recombinant scFv vaccine emulsified with Freund's incomplete adjuvant (FIA) was developed. The recombinant anti-idiotypic antibody scFv, which lacks Fc domain, resulted in efficient protection against infection by different serotypes of *E. tarda* [84]. A comparative study of the effects of FIA and aluminium adjuvants was made to study the immune response to an *E. tarda* major antigen, and he found that FIA was immunologically more potent than aluminium-based adjuvants [36]. A D15-like surface antigen, Esa1, when used as a recombinant subunit vaccine, was able to induce protective immunity in Japanese flounder against *E. tarda* challenge [97]. A DNA vaccine was developed, pCEsa1, based on the D15-like surface antigen Esa1 derived from *E. tarda*, which afforded 57% protection [100]. Japanese flounder with live DH5α/pTAET21 *(E. coli* DH5α harbouring plasmid pTAET21) elicited immune protection that was significantly higher in level than that induced by vaccination with purified recombinant Eta21 (*E. tarda* antigen). Vaccination with DH5α/pTAET21 and recombinant Eta21 both induced the production of specific serum antibodies at four to eight weeks post-vaccination. Eta21 delivered by DH5α/pTAET21, is an effective vaccine candidate against *E. tarda* infection [37]. Eta2 is a protective immuno gen that induces different immune responses as a purified recombinant subunit vaccine and as a DNA vaccine. High level of protection was observed in rEta2

and it may be due to the stimulating effect of the aluminium hydroxide (AH) which was added in the rEta2 vaccine formulation. The relative percent of survival calculated with PBS + AH (for subunit vaccine) is 83% [99]. Purified recombinant DnaJ induced protective immunity in Japanese flounder when used as a subunit vaccine, and aluminium hydroxide was used as an adjuvant [14].

Recombinant outer membrane protein A (OmpA) of *E. tarda*, could act as a potential vaccine candidate for common carp [70]. *P. olivaceus* immunized with recombinant strain BL21 (DE3) Pet-28a-OmpS (2) showed a protective ability of 70% to *E. tarda* [134]. OmpC (Outer membrane protein C) of *E. tarda* is an immunogenic surface protein, which induces an innate and humoral immune response in flounder and evokes highly protective effects for *E. tarda* challenge when used in the form of a recombinant protein [62].

Fusion protein Sia10-DnaK (*S. iniae* antigen Sia10 and *E. tarda* heat shock protein rDnaK) was expressed in *E. coli* DH5α via the plasmid pTDK, and when flounder fish were vaccinated with live DH5α/pTDK, strong protection was observed against *E. tarda* with an RPS rate of 74% against *E. tarda*. The rDnaK is an intrinsic ATPase with immuno protective property, and that Sia10-DnaK delivered by a live bacterial host is an effective bivalent vaccine candidate for *E. tarda* and *S. iniae* infection [31]. Recombinant NanA, when introduced into flounder as a subunit vaccine, produced a protection rate of 69% for lethal *E. tarda* challenge, suggesting that rNanA is a protective immunogen [40]. Recombinant Inv1 as a subunit vaccine induces strong protective immunity in flounder against *E. tarda* infection, with the induced protection rate of 88.9% [55].

Recombinant GAPDH could be a broad-spectrum vaccine candidate against polymicrobial infections in aquaculture, and a high level of RPS (at least 60%) was achieved in turbot against *E. tarda* [58]. Vaccination of turbot (*S. maximus*) with Δ*ugd* by intraperitoneal injection elicited significant protection against the wild-type *E. tarda* strain with an RPS of 76.70% [69]. Recombinant Eta1, when used as a subunit vaccine, induced a protection rate of 83.3% upon lethal *E. tarda* challenge in flounder [101]. Flagellar proteins are potential vaccine candidates. After expression in *E. coli* and purification, the recombinant FlgD was evaluated in zebrafish and turbot by intramuscular injection and found to lead to a high RPS for *E. tarda* EIB202 challenge, making recombinant FlgD a promising candidate vaccine against edwardsiellosis [133]. The adjuvant effects in the immunity of two forms of flounder IL-6 (rIL-6 and pcIL-6) were evaluated and comparatively analysed on *E. tarda* subunit vaccine rOmpV in flounder following the vaccination. The rIL-6 could induce much stronger humoral and inflammatory immune responses, whereas the cellular immunity enhanced by pcIL-6 was much stronger than rIL-6 [25]. When NADP-dependent isocitrate dehydrogenase (IDH) of *E. tarda* was recombinantly expressed, and the vaccine potential of rIDH was tested in a flounder model, and the results showed that rIDH produced a RPS of 73.3% and induced both Th1 and Th2 type of immune responses [108]. The adjuvant effects of four recombinant Th0 cytokines including IL-1β, IL-8, TNF-α, =and G-CSF on *E. tarda* subunit vaccine rOmpV were comparatively investigated in flounder, and he reported that rIL-1β and rIL-8 could be promising adjuvants for subunit vaccines against *E. tarda* as they produced higher RPS of 75% and 68%, respectively [26].

### 4.5 DNA vaccines

TX5RMS10 is a genetically assembled vaccine that possesses the combined advantages of an attenuated live bacterial vaccine (*E. tarda*) and a DNA vaccine (*S. iniae*). When flounder were vaccinated with TX5RMS10 via oral and immersion routes, the vaccinated fish exhibited relative percent survival rates of 69–83% [96]. DNA vaccines based on *E. tarda* antigens were constructed and the immune protective efficacies of the vaccines were studied in Japanese flounder model. The DNA vaccines were based on antigens Eta6 and FliC in the form of plasmids pEta6 and pFliC and chimeric DNA vaccine, pCE6 (which encodes Eta6 fused in-frame to FliC), and pCE18 (which expresses FliC fused to *E. tarda* antigen Et18) [39]. A multivalent vaccine candidate WEDDeltaasdB/pUTa4DGap, was developed which combined a diaminopimelic acid (DAP)-dependent mutant (WEDDeltaasdB) with a non-antibiotic resistant vector containing asdB gene and expressing a protective antigen gene gapA34 from *Aeromonas hydrophila* LSA34. The vaccine was shown to evoke an effective immune response against both *E. tarda* and *A. hydrophila* LSA34 and has great potential for broad applications in aquaculture [129].

GroEL of *E. tarda* is a strongly immunogenic protein that exists both in the outer membrane and the secretome. It produced an RPS of 60% when formed as a DNA vaccine, which indicated that GroEL could be a vaccine candidate for *E. tarda* infection. Moreover, pCG-GroEL could induce a strong innate immune response, humoral immune response, and cellular immune response, which were essential for combating both extracellular and intracellular *E. tarda* [61].

A bicistronic DNA vaccine was constructed in pIRES plasmid and designed as pGPD+IFN. The vaccine contained an immune adjuvant gene as interferon gamma gene of *L. rohita* and an antigenic gene which was GAPDH of *E. tarda.* The immunized fish exhibited an RPS of 63.16% [48]. Nano conjugated bicistronic DNA vaccine using chitosan nanoparticle (CNPs-pGPD+IFN) was designed against *E. tarda* infection in *L. rohita.* This vaccine can be administered by oral or immersion route with maximum effectiveness [45].

A bicistronic DNA vaccine macromolecule complexed with poly lactic-co-glycolic acid (PLGA) and PLGA chitosan nanoparticle (Chit NPs) was developed. PLGA has an adjuvant property. This vaccine could enhance the mucosal immunity of *L. rohita* against *E. tarda* infections [52]. Species-specific IgM antibodies was developed using IgM antibodies of *L. rohita* and enhanced the mucosal immune response of *L. rohita* using the recombinant bicistronic nano DNA vaccine (RBND Vac). The immunoglobulin in *L. rohita* primed with RBND Vac complex provided 64.70% protection against *E. tarda* [53]. Co-vaccination with rOmpV with rIL-2 or pcIL-2 could induce stronger immune responses and could evoke increased immune protective efficacy against *E. tarda* infection in flounder [107].

## 5. Conclusion

*E. tarda* is considered as a threatening pathogen to aquaculture as it has resulted in devastating outbreaks and economic losses in fish farming. The aim of this review is to gather knowledge about the bacterial pathogenesis and virulence factors and to have a better understanding about various vaccination strategies that have been employed so far in order to come up with successful commercial vaccine for use in aquaculture.

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