

Edwardsiella Species Infection in Fish Population and Its Status in Ethiopia

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Abstract

The genus *Edwardsiella* (E.) is a Gram-negative, glucose fermentative, catalase positive, and capable of producing H₂S and indole (except *E. ictaluri*). It comprises the three previously isolated *E. tarda*, *E. hoshinae*, *E. ictaluri* and the two recently isolated *E. piscicida*, *E. anguillarum* species. Except *E. hoshinae*, other *Edwardsiella* spp. are pathogenic to fish, and only *E. tarda* is known to cause human infection. Pathogenic *Edwardsiella* spp. uses the virulence factors that include flagellin, adhesin, hemolysin, type III and type VI secretion systems to gain entry into and survive within the host. The status and distribution of edwardsiellosis in fish and its public health significance in Ethiopia is poorly understood, and thus needs further studies as aquaculture is growing in the country. Since differentiation of *Edwardsiella* spp. based only their phenotypic characteristics is less discriminatory than their genotypic characteristics, thus phenotypic studies should always be followed by genetic analysis of the bacterium. Therefore, the objective of this seminar paper is to review *Edwardsiella* infections in fish, its zoonotic importance and status in Ethiopia.

Keywords: *Edwardsiella*; Ethiopia; Fish; Human; Infection

Introduction

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world [1], although fish are susceptible to a wide variety of bacterial pathogens especially when they are subjected to stressors, i.e., oxygen depletion, poor water quality and overstocking. Infectious diseases are the main cause of economic losses in the aquaculture industry, which is negatively impacted by various bacterial pathogens. One of the important bacterial diseases in tropical fish culture systems is edwardsiellosis associated with *Edwardsiella* spp. [2,3]. Edwardsiellosis is a septicemic disease, that is caused by *Edwardsiella* spp., characterized by extensive lesions in the skin, muscle, and internal organs that infects commercially important fish including eels, channel catfish, mullet, chinook salmon, flounder, carp, tilapia, and striped bass.

The genus *Edwardsiella* (E.) consists of five species; *E. tarda* [4], *E. ictaluri* [5], *E. hoshinae* [6], *E. piscicida* [7] and *E. anguillarum* [8], which all are Gram-negative rods and belongs to the family Enterobacteriaceae [9]. The genus is far more notorious from its fish pathogenic members and it is responsible for heavy losses in aquaculture [10,11]. The genus originally has only one species, *E. tarda*, reported as a pathogen of aquatic animals and humans. However, the public health importance of *E. piscicida* [7] and *E. anguillarum* [8] are not yet been studied. *E. ictaluri* is the causative agent of Enteric Septicemia of Catfish, one of the most important infectious diseases in the United States and Asian aquaculture industry although *E. tarda* and *E. ictaluri* are reported in other countries including Ethiopia [12-14]. The recently identified species *E. piscicida* and *E. anguillarum* are also reported as a causative agent of *Edwardsiella* infection in fish species [7,8] which are reported in more than 20 fish species mainly in

Asia, although the disease has been reported in Europe and more recently in the Mediterranean region, but not yet in Ethiopia [13,15].

In Ethiopia, the significance of fish infection due to *Edwardsiella* spp. is not well investigated except for few reports on the isolation *E. tarda* and *E. ictaluri*, from some freshwater fish species of Lake Tana [12], Langano, Zeway [14] and Hawassa [13]. Few of the studies so far done indicated the significance of the bacteria as a fish pathogen and public health threat. However, information on the safety of fish products harvested for consumption with regard to contamination with *Edwardsiella* spp. is lacking. Such information would otherwise be useful due to the potential threat of the bacteria for future aquaculture practices as well as public health owing to the practice of consuming raw or partially cooked fish meals in Ethiopia.

Therefore the objective of this seminar paper is to review *Edwardsiella* infections in fish, its zoonotic importance, and its status in Ethiopia.

The Genus *Edwardsiella*

Taxonomic classification

The genus *Edwardsiella* is named in 1965 after P.R. Edwards (American microbiologist) to honor his numerous contributions to the field of enteric bacteriology. In the same year Ewing et al. [4] and his colleagues proposed a creation of a new species called *E. tarda* from human feces [4] and it was considered as a single species for a genus until *E. ictaluri* is isolated from channel catfish by Hawke et al. [5]. Three years later in 1980 third *Edwardsiella* spp. is also discovered from reptiles and birds by Grimont et al. [6]. It is called *E. hoshinae* which name was proposed in honor of the Japanese bacteriologist Toshikazu Hoshina. Then after *E. hoshinae* added the genus *Edwardsiella* known to be containing only three species for a long period (1980-2013) due to its most similar biochemical characteristics

[7]. However, with the increasing understanding of *Edwardsiella* and the development of knowledge about species specific molecular technique usage helps to differentiate them based on its gene sequence reveals the identification of the fourth and the fifth species of *Edwardsiella* by Abayneh et al. [7,8] respectively. This recently added species are called *E. piscicida* and *E. anguillarum*. Once identified both are reported as the main problem for fish aquaculture [15].

Phenotypic and genotypic characteristics of the bacterium

Edwardsiella spp. are Gram-negative, small, straight rods and usually motile with peritrichous flagella [7,11]. All species are able to grow aerobically and anaerobically [8]. Biochemically all are unable to utilize citrate or deaminate phenylalanine, negative for vages proskauer and oxidase test, and unable to ferment sugars such as lactose, sucrose, L-arabinose, D-sorbitol, mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, arabinose, raffinose, and rhamnose. They exhibits positive reaction for motility, catalase, methyl red, indole tests, production of acid and gas from glucose, maltose and fructose, hydrogen sulfide production on Triple sugar iron agar, decarboxylation of lysine and ornithine [7,16,17]. However, several variations of biochemical tests have been found between *Edwardsiella* spp. on ornithine decarboxylase, citrate utilization, indole and hydrogen sulfide production, and fermentation of mannitol and arabinose [5,7].

Edwardsiella spp. show 99.35% to 99.81% similarities to each other [7]. However, they are differing in chromosomal gene sequence, guanine and cytosine (G+C) content of the chromosome and extrachromosomal element [18-22]. Unlike *E. ictaluri* the other *Edwardsiella* spp. chromosome have longer base pair (bp), that is, *E. ictaluri* has 43,378 bp [20], *E. tarda* (3,857,0403 bp) [19], *E. hoshinae* (3,811,650 bp) [22], *E. piscicida* (3,934,167 bp) [21]. All *Edwardsiella* spp. genome consists of one circular chromosome with respect to their G+C content. The G+C content of *E. tarda*, *E. ictaluri*, *E. hoshinae*, *E. piscicida* and *E. anguillarum* were identified as 59.6% [19], 52.6% [20], 56.9% [22], 59.1% [21] and 58.72% [8], respectively. As Reichley et al. [21,22] reported that *E. hoshinae* isolated from Lizard and *E. piscicida* isolated from diseased Grouper have no extrachromosomal plasmid in there chromosome. However Yasuike et al. [20,23] had reported extrachromosomal plasmid in *E. tarda* and *E. ictaluri* respectively.

Edwardsiella Infection

In fish

The genus *Edwardsiella* is a causative agent of Edwardsiellosis in both fish and human [24] and that causes a serious devastating economic losses in worldwide aquaculture industries [11,15]. It was initially found to cause disease in eels (*Anguilla japonica*) in Japan [25], and in channel catfish (*Ictalurus punctatus*) in the United States [26]. Currently, the disease known as edwardsiellosis occurs with high frequency in fish species like Japanese eel (*A. japonica*), Channel catfish (*I. punctatus*), Chinook salmon (*Oncorhynchus tshawytscha*), Rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), turbot (*Scophthalmus maximus*) and Olive flounder (*Paralichthys olivaceus*) [11,27-30] which are intestinal carriers and constitute the natural habitat of *Edwardsiella* spp. The bacterium is commonly isolated from intestinal samples of commercial fish although they can be isolated from different part of fish like liver, kidney, mucous and blood, which make possible the contamination of fish carcasses during fish processing which can be a possible risk for human contamination [14,31]. The disease outbreak aggravated when

fish are living under imbalanced environmental conditions, such as high water temperature, poor water quality, and high organic content [32].

Edwardsiella infection is the most widely spread disease which have been reported from over 20 species of freshwater and marine fish elsewhere in the world [15,23]. Surprisingly many reports suggested that species of *Edwardsiella* have different distribution in environment as well as in host [11,14,17]. Despite *E. hoshinae*, which is nonpathogenic and reported only in reptiles and birds, the most studied species *E. tarda* following *E. ictaluri* and the newly identified *E. piscicida* and *E. anguillarum* are reported as pathogenic for fish [4-9]. Among which *E. tarda* is the most studied and known to infect reptiles, amphibians, marine mammals and other warm blooded animals including human beings [24], while *E. ictaluri*, *E. piscicida* and *E. anguillarum* are reported as pathogenic for fish [7-9]. Moreover, the infection of *E. ictaluri* and *E. tarda* has been more reported as compared to other species of *Edwardsiella*. However, the geographic range of *E. tarda* is worldwide, whereas that of *E. ictaluri* is mostly confined to catfish growing areas [11].

Dvorak [33] suggested that fish disease can be spread by direct contact with water sources and fomites, via ingestion, and/or through vectors. Direct contact is one of the most common routes of disease transmission in aquaculture. This involves the transfer of disease causing agents through direct contact with infected fish. Entry may occur through the skin, open wounds, mucous membranes, or gills. While water sources can also serve to transfer disease causing organisms that is contaminated by the urine, faeces, reproductive fluids, and mucus of diseased fish. The movement of contaminated water during the transport of fish can spread pathogens to new locations. A few fish pathogens have been found to spread via aerosols, sprays or splashes between tanks, although less common and typically requiring close proximity of sources. Moreover, vectors are living creatures, such as fish preying birds that can spread pathogens. These animals may transfer fish diseases between locations by carrying the pathogen on their body or feet, or by dropping fish or fish parts at other locations. Rodents and birds may carry some fish pathogens in their faeces or urine, contaminating the environment or fish feeds. People may serve as vectors, transferring pathogens to fish during handling (e.g., hands). Furthermore, Disease organisms can be transmitted orally by consumption of contaminated feed, infected live or frozen fish, or cannibalism of dead or dying fish from the same unit. Ingestion of water contaminated with waste products from infected fish also may serve as a transmission route.

Fish infected with *Edwardsiella* spp. show abnormal swimming behavior, including spiral movement and floating near the water surface or hang head up in the water column disoriented behaviors characterized by a corkscrew swimming motion [34,35]. Although clinical signs vary after onset, fish infected with this bacteria show loss of pigmentation, exophthalmia, opacity of the eyes, swelling of the abdominal surface, petechial hemorrhage in fin and skin, ulceration, enteritis in acute disease, a soft, fluctuant red swelling on the dorsum of the head in chronic disease and swollen anus due to the accumulation of ascitic fluid [34,36-38].

In human

The most commonly isolated *Edwardsiella* spp. in human is *E. tarda* that causes intestinal like gastroenteritis and extra intestinal disease such as myonecrosis, soft tissue infection, and meningitis, peritonitis with sepsis, bacteremia and wound infections [17,36,39,40]. This is

usually more severe in immunocompromised or immunodepressed patients, older adults and children characterized by diarrhea, gastroenteritis, wound infection and even death [41]. The intestinal disease in neonates and young infants is characterized by prolonged or intermittent watery to bloody diarrhea, anorexia and vomiting which are particularly susceptible to the infection due to its immature immunity [42]. Patients receiving immunosuppressive therapy during organ transplantation are also susceptible to develop gastroenteritis due to the bacterium [31,43]. Likewise, extra-intestinal infections which usually originate from wounds associated with fishing, diving or swimming or abdominal trauma [40], with subsequent escape of the bacteria from the bowel and spreading into adjacent tissues, causing peritonitis, multiple liver abscesses, cholangitis, meningitis, cholecystitis, salpingitis, skin and genitourinary tract infections [32,40]. It can also colonize the maternal birth canal and uterus that often progress into necrotizing soft tissue and neonatal infection particularly meningitis in newborns that could progress into septicemia [43] or multiple neonatal brain abscesses, if not properly diagnosed and treated [32].

Transmissions of *Edwardsiella* infection from fish or aquatic environment to humans are quite common. These may be due to the consumption of raw or partially cooked fish meals, handling of fish, during filleting, swimming in contaminated water and the immune system status of the exposed individual [14,32,33,44]. In other means ingestion of contaminated food or water introduces the bacteria into the gastrointestinal tract, after which the bacteria becomes invasive and infects epithelial and other cells in the host. The reservoir and source of infections are usually linked to aquatic habitats and aquatic animals [33]. Those most affected tend to be compromised individuals with underlying diseases such as hepatobiliary diseases, malignancy, and diabetes [32,42,45].

Virulence factor in *Edwardsiella* infection

The infection pathway of *Edwardsiella* may be different. In the first way it may use its flagella to approach and attach to the host's epithelial cells. Flagellin, the principal component of the flagella, was identified as a significant virulence factor when the extracellular protein of avirulent and virulent strains of *Edwardsiella* was compared [46,47]. Ling et al. [48] reported that the epithelia of the gastrointestinal tract, gills, and body surface are the main sites of bacterial attachment. In order to bind to a host surface and initiate an infection, it may follow the second way that the bacterium may utilize adhesins like fimbrial protein [47], afimbrial hemagglutinin or autotransporter adhesion [46]. Upon binding to a host cell, the bacterium is assisted by invasion like proteins called hemolysin that allow the bacterium to invade or be internalized into host tissues and cells such as epithelial cells [49].

Once *Edwardsiella* is inside the epithelium of the host, it develops third strategy that could be the bacterial type III and type VI secretion systems (T3SS and T6SS). These are believed to play an essential role to allow a bacterial to be internalized, replicate intracellularly [50,51], and may spread to adjacent epithelial cells as is seen in a typical case of gastroenteritis. When the number of bacteria increases and the host cannot fight off the local infection, *Edwardsiella* can spread and invade deeper tissues and reach the blood and lymph. During this stage of infection, the bacteria may encounter phagocytic cells and be engulfed by them. At this point the T3SS and T6SS virulence mechanisms may help the surviving and replicating of *Edwardsiella* inside the phagocytes [31,52].

Once inside phagocytic cells or during the process of penetrating deeper into host tissues, the bacterium may have to oppose with host defences such as phagocytes and serum-mediated killing. This is achieved by activating acid neutralizing genes to protect the bacterium from the acidic environment and phagosomes while genes code for catalase and superoxide dismutase also allows the bacterium to break down the reactive and damaging oxygen components such as hydrogen peroxide [53,54]. During intracellular survival inside macrophages, expression of T3SS proteins is significantly increased, while production of flagellar proteins and invasive proteins are greatly decreased because motility and invasion are not needed for intracellular living [55].

As infection progresses, it follows another mechanism, *Edwardsiella* needs to acquire nutrients within the host for growth and proliferation. Nutrient depletion could also be sensed by the bacterium as a clue that it is inside the host. To obtain essential nutrients such as phosphate and iron, high affinity phosphate transport genes and siderophore producer genes are activated [50,55] and contribute to bacterial virulence inside the host. At the same time, extracellular enzymes such as hemolysins, chondroitinase, collagenase and proteases may aid the bacteria to spread during the systemic infection stage [50,56,57]. Deletion of T6SS genes is found to decrease proliferation of *Edwardsiella in vivo* suggesting that T6SS may also be important in systemic infection [47].

Isolation and Identification of the Bacterium

Edwardsiella spp. are a type of fish affecting bacteria that requires isolation and identification to know the exact disease causing pathogen for control and prevention of disease [58]. Hence, *Edwardsiella* spp. could be isolated from fish and humans samples cultured on trypton soya agar and produce a colony with characteristics of round or circular, gray or grayish white, and pigmentation after 24 or 48 hours incubation at 20°C-30°C [6-8,14,35]. Generally as Abayneh et al. [7,8] demonstrated that all *Edwardsiella* spp. are capable of grow at 25°C, 30°C and 37°C but not at 12°C and 42°C and they are also capable of grow at sodium chloride concentration of 1%-5%.

Shao et al. [8] investigated that, biochemically all species have the typical biochemical features of the genus *Edwardsiella* with respect to the commonly used biochemical tests. All *Edwardsiella* spp. are unable to ferment inositol, sorbitol, rhamnose, saccharose, melibiose, inositol, sbitol, amygdalin, lactose and trehalose. All species are also negative for production of L-galactosidase, arginine dihydrolase, urease, tryptophan deaminase, gelatinase and unable to degrade acetic acid, formic acid, α -ketobutyric acid, α -ketovaleric acid. However, all species are able to produce lysine decarboxylase, acid from glucose and D-galactose and able to degrade D-glucosaminic acid, L-asparagine, L-aspartic acid, L-serine and uridine. Only *E. anguillarum* is able to ferment mannitol and arabinose and degrade Simmon's citrate and *E. piscicida* can also degrade quinic acid. Furthermore, all species are capable of producing hydrogen sulfide and indole except *E. ictaluri* [7,8]. *E. tarda* is able to degrade β -methyl-D-glucoside, tween 80, L-fucose, bromosuccinic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, glycerol and d, L- α -glycerol phosphate while there are variable reactions between *E. anguillarum* and *E. piscicida* [7].

Prevention and Control of *Edwardsiella* Infection

An outbreak of edwardsiellosis is often resolved by itself if environmental stress factors such as overcrowding, low oxygen, rough handling, malnutrition, sudden change of water temperature or pH

and good hygiene are controlled or removed [1]. This may be achieved by practicing a periodic drying of ponds, providing of anti-stress substances such as probiotics, ascorbic acid, lipopolysaccharides adding with fish feed [59] and disinfection of aqua farming areas if the farm lands at confined place which can be easily managed [60] otherwise it is difficult to control and prevent the distribution of the disease [1]. Minimizing of disease transmission way may also help to control the circulation of the *Edwardsiella* infection through practicing of biosecurity measures, intermittent examination of water and its organic material for the presence of pathogens [61]. The newly introduced fish stock has to be purchased from known and trusted suppliers. These fish should be inspected and found free of important diseases. Limit the number of sources that fish are purchased and a frequency of new fish introductions into the farm. When possible, purchase eggs or fish from certified disease free brood stock. Eggs have to be disinfected upon arrival. When appropriate, vaccinate newly acquired fish for diseases [33,62]. By default this measure helps to reduce human infection and avoiding of eating improperly cooked fish meal also important to control human infection [32].

Although the uses of antibiotics are cause for the emergence of drug resistant bacteria, antibiotics have been also used to prevent *Edwardsiella* disease outbreaks and control proliferation of the pathogens for a long period. Moreover, four tetracycline resistant determinant genes, namely tet-A, tet-D, tet-B and tet-G have been reported in *Edwardsiella* spp., of which the first two types are present on the mobile plasmid [63]. Earlier studies indicated that *Edwardsiella* spp. is susceptible to various antibiotics such as the aminoglycosides, cephalosporins, penicillins, aztreonam, ciprofloxacin, sulphamethoxazole/trimethoprim, nitrofurantoin, the quinolones and antibiotic beta lactamase inhibitor agents [32,64]. Although a good number of antibiotics such as norfloxacin, ciprofloxacin, oxytetracycline, gentamicin, chloramphenicol [32], cefazolin [65] and aztreonam [66] are used for controlling of fish infection, these have their own disadvantages like the development of disease resistant strains, high cost and dose problems, as well as indiscriminate use by aqua farmers. Thus, the overuse of antibiotics and other chemicals needs to be checked and the use of alternative method have to be focused [59].

The Status of *Edwardsiella* Infection in Ethiopia

The status of *Edwardsiella* infection in Ethiopia is not much clear except for few reports on the isolation of the bacterium from some freshwater fish species of Lake Tana [12], Hawassa [13] and Zeway, Langanu [14]. Among *Edwardsiella* spp. *E. tarda* and *E. ictaluri* are isolated from a freshwater fish in Lake Tana with the prevalence of 2% and 1.2% respectively [12]. Other studies also reported that *E. tarda* is isolated from fish found in Lake Zeway, Langanu [14] and Hawassa [13]. Both *Edwardsiella* spp. were isolated using biochemical tests [12-14] which implied that the isolated species can be presumptively identified as *E. tarda* like species. Due to the fact that it is impossible to differentiate them from phenotypically similar species *E. piscicida* [7] and *E. anguillarum* [8] from *E. tarda* using phenotypic isolation techniques [13] because of the difficulty of identifying *E. tarda* from the two recently described new species using phenotypic characteristics, molecular based work has to be employed to differentiate them [13] and to know their relative distribution with the potential threats of fish production and human health.

Conclusions and Recommendations

Genus *Edwardsiella* is a Gram-negative, glucose fermentative, catalase positive, and capable of producing hydrogen sulfide and indole (except *E. ictaluri*). It comprises the three previously isolated *E. tarda*, *E. hoshinae*, *E. ictaluri* and the two recently isolated *E. piscicida*, *E. anguillarum* species. Among *Edwardsiella* spp. *E. hoshinae* is not cause of *Edwardsiella* infection in fish; the remaining four species are pathogenic to fish and can be a cause of fish disease whereas up-to-date the known pathogenic *Edwardsiella* spp. to cause human infection (such as extra intestinal and intestinal infection) is *E. tarda*. However, the zoonotic importance of the two recently isolated *Edwardsiella* spp. are not yet been confirmed.

In Ethiopia *E. tarda* and *E. ictaluri* are the only isolated *Edwardsiella* spp. using biochemical tests. This species probably similar to recently identified species of *E. piscicida* and *E. anguillarum*, because of the most similar phenotypic characteristics of the species. So it is doubtful to conclude confidentially that the isolated *Edwardsiella* spp. in Ethiopia are really *E. tarda* and *E. ictaluri*.

Therefore, based on the above conclusion the following recommendations are forwarded:

- Future investigation should be applied to know the public health importance of the two recently identified *Edwardsiella* spp.
- Further molecular identification work should be essential to confirm the available *Edwardsiella* spp. in Ethiopia.

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