Received: 5 December 2021 | Revised: 4 May 2022 | Accepted: 10 August 2022

DOI: 10.1111/raq.12743

REVIEW

REVIEWS IN Aquaculture

Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance

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Abstract

Tilapia culture is an important source of income and nutrition to many rural families. Since 2000, the production of tilapia increased and reached domestic and global markets. Major farmed species is Nile tilapia (*Oreochromis niloticus*), in earthen ponds and cage cultures. Intensification contributed to global tilapia disease outbreaks, with bacterial infections causing mortalities and morbidities, threatening sustainable production. At tilapia farms, high nutrient concentrations, water temperature and fish

Funding information
Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO); Norwegian Agency for Development Cooperation

densities enhance bacterial growth including virulent bacterial clones and potential zoonotic bacteria. Global warming favours this. This review respectively provides a comprehensive overview of the most common and emerging bacterial pathogens, diseases, clinical presentations and diagnostics of tilapia, including bacteria and diseases with zoonotic potential. First, common bacterial disease outbreaks, including streptococcosis, motile *Aeromonas* septicaemia, francisellosis, columnaris disease and vibriosis are described. Then, information on emerging bacterial infections of concern for tilapia, like edwardsiellosis through *Edwardsiella ictaluri* and *E. tarda*, as well as *Aeromonas schubertii* is provided. Reports of infectious bacterial tilapia disease outbreaks from other bacteria, including *Lactococcus garvieae*, *Aerococcus viridans*, *Pseudomonas* spp., *Mycobacterium marinum* and *Chlamydia* spp., and others are reviewed. Furthermore, bacteria with zoonotic potential, like *Streptococcus agalactiae* ST283, *S. iniae*, *Aeromonas* sp., *E. tarda*, *Vibrio vulnificus* pathovar (pv) *piscis* and *M. marinum* are included in the review, to provide the most current overview of the disease risks affecting production and post-harvest stages. Additionally, the status and risks of antimicrobial resistance in bacteria from tilapia and other cultured fish through imprudent use of antibiotics, and its future at a global level are provided.

KEYWORDS
AMR, bacterial disease, diagnosis, tilapia, zoonosis

1 | INTRODUCTION

Diseases of aquatic organisms seriously constrain the expansion and development of sustainable aquaculture. Globally, in aquaculture, the trend is that a previously unreported pathogen that causes a new and unknown disease will emerge, spread rapidly, including across national borders, and cause major production losses approximately every 3–5 years.[1]

The capability to manage health of aquatic organisms has significantly increased during the last three decades. However, such capacity did not match the rapid growth of the aquaculture sector.[2] Many of the most serious infectious disease agents affecting cultured species in aquaculture are bacteria. Because they rarely act as primary pathogens and they occur most commonly as opportunistic pathogens in already damaged or severely immunocompromised hosts, there is low attention given to this pathogen group. In fact, in the OIE (now known as WOAH) list of notifiable aquatic animal diseases, there are very few bacterial pathogens.[3]

However, bacteria may cause severe losses in tilapia farming. Bondad-Reantaso et al.[4] compiled a list of bacterial species or species groups affecting cultured finfish, crustaceans and molluscs. Their importance is growing, thus the need to pay more attention is there, not only in the context of its impact on production, but also of its zoonotic potential and contribution to development of antimicrobial resistance (AMR) through misuse of antibiotic treatments.

Farming of tilapia is primarily done in Asia; additional production comes from Africa and the Americas. The most predominant species is Nile tilapia (*Oreochromis niloticus*) with a 2019 production of 4.6 million tonnes.[1] From subsistence farming, tilapias are now commercially produced and tilapia products are traded globally. At a global level, the top three producers in 2019 are (i) China (1.6 million tonnes), (ii) Indonesia (1.3 million tonnes) and (iii) Egypt (1.1 million tonnes).

This article is part of a compendium of papers of a Special Issue in *Reviews in Aquaculture* which resulted from a virtual webinar event: 'Tilapia health: *quo vadis*', organized by the Food and Agriculture Organization of the United Nations (FAO), held from 1–3 December 2021. The objective is to review the most important bacterial pathogens and bacterial diseases affecting tilapia, including those that have zoonotic potential and understand ways to reduce bacterial disease risk for both fish and humans, with general recommendations of therapeutic and prevention strategies against the related pathogens, and pointing to the risk of development of antimicrobial resistance through imprudent use of antibiotics.

For this literature review, the authors used a systematic approach to the review, which included the use of relevant keywords (e.g. streptococcosis and tilapia) in the following databases of literature: *Web of Science*, *Scopus*, *PubMed*. The scientific literature included peer review journals, book chapters, health organism's reports, and so forth, with an initial search covering the last 10 years. Where little data was available, the temporal search was expanded as appropriate. An inclusive approach was adopted, where each of the authors took responsibility for a section and worked with those that had most expertise/experience in each of the sections or bacterial species. This was then shared with the authors and cross-revised accordingly. Preference was given to literature that included tilapia, and other fish species were included where data in tilapia was more limited.

The review work was divided among the authors, per expertise. Each expert read their database-acquired collection of papers and made

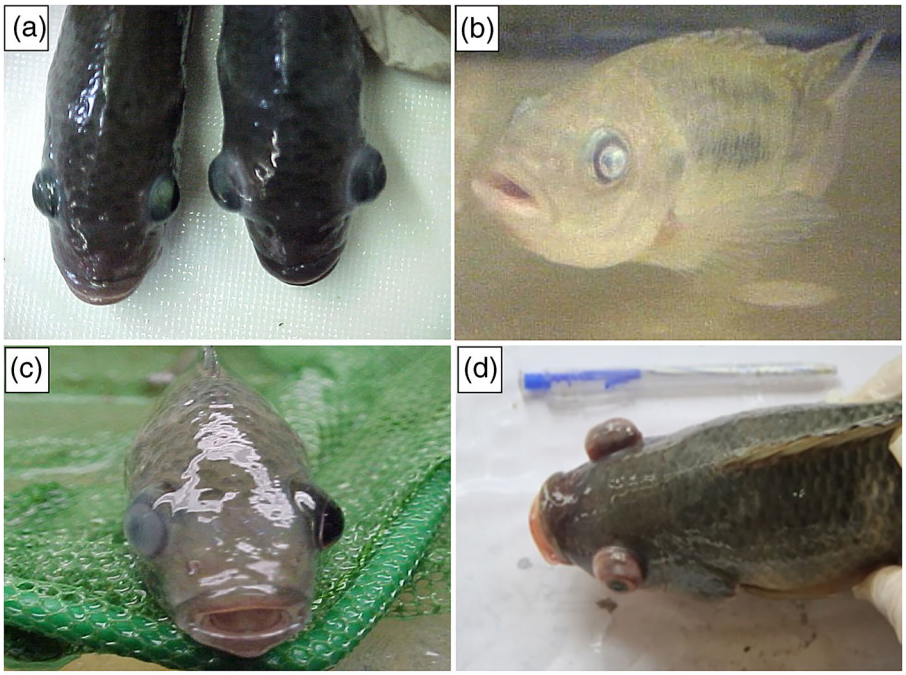

FIGURE 1 (a–c) Streptococcosis by *S. iniae*/*S. agalactiae* in diseased tilapia in the USA. The tilapia shows exophthalmos and cataract. They may have a C-shaped body, which causes them to swim spirally (b). Pictures (a–c): Courtesy Dr Joyce Evans, USDA-ARS, Aquatic Animal Health Research Unit, Auburn, Alabama, USA. Picture (d) Diseased tilapia from an outbreak of *S. agalactiae* in tilapia in Vietnam. The fish shows exophthalmos and a congested belly from full sepsis. Courtesy Dr Truong Dinh Hoai (co-author)

a draft text, which was altogether reviewed by the co-authors. Although most of the information was found on bacterial diseases of other fish than tilapia, we focused as much as possible on tilapia bacterial diseases.

2 | REVIEW

In total, 370 references have been cited for this review paper, from the years 1970–2022.

2.1 | Current bacterial diseases of significant importance

Tilapia may be infected with various bacteria, including species of the genera *Vibrio*, *Aeromonas*, *Pseudomonas* and *Streptococcus*,[5] whereas some genera may be present also on healthy fish, like species of the genera *Pseudomonas Aeromonas*, and *Plesiomonas*.[6] In general, most fish diseases are induced by a stress factor, like a suboptimal environment, for instance, bad water quality, and this allows opportunistic bacteria including *Aeromonas hydrophila* to infect tilapia and cause disease.[7–9] Moreover, many bacterial diseases are multifactorial.[10] We should keep this in mind, when trying to understand the cause of and finding a way to cure a bacterial fish disease.

The current bacterial tilapia diseases of significance (related to fish-welfare, economy and society) are streptococcosis, aeromonasis, francisellosis, columnaris disease and vibriosis.

To compare economic losses in USD due to bacterial disease in tilapia farming with those in other fish culture species is difficult, as costs are dependent on the value of the fish species, the production system, the country, the currency and so forth. A comparison in terms of % of fish production lost might be meaningful but there is not sufficient data that is collected in a consistent manner to allow for such comparisons across studies, countries, fish species and production systems.

2.1.1 | Streptococcosis

Outbreaks of streptococcosis have been widely reported in farmed tilapia species globally,[11–13] described as a septicaemic infection due to the bacterial species of *S. iniae* or *S. agalactiae*.[14] These facultatively anaerobic, Gram-positive bacteria are described as non-motile and non-spore forming, presenting with varied degrees of haemolysis dictated by species and strain variation.[15] In cultured tilapia, high prevalence of *S. iniae* and *S. agalactiae* infection was usually observed during hot and dry seasons when the water temperature is ≥27°C.[16,17]

Streptococcus agalactiae may cause acute[15] or chronic disease[18] in tilapia. Clinical presentations of the acute form include, but are not restricted to erratic swimming, c-shaped body of the fish, uni- or bi-lateral exophthalmia (with or without corneal opacity), distended abdomen and haemorrhages[15] (Figure 1). Meningoencephalitis has been reported in infected tilapia, as the bacteria cross the blood brain barrier[19,20] and similar clinical signs of disease were reported[21] in

tilapia both naturally and experimentally infected with either *S. iniae* or *S. agalactiae*. In the chronic form, yellow or dark red nodules were seen in the musculature near the vertebra of Nile tilapia.[18] An outbreak or cumulative mortality during chronic, persistent streptococcosis in tilapia can reach 80%,[22] while monthly prevalence of isolation ranged from 0% to 32% throughout the year.[23] Since *S. agalactiae* and *S. iniae* may be zoonotic,[24,25] in case of a chronic infection, the fish farmers may have a longer exposure to the bacterium, without very clear clinical signs.[18] This imposes a risk for the fish farmers, the fish processors, and the consumers.

Concurrent *Streptococcus* infection with other bacteria and tilapia lake virus (TiLV) has been reported in cultured tilapia.[26,27] The estimated economic impact of *S. iniae* and *S. agalactiae* infections in tilapia was around USD 150 million annually in 2000 and further increased to USD 250 million annually in 2008, representing approximately 5.7% and 6.7% of the total global value of tilapia, respectively.[14] However, no updated value on the economic impacts of streptococcosis in cultured tilapia is available.

Streptococcus isolated from fish are identified using a combination of phenotype (biochemical tests), serotype (agglutination test) and genotype (PCR, multi-locus sequence typing and whole genome sequencing). Barnes et al.[28] serologically and morphologically typed *S. iniae* isolates from tilapia (*Oreochromis* sp.) and hybrid striped bass (*Morone saxitilis* × *M. chrysops*) from the USA. Serologically distinct isolates of *S. iniae* identified as serotype I (ADH + ve) and II (ADH-ve) were isolated from natural disease infections in Thai tilapia farms.[29] Imperi et al.[30] reported 10 serotypes of *S. agalactiae* based on the composition of the capsular polysaccharide, where serotypes Ia, Ib and III are the most commonly reported strains in global tilapia outbreaks.[31,32] Genotyping studies using multi-locus sequence typing and whole genome sequencing have improved the understanding of pathogenesis of both *S. iniae* and *S. agalactiae*.[33]

In piscine streptococcosis, three major factors influence the pathogenesis; the virulence of the agent, the environmental stressors and the susceptibility of the host. Genetic virulence associated with genes that encode several protein molecules have been identified.[34] Buchanan et al.[35] identified the enzyme phosphor-glucomutase as the virulence factor for *S. iniae*. This enzyme inter-converts glucose-6-phosphate and glucose-1-phosphate, which play important roles in the production of polysaccharide capsule of *S. iniae* that enhances the bacterial virulence. In *S. agalactiae*, virulence gene profiles revealed that *S. agalactiae* serotype Ia ST7 lacked *lmb*, *scpB*, *pavA*, *fbsB*, *cyl*, *bca*, *cspA* and *bac* genes, which were present in serotype III ST283.[36] Varied routes of transmission have been reported in tilapia infections including cohabitation of infected and non-infected fish.[16] Transmission of *S. agalactiae* from a hatchery to a grow-out farm also has been documented.[23] Pradeep et al.[37] reported the first evidence demonstrating parents-to-offspring, vertical transmission of streptococcosis in tilapia.

Regarding vaccination, Shelby et al.[38] tested passive immunization of tilapia (*O. niloticus*) with intraperitoneal (i.p.) injection of anti-*S. iniae* whole sera, and this proved to be highly effective. Evans et al.[39] produced a *S. agalactiae* (Group B) vaccine for tilapia, which worked best after i.p. injection. Vaccination through i.p. injection with a re-attenuated strain of *S. agalactiae* (TFJ-ery), from the natural low-virulence *S. agalactiae* strain TFJ0901 as basis, gave almost 100% protection of tilapia.[40]

Regarding genetic resistance it is difficult to disentangle the role of tilapia species or strain, environmental conditions, pathogen prevalence and fish husbandry in susceptibility to different pathogens because most descriptions of disease are observational and not based on systematic comparison under controlled condition. Hence, any apparent association with species or breed may be due to underlying, uncontrolled, risk factors. There is, however, opportunity to breed for resistance to certain pathogens, as demonstrated recently for *S. agalactiae*, where a reduction in mortality of >50% could be achieved.[41,42] The impact of breeding for disease resistance on other desirable traits, for example, growth rate or flesh quality, is yet to be assessed.

2.1.2 | Aeromoniasis

Aeromonas spp. are ubiquitously found in freshwater environments and are described as infectious and opportunistic organisms, which may cause fish disease when stress factors are present in a diverse range of aquatic farming systems.[8] It has been shown, that *A. hydrophila* is one of the main pathogenic bacteria in tilapia culture, which not only causes high mortality and disease to cultured fish, but also causes similar problems to wild fish, resulting in huge economic losses, to both tilapia and wild fish.[43–45] It has been reported that aquatic animals infected with *Aeromonas* may suffer acute and chronic diseases, including haemorrhagic septicaemia, skin ulcers, and enteritis, with an average mortality rate of 30%.[46,47]

The taxonomy of the genus *Aeromonas* is subject to constant change, currently comprising 36 recognized species. The aeromonad fish pathogens are all motile with the exception of *A. salmonicida* subspp.[44] Generally, they are all described as Gram-negative, oxidase positive, facultative anaerobes.[48–50] They are non-spore forming, rod-shaped bacteria of approximately 1–3 μm[51,52] in length, capable of fermenting glucose and characterized by tolerating increasing concentrations of NaCl varying from 0.3% to 5%.[51]

A diverse range of motile aeromonads are reported as opportunistically pathogenic, especially under stressful environmental circumstances, resulting in clinical disease outbreaks leading to high levels of morbidity and mortality in a wide range of tilapia farming systems.[43,45] The most common species associated with natural disease outbreaks in farmed tilapia include *A. hydrophila*,[27,53–55] *A. sobria*,[56] *A. dhakensis*,[57–59] *A. veronii*[26,60,61] and *A. jandaei*.[60] The *A. hydrophila* and *A. veronii* had the highest prevalence of bacteria isolated from the liver, spleen, and other organs of infected tilapia.[60,62,63] Tilapia infected by these two species of bacteria showed lethargy, and apathy, ulcerations, pale spots, and haemorrhages along their body.[43,45,60,63] In addition, co-infections of *Aeromonas* with other bacteria is one of the important reasons for mass mortalities of tilapia, such as co-infection with *A jandaei* and *A. veronii*[60] (Figure 2),

FIGURE 2 Nile tilapia (*Oreochromis niloticus*), co-infected with *Aeromonas veronii* and *A. jandaei*. Courtesy Dr H. T. Dong (co-author)

Aeromonas sp. and *Streptococcus* sp.,[64,65] and of *A. veronii* and *F. columnare*.[26] Furthermore, co-infections with TiLV,[66] and with *S. agalactiae* and TiLV[27] (Figure 3) have been described.

The non-motile *A. salmonicida salm.* may cause furunculosis in salmonids and the atypical *A. salmonicida* is known to cause ulcer disease or erythrodermatitis in cyprinids[67] and in marine flatfish.[44] Experimentally induced infection of tilapia of 40 g through i.m. and i.p. injection of tilapia with atypical *A. salmonicida* at 28°C caused darkening, ulcers on the dorsal musculature and trunk region, gill congestion, exophthalmus and haemorrhages in the eyes, and reached 100% mortality at an i.m. dose of $\sim 1 \times 10^8$ CFU/fish. Internally, a congested liver and kidney were recorded.[68] Atypical *Aeromonas salmonicida* has been isolated from tilapia in Oman, but experimentally induced infection by intraperitoneal (i.p.) and intramuscular (i.m.) injection of 0.1×10^8 colony forming units (cfu) per 30 g tilapia at 26°C did not cause any disease or mortality.[69] In Bangladesh, a study was done on the presence of typical *A. salmonicida* in swamp water where tilapia is cultured, and its pathogenicity to tilapia of 10g after i.p. injection.[70] Results indicated, that the swamp water contained on average 3.3×10^6 CFU/ml. The injected tilapia showed 20% mortality at an i.p. dose of 3.3×10^6 CFU/g, and up to 80% mortality at an i.p. dose of 3.3×10^8 CFU/g at 20–25°C. They concluded, that natural average bacterial load of 3.3×10^6 CFU/ml or below in tilapia culture water did not produce significant mortality in *Oreochromis mossambicus*.[70] Overall, *A. salmonicida* may be harmful, but, like with motile aeromonads especially to injured tilapia under stressful conditions.

Identification of *Aeromonas* strains to species level is still a challenge because of the genetic heterogeneity of this genus.[71] Phenotypic identification of *Aeromonas* strains is done by physiological, morphological and biochemical characteristics.[48,72,73] Classic phenotypic characteristics that identify the genus *Aeromonas* are Gram-negative staining, the presence of cytochrome oxidase, and growth in nutritive broth at 0% NaCl in the presence of the vibriostatic factor O/129.[48,73] Commercial, fast identification systems, such as API 20E, Vitek, BBL Crystal, MicroScan W/A and others, have commonly been used to identify *Aeromonas* spp.[74] However, conventional methods based on the phenotypic properties and automated systems are of limited utility in identifying some *Aeromonas* spp.,[73] and their accuracy is affected by constant reclassification among components of this genus.[75]

Molecular biological techniques are the best option for the precise identification and taxonomic classification of the genus *Aeromonas*, through amplifying constitutive housekeeping genes (*gyrB* and *rpoD*) genes through polymerase chain reaction and sequencing the amplified products.[75] The 16S rRNA typing method, generally used in bacteriology[76,77] is also accurate for identification of *Aeromonas* spp.[78–80] Dong et al.[60] identified *A. jandaei* and *A. veronii* based on phenotypic features and homology of 16S rRNA. However, for certain species of *Aeromonas*, 16S rRNA alone will not adequately distinguish them, as additional sequencing of housekeeping genes such as *gyrB* is needed.[81]

Nile tilapia juveniles, after being exposed to transport-induced stress, appeared to have 19 responsible isolates of *A. hydrophila* in their body, as identified by 16S rRNA testing.[9] The *A. dhakensis* was firstly identified by phenotypic and 16S rRNA sequencing from diseased Nile tilapia.[57] Additionally, other molecular methods, such as the enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), and the amplified fragment length polymorphism (AFLP) are also used for identification and genotyping of *Aeromonas*.[82–86] The ERIC-PCR is one of the most popular methods for genotyping *Aeromonas* because it is easy to carry out, does not require any expensive equipment, and is highly reproducible.[87]

Aeromonas virulence is complex since several factors contribute significantly to the development of the infection process.[88,89] These virulence factors such as structural components, extracellular products, secretion systems and proteins associated with metals acting jointly or individually enable the microorganisms to adhere to and invade host cells, evade host immune defences and compete for nutrients, resulting in an infection that generates the disease.[46,48,71,90–94] Four secretory systems have been reported in the genus *Aeromonas*, being types II, III, IV and VI. They are responsible for releasing virulence factors produced by bacteria into the extracellular environment or even directly into the host cell, which is extremely relevant to the host cell damage and infection processes.[46,50]

At present, there are no specific data on the transmission mechanism of *Aeromonas* in fish, but there are data on its transmission in humans. Holmberg et al.[95] studied the clinical and epidemiological characteristics of human enteritis caused by *Aeromonas* and believed that drinking untreated water was the most likely mode of infection for patients, supported by Moyer[96] in a study of *Aeromonas* isolated from diarrhoea patients. Ghenghesh et al.[97] proposed water and food transmission in their research on *Aeromonas* infections in humans in developing countries, which has certain limitations, compared to in fish. However, overall, it is recognized, that the transmission routes of *Aeromonas* are horizontal, via water, food and faeces.

Certain *Aeromonas* strains are serious pathogens of tilapia, devastating this industry worldwide. Therefore, proper preventive and control measures are necessary. Generally, antibiotics are the most effective and often the main option for tilapia farmers. An example of antibiotic susceptibility was published for tilapia in Ethiopia.[5] However, antibiotic therapy should always be based on an antibiogram, to be sure, the therapy is effective. Moreover, frequent use of antibiotics results in development of antibiotic resistant strains, bio-accumulation, changes in the physiochemical properties of water and imbalance of bacterial microbiota in the fish bodies or the habitat.[63,98,99]

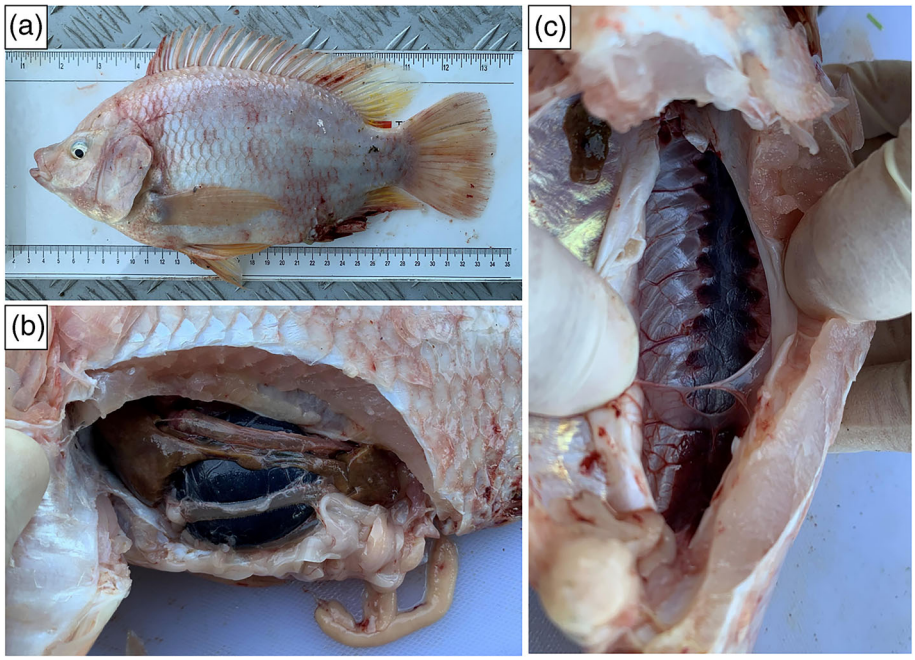

FIGURE 3 Clinical signs and gross lesions of red hybrid tilapia naturally co-infected with *Aeromonas hydrophila*, *Streptococcus agalactiae* and tilapia lake virus (TiLV). (a) Red skin with haemorrhages in the operculum, body and base of anal fin. (b) Enlarged gall bladder and brownish liver. (c) Haemorrhages of kidney. Photos: Courtesy: Mohammad Noor Amal Azmai (co-author)

Good Aquaculture Practice for tilapia,[100] and more specifically, vaccination may be the choice for prevention and treatment of *Aeromonas* infections. Formalin whole cell inactivated live vaccine was successfully used for the first time in tilapia in 1986, and the relative protection level of the vaccine was 100%, within 2 weeks after inoculation.[101] Since then, many researchers have been engaged in the research of fish vaccine against *Aeromonas* and obtained many achievements. Pridgeon and Klesius[102] prepared live vaccines against different virulent strains of *A. hydrophila*, with 100% protection at 14, 28 and 56 days post-vaccination (dpv). Pridgeon et al.[103] attempted live vaccines against *A. hydrophila*, *E. tarda*, *S. iniae* and *S. agalactiae* in tilapia and catfish. After bacterial challenge, the relative percentage of survival (RPS) of tilapia inoculated at 14 and 28 dpv were 100% and 92%, respectively. Aly et al.[104] developed an inactivated *A. hydrophila* vaccine for tilapia. An effective bivalent inactivated vaccine for tilapia brood stock against *S. agalactiae* and *A. hydrophila* resulted in 73.81% RPS after challenge by *A. hydrophila*.[105] Monir et al.[106] proposed an alternative method to reduce the main infectious diseases of tilapia, namely feed-based vaccination, and conducted experiments with four different forms and control groups of bivalent inactivated vaccines against *S. iniae* and *A. hydrophila* of hybrid red tilapia. The results showed that bivalent vaccines caused significant non-specific and specific immune responses to hybrid red tilapia, and had a high protective effect. This newly developed feed-based bivalent vaccine is an effective and large-scale fish immunization technique in aquaculture.[106] Some researchers developed recombinant fish vaccines to solve the serotype specificity issue.[107] The surface proteins Omp38 and OmpF of *A. hydrophila* were presented as vaccine candidates against *A. hydrophila*.[108] An S-layer protein-based vaccine for tilapia demonstrated a high protection against *A. hydrophila*.[109] Although some recombinant vaccines have been developed, these vaccines induce lower protection than whole-cell killed vaccines under the same conditions.[107]

Therefore, further works on recombinant vaccines should focus not only on optimizing and improving the protective efficacies, but on cost-effectiveness for commercial-scale to enable it as a viable solution to motile aeromonad septicaemia. At present, some studies have found that adding specific plant extracts to feed can prevent and treat some bacterial diseases in fish. Hardi[110] found that when combined extracts of *Boesenbergia pandurata* (BP), *Solanum ferox* (SF) and *Zingiber zerumbet* (ZZ) were added to fish diets, in particular, SF50/ZZ50 (50 mg SF extract/kg feed with 50 mg ZZ/kg feed) had positive effects on the immune system of tilapia in the treatment and prevention of bacterial infection. Adding ZLP (*Ziziphus mauritiana* leaf powder) into the tilapia diet enhanced the immune and antioxidant capacity to effectively control *A. hydrophila* infection of Nile tilapia.[111] Plant extracts carvacrol and cymene at concentrations of 100 or 200 ppm were used as effective oral treatment of experimentally infected *Oreochromis niloticus* with atypical *A. salmonicida*.[68] Kuebutornye used *Bacillus* isolated from tilapia, and Phumkhachorn used bacteriophages to control *A. hydrophila* infections in tilapia (*O. niloticus*).[54,112]

2.1.3 | Francisellosis

Francisella orientalis, formerly known as *F. noatunensis* subsp. *orientalis*,[113,114] has been recognized as one of the most serious pathogens of tilapia (*Oreochromis* spp.) and other fish species such as three-line grunt (*Parapristipoma trilineatum*) and hybrid striped bass

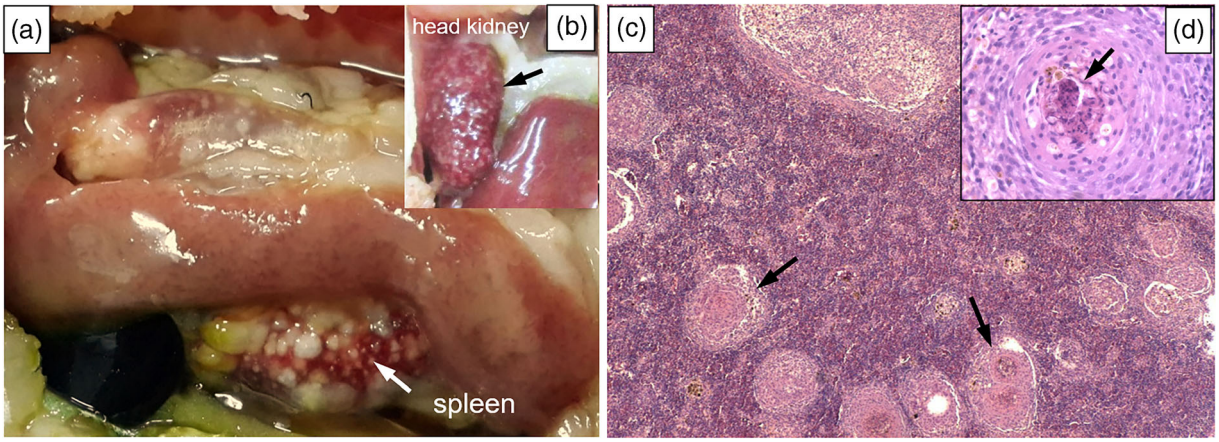

FIGURE 4 Francisellosis in tilapia. (a) Granuloma in head kidney of *F. orientalis* infected tilapia. (b) Same fish: Granuloma in spleen. (c, d) Haematoxylin–Eosin stained histological sections of the spleen of a tilapia from indoor recirculation aquaculture in the Netherlands showing granuloma from a systemic and chronic *Francisella*-infection. (c): 40× magnification. (d): 400× magnification. Pictures (a, b): courtesy Dr H. T. Dong (co-author); (c, d): courtesy Dr O. Haenen (leading author)

(*Morone chrysops* × *M. saxatilis*), both farmed and wild, from various geographical regions worldwide.[115–120] Occurrence of francisellosis in farmed tilapia has been documented in Brazil,[121] China,[122] Costa Rica,[123,124] Indonesia,[125] Taiwan Province of China,[126] Thailand,[127] United States[128] and United Kingdom.[129] Initially considered a *Rickettsia*-like[117,126,130] or *Piscirickettsia*-like organism,[130] the pathogen was later confirmed as a ɣ-Proteobacteria in the family *Francisellaceae*, order *Thiotrichales*.[131]

The typical gross pathological signs of francisellosis in tilapia and other species such as three-lined grunt and hybrid striped bass have been commonly manifested as granulomatosis (Figure 4) causing renomegaly and splenomegaly typically ascribed to multiple whitish nodules with comparable lesions in the gills, muscle or liver.[126,127] Furthermore, pale body coloration, the presence of numerous white granulomas on gills and internal organs including the spleen, liver, kidney and intestine have been noted in tilapia infected with *F. orientalis*.[127] Francisellosis could induce 50%–60% mortality in cultured tilapia which usually occurs in cool season, that is, when water temperature ranges from 23°C to 26°C.[127] Notably, coinfection of *F. orientalis* and the ciliate parasite *Ichthyophthirius multifilis* could lead to more severe mortality compared to the single infection with either *F. orientalis* or *I. multifilis*.[132]

Francisella spp. are strictly aerobic, facultatively intracellular, nonmotile, Gram-negative coccobacilli to pleomorphic spherical measuring 0.1–1.5 μm in size.[131] Members of the genus *Francisella* are fastidious in their requirements for growth on laboratory media and require specific media for in vitro culture. Isolation of *F. orientalis* from the blood, spleen, kidney or granulomatous lesions of infected fish has been successfully attained using enriched blood agar plates supplemented with 0.1% cysteine and 1% glucose, cysteine heart agar with 5% sheep blood (CHAB) or cysteine heart agar with 1% haemoglobin (CHAH) or Thayer–Martin Media,[118,124,131] with optimal growth of *F. orientalis* on these enriched blood agar plates observed at 28–30°C.[124] The addition of polymyxin B (100 μg/mL) with or without ampicillin (50 μg/ml) to selective agars was successfully used for the isolation of *F. orientalis*.[124] Additionally, nucleic acid-based detection methods including conventional polymerase chain reaction (PCR),[113,115,124,127,133] quantitative real-time PCR (qPCR),[133–137] duplex PCR, *in situ* hybridisation,[138] recombinase polymerase amplification (RPA),[139] and loop-mediated isothermal amplification (LAMP)[37] have been applied for the detection of *F. orientalis* in tilapia.

Nile tilapia experimentally infected with *F. orientalis* via immersion challenge exhibited the highest number of bacteria, that is, quantified as *F. orientalis* genome equivalents by qPCR, in their surface mucus at 3 h post-infection. Moreover, at 96 h post-infection, septic fish had marked increases of *F. orientalis* genome equivalents in their gills, anterior and posterior kidney, spleen, liver, heart, gastrointestinal tract and gonads which corresponded with the appearance, size and number of granulomas typical of francisellosis.[140] Homologues of virulence genes associated with the serious, zoonotic pathogen *F. tularensis*, detected in various cold and warm-blooded animals and humans,[141] have also been identified in *F. orientalis* including the intracellular growth locus (IGL; *iglA*, *iglB*, *iglC* and *iglD*) genes associated with the type 6 secretion system present on the *F. tularensis* pathogenicity island.[142] Soto et al.[142] reported that a functional *iglC* gene of *Fno* was crucial for intramacrophage survival, although *iglC* gene played no role in protection from serum killing. The *iglC* gene is by far one of the most extensively studied genes within the *Francisella* pathogenicity island owing to its marked expression during intracellular growth, demonstrating its significance for pathogenicity and virulence.[143] Also, serum complement and host cell mannose receptors have been recognized as vital for internalization of *F. orientalis* in macrophage.[130] Horizontal transmission of *F. orientalis* via the waterborne route has been demonstrated by Soto et al.[140] in Nile tilapia fingerlings under experimental condition.

Additionally, Pradeep et al.[37] documented that apparently healthy red tilapia (*Oreochromis* spp.) broodstock who were asymptomatic

carriers of *F. orientalis* could vertically transmit the pathogen to the fertilized eggs. Evidence of vertical transmission was subsequently confirmed in a controlled laboratory challenge.[144] Therefore, utilization of *F. orientalis* negative tilapia broodstock is an important strategy to prevent vertical transmission of *F. orientalis* to their offspring.

Although commercial vaccines are currently unavailable, there are promising results from research. In 2019, developed *F. noatunensis* subsp. *orientalis (Fno)* whole-cell vaccines were developed for tilapia.[145,146] A whole-cell formalin-inactivated autogenous vaccine was developed using the highly virulent isolate STIR-GUS-F2f7 and the oil-based adjuvant Montanide™ ISA 763A VG showing 100% RPS (relative percentage of survival) rates in red tilapia after i.p. injection with 4.0×10^3 CFU/fish.[145] Shahin et al.[146] compared a 100% RPS giving *Fno* vaccine with inactivated whole-cell injection vaccines of *Fno*, using bacterial strains from various geographical regions in heterologous and homologous infection trials by i.p. injecting nile tilapia. They found RPS values of 65.9%–82.3%, with the highest in homologous trials.[146]

Pulpipat et al.[147] demonstrated recently the efficacy of a formalin-killed *F. orientalis* vaccine in cultured tilapia via intraperitoneal injection. Vaccinated fish experimentally challenged with *F. orientalis* via intraperitoneal injection and immersion at 6 weeks post-vaccination led to production of potent antibodies and relative percent survival (RPS) of 71% and 76%, respectively. Transcripts of proinflammatory cytokines and immune-related genes, including interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNFα), C-X-C motif chemokine ligand 8 (CXCL8) and interleukin-17C (IL-17C), were significantly upregulated after vaccination. Additionally, vaccinated fish had lower bacterial loads in the blood and lower granuloma intensities in the kidney, spleen, liver and gill compared with the unvaccinated fish. Antibiotic administration of in-feed oxytetracycline and florfenicol to naturally and experimentally infected tilapia resulted in lower mortalities[148] suggesting efficacious antibiotic treatment. Furthermore, antibiotic treatment was particularly noted to be effective during the acute stage of infection.[148] Accordingly, in the event of an outbreak, it is prudent to depopulate fish and disinfect the facility with disinfectants that are effective against planktonic and biofilm forms of *F. orientalis*.[149]

2.1.4 | Flavobacteriosis

Flavobacteriosis, and in this case, columnaris disease caused by *F. columnare* (also known as myxobacterial disease, peduncle disease, saddleback, fin rot, cotton wool disease or black patch necrosis) is one of the oldest known diseases of freshwater fish species worldwide.[150,151] The *F. columnare* associated with (or isolated from) tilapia was recently renamed to *F. oreochromis*.[152]

The disease affects various fish species culturing in both cold and warm water, including tilapia (*Oreochromis* spp.).[153–155] The earliest report of columnaris disease in farmed Nile tilapia was documented in Egypt[156] but remained relatively unrecognized until recent reports in Brazil[154] and Thailand.[155,157] The disease affects fish in both hatcheries and grow-out systems, and resulted in 10%–70% cumulative mortality in natural outbreaks.[155] Experimental challenge resulted in variable levels of mortality ranging from 0% to 100% in hybrid red tilapia (*Oreochromis* sp.) fry and juveniles.[157,158] Major gross signs of disease fish were discoloration, fin and skin erosion and gill necrosis[155–157] (Figure 5).

Flavobacterium columnare is a Gram-negative, slender filamentous bacterium. This bacterium produces flexirubin pigment and forms yellow rhizoid colonies on culture media due to the characteristic of gliding motility on solid surface.[150,153] Dong et al.[155] reported that the isolates from tilapia exhibited homologous phenotypic characteristics, but high genetic diversity. Based on the restriction fragment length polymorphism of the 16S rRNA gene (16S-RFLP), a scheme for genetic typing *F. columnare*,[159] the isolates from tilapia were classified into three genomovars (I, II and I/II) with predominance of genomovar II.[155,160] Phylogenetic analysis based on the 16S rRNA suggested that majority of tilapia isolates belong to a unique genetic group.[155,161] Comprehensive genomic comparison of *F. columnare* isolates derived from different host species revealed extensive sequence diversity where the unique strains from tilapia were thought to represent the forthcoming novel taxa or subtaxa in the genus *Flavobacterium*.[162] In 2022, this was confirmed, as many *F. columnare* strains were genetically reclassified by phylogenetic analyses of 16S rRNA and *gyrB* genes, and this resulted in four genetic groups, with proposed names of 4 species: Genogroup 1 = *F. columnare*, Genogroup 2 = *F. covae* sp. nov. (AL-02-36$^{\text{Type strain}}$), Genogroup 3 = *F. davisii* sp. nov. (90-106^{T}), and genogroup 4 = *F. oreochromis* sp. nov (Costa Rica 04-02-TN^{T}), with at least the last species being a tilapia pathogen.[152]

Apart from gross pathological signs, examination of long rod-shaped filamentous bacteria through wet-mount and/or rapid Gram-staining for smeared lesions are useful for presumptive diagnosis of columnaris disease in tilapia. Bacterial isolation was successful using selected media such as Anacker and Ordal's agar (AOA), modified Shield agar (MSA) or tryptone yeast extract salts (TYES) agar supplement with antibiotics either tobramycin or neomycin and polymyxin B.[150,155] Specific PCR,[163,164] LAMP,[165] and *F. columnare*-monoclonal antibodies[166] have been used for rapid diagnosis of *F. columnare* from clinical samples and bacterial culture. Sequencing of 16S rRNA and/or whole genome represents common approach for identification and characterization of this bacteria.[155,161,162]

The tilapia isolates form two different colony morphotypes (rhizoid *vs.* non-rhizoid). The rhizoid morphotype is highly pathogenic while the non-rhizoid morphotype has non- or low pathogenicity.[157,158] Comparative studies of *F. columnare* revealed that the adhesion ability to the gill surface, biofilm formation and the production of capsular polysaccharide are significantly associated with the highly pathogenic strain of *F. columnare*.[157] Like other *F. columnare* infections, the disease in tilapia affects the skin, gills and muscle and is rarely found in the internal organs.[26,155,167] Coinfections of *F. columnare* and other pathogens have been recorded which may contribute to increasing disease severity.[26,158,168,169] Horizontal transmission through waterborne routes have been demonstrated by experimental immersion studies for both Nile tilapia and hybrid red tilapia.[138,156,157] It is unclear whether *F. columnare* transmits vertically. However,

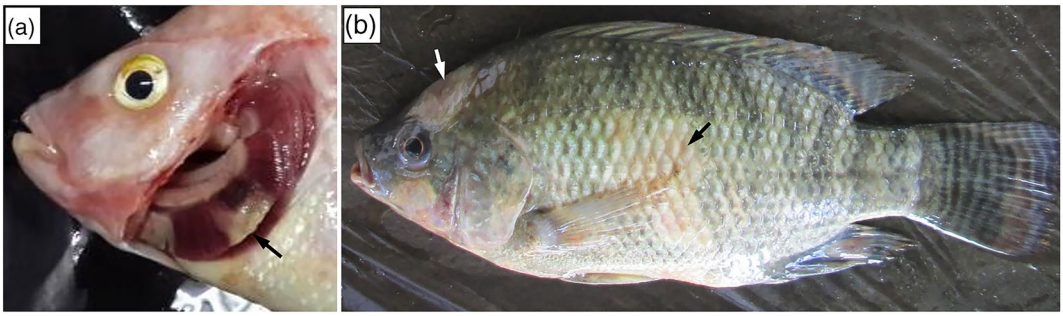

FIGURE 5 Tilapia (*Oreochromis* sp.) infected with *Flavobacterium columnare* (new, proposed name *F. oreochromis*[152]), showing (a) Gill necrosis (arrow), and (b) Superficial skin necrotic lesions all over the body, with deslimed areas (arrows). Pictures courtesy Dr H. T. Dong (co-author)

detection of *F. columnare* in reproductive organs of apparently healthy tilapia broodstock, fertilized eggs and newly hatched fry suggested possible maternal transmission.[165]

Effective antibiotic therapy against flavobacteriosis in tilapia is difficult, as mostly other factors, like stress play a role in the disease. Moreover, findings on antibiotic susceptibility differ. Various fish strains of the salmonid pathogen *F. psychrophilum* were found susceptible to ampicillin, erythromycin, streptomycin, tetracycline, trimethoprim-sulphate, with resistance against neomycin and polymyxin.[170] The oxytetracycline-treated group showed significant reduction in these lesions and the treated fish appeared normal. Use of a probiotic, *Bacillus subtilis* was tested in water and in fish feed as prophylaxis and was effective in amelioration of lesions caused by *F. columnare* in Egyptian freshwater fish.[171] They also stated that oxytetracycline was effective to treat columnaris disease.[171] In an Egyptian Master thesis[172] strains of *F. columnare* were found susceptible to tetracycline, nalidixic acid, trimethoprim, erythromycin, streptomycin and doxycycline with high resistance to neomycin. Studied 20 strains of *F. columnare* of Nile tilapia were tested for *in vitro* susceptibility to amoxicillin, amoxicillin, clavulanic acid, amikacin, cefixime, ciprofloxacin, novobiocin, neomycin, norfloxacin, nitrofurantion, poly mixin B and tetracycline: They found multi-resistance in >18/20 strains.[173] A paper on the development of genetic-resistant strains of Nile tilapia against *F. columnare* presented promising results as a longer-term alternative to antibiotic treatment.[174]

2.1.5 | Vibriosis

Fish vibriosis is referred to as a systemic infection caused by a number of *Vibrio* spp., including *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum* and *V. vulnificus*.[175,176] The genus includes Gram-negative, oxidase-positive rod-form bacteria with polar flagella, ubiquitous in marine and estuarine ecosystems. Although vibriosis has multiple clinical manifestations, depending on the host and bacterial species, in all cases the acute form is a septicaemia that can lead to death, mainly in immunocompromised hosts.[177–179]

Vibriosis is commonly associated with brackish and marine aquaculture, and therefore, tilapia cultured in these environments are

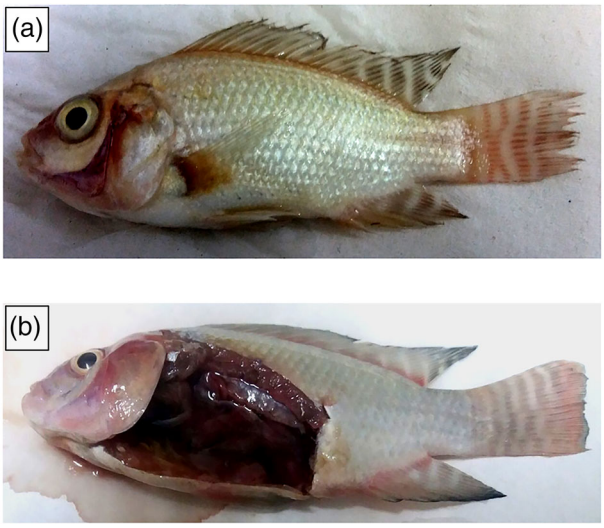

FIGURE 6 Vibriosis caused by *Vibrio vulnificus* pathovar *piscis* in Nile tilapia *(Oreochromis niloticus)*. Images correspond to moribund tilapia after being challenged by immersion. Clinical signs mirror those of the natural disease, a septicaemia characterized by haemorrhages in (a) the mouth, head and fins and in (b) the intestine, abdominal cavity and muscle. Pictures courtesy of Dr B. Fouz (co-author)

susceptible. Although sporadic cases of some and related *Vibrio* spp. have been isolated from diseased tilapia (*V. parahaemolyticus* or *Photobacterium damselae* subsp. *damselae*, *Phdd* [formerly *V. damsela*]),[180,181] *V. vulnificus* is the major pathogenic *Vibrio* spp.[175,179] It is important to highlight that, within this species, only pathovar *piscis* (pv. *piscis*; formerly Biotype 2) is considered as fish pathogenic,[182] and the disease is known as warm-water vibriosis (WWV).[179,183]

Pv. *piscis* strains possess a conjugative fish virulence plasmid (pFv) absent in other strains of the species, and group in several clades/serovars, Ser E and the recently described Ser T proving zoonotic potential.[184,185] Different authors have reported *V. vulnificus* as the causative agent of infectious episodes/outbreaks in Japan,[186] Taiwan Province of China,[187] Bangladesh,[188] India,[189] or eastern Mediterranean farms.[185,190] In all cases, the bacterium was mostly isolated from the diseased fish blood, kidney, liver, spleen, and brain. Diseased fish showed dark coloration, external haemorrhagic areas, exophthalmia and skin ulcers. Internally, a pale liver

with haemorrhagic lesions, oedematous brain or splenomegaly were observed. Moreover, some authors have experimentally induced infections and disease in Nile tilapia after challenges with pv. *piscis* strains (specifically serovars/clades E, A and the new one described T)[185,191–193] (Figure 6).

Simple and rapid methods to identify *Vibrio* spp. causing disease in cultured fish are essential in order to take fast preventive and curative decisions. Individuals with clinical signs of septicaemia compatible with vibriosis should be analysed microbiologically by bacterial isolation, using a general medium such as TSA-1 (1% NaCl concentration), together with thiosulfate-citrate-bile salts-sucrose (TCBS) and/or *V. vulnificus* medium (VVM) agar.[179] However, since *V. vulnificus* is recovered as a pure culture from diseased tilapia, also media, like sheep blood agar plates may be used to isolate *V. vulnificus*. Pure cultures could be tentatively identified to species level using the commercial phenotypic API 20E system (bioMérieux). Afterwards, PCR- or protein-based (like MALDI-TOF) methods should be used to confirm species[194] or subspecies identification.[195]

In the case of *V. vulnificus*, PCR targeting *vvhA*, *fpcrp* and *seq61* genes allows to identify strains to species, pv. *piscis*, and zoonotic Ser E, respectively.[196] *V. vulnificus* strains could be subtyped for public health hazard by a PCR that amplifies a variable region located within the gene *pilF*.[197] Although *V. vulnificus* is generally sensitive to most antimicrobials permitted on fish farms in the EU and the USA, an antibiogram must be performed to select the most effective antibiotic to start the treatment as soon as possible.

Fish pathogenic *Vibrio* spp. exhibit different virulence factors such as capsular polysaccharides, adhesive factors, cytotoxins, lipopolysaccharides and flagella.[198] In bacterial pathogenesis, the adherence to the host surface is considered a critical step and can be favoured by flagella, capsules or loose slime. Resistance to phagocytosis and complement-mediated killing together with efficient iron acquisition systems allow bacteria to colonize the host and multiply. Moreover, toxins and exoenzymes are responsible for host lesions. *V. vulnificus* pv. *piscis* initially colonizes the gill/skin mucus, being protease VvpE and the capsule involved in this process and invasion is favoured by local damage and destruction of phagocytes by excreted toxins (mainly toxin RtxA1).[179] When bacteria enter the bloodstream of the fish, they are able to survive, proliferate, and therefore, induce the fatal septicaemia. Under iron restriction, the bacterium over-expresses the haemolysin VvhA and RtxA1 toxins as well as the outer membrane proteins Fpcrp (fish phagocytosis and complement resistance protein) and Ftbp (fish transferrin-binding protein), which constitute a 'survival in fish blood kit',[199] encoded by plasmidic genes. pFv and closely related plasmids have probably been acquired in fish farms by different clones which have been amplified after successive outbreaks.[182,185]

Vibriosis is a water-borne infection, meaning that the etiological agent uses the water column as its natural transmission medium. In fact, experiments with eels and tilapia artificially infected with pv. *piscis* by different routes revealed that immersion in water followed by ingestion is the primary route for the transmission of WWV.[183,185,191–193] The virulence of the strain is strongly dependent on the water salinity (maximum at 0.5%–1.5%, depending on the serovar) and temperature (maximum at 28°C).[183,193] Similar observations were reported in transmission of vibriosis caused *by Phdd*,[200] another potential pathogen for Nile tilapia. Therefore, since *Vibrio* spp. can be transmitted horizontally, either from open lesions or as secretion in the faeces of infected fish and carriers, pathogenic strains can be easily transferred among fish in the nearby area using water as transport medium.

Finally, efficient preventive measures in tilapia farms against *V. vulnificus* pv. *piscis* are considered necessary, including both manipulation of physicochemical parameters (use of freshwater and temperature below 26°C) and specific vaccination. In fact, a patented vaccine called *Vulnivaccine* has proven to be highly effective against WWV at eel farms.[179]

2.2 | Emerging bacterial diseases of concern

2.2.1 | Edwardsiellosis

Edwardsiella is well known as a genus hosting severe pathogenic bacteria affecting global aquaculture with various fish species, including tilapia.[201–203] The genus comprises Gram-negative, rod-shaped bacteria belonging to the family Enterobacteriaceae and the order Enterobacteriales.[204] The bacterium is a facultative intracellular pathogen that can survive inside fish phagocytes such as macrophages and neutrophils.[205,206] Since recently, the genus comprises five species, and three of them have been reported to infect and cause mortality in Tilapia including *E. ictaluri*, *E. tarda* and *E. anguillarum*.[201,202,203,207,208]

2.2.2 | *Edwardsiella ictaluri*

Edwardsiella ictaluri, is the causative pathogen of enteric septicemia in channel catfish[209] and freshwater catfish species *Pangasianodon hypophthalmus*.[210] It now has a less restricted host range causing disease in various catfish species[167,211–215] and non-catfish species such as zebrafish, and wild ayu in Japan.[216–218] Natural disease outbreaks reported in several fish species showed that this pathogen produced 40%–90% mortality,[207,219] while experimental infection resulted in up to 100% mortality,[207,216,220,221] indicating that *E. ictaluri* is a pathogenic bacteria of multiple freshwater fish species. The first detection of *E. ictaluri* in tilapia was in the Western hemisphere.[203] Natural disease cases of *E. ictaluri* in red tilapia raised in open floating cages were first detected in Southeast Asia in 2016,[207] and truly have become an emerging disease, widespread to a large region in Vietnam, with high risk of further national and international spread.[208] *E. ictaluri*-affected tilapia did not exhibit recognizable external signs, causing misleading presumptive disease diagnostics and untimely treatment efforts under active surveillance. Early diagnostic screening and biosecurity measures are highly recommended to prevent for transboundary spread and negative impact of this pathogen.

Gross signs with white spots appearing on the spleen and head kidney are critical features for the first detection (Figure 7). In

FIGURE 7 Diseased tilapia by *Edwardsiella ictaluri* from an experimentally induced infection. Courtesy Dr Truong Dinh Hoai (co-author)

addition, pale gills due to anaemia and the liver due to the reduced fat reserve in the liver are also helpful for screening affected fish.[203,207,208] Wet-mount with gram staining with the presence of Gram-negative, rod shape, the intracellular bacterium could be the first step to confirm the presence of *E. ictaluri* from fresh fish tissue such as kidney, spleen. A distinguishing test should be performed between francisellosis through *F. noatunensis* and *F. orientalis*, and edwardsiellosis through *E. ictaluri* because the clinical signs of visceral white spots had always been linked to these diseases. PCRs should be developed, but currently, the wet-mount technique could help to distinguish them, since *F. noatunensis* and *F. orientalis* have a different shape as coccobacillus bacteria.[133] *E. ictaluri* grows as typical whitish pinpoint colonies on culture media. Biochemical characteristics of *E. ictaluri* from tilapia were identical to a strain isolated from catfish, except for the Voges–Proskauer test which was variable among isolates.[207,208,210] Thus, the combination of sequencing of 16S-rRNA, house-keeping genes such as *gyr*B for phylogenetic analysis[222,223] and specific PCR-based assay[224] were accurate for identifying *E. ictaluri*. To discriminate *E. ictaluri* from tilapia from other different hosts and geographic origins, parallel and combined techniques such as rep-PCR, 16S, *gyr*B and sequencing plasmid or whole-genome has been recommended.[225,226]

Regarding pathogenesis, varieties of virulence factors for *E. ictaluri* have been identified, such as extracellular capsular polysaccharide, fimbriae-like structures, chondroitinase, lipopolysaccharides O side chain and outer membrane protein. Other known pathogenicity islands such as the type III secretion system (T3SS) gene *esrC*, the putative T3SS effector *eseI* and its chaperone *escD*, the type IV secretion system (T4SS) gene *virD4*, the type VI secretion system (T6SS) gene *evpC* and *ureA-C* of the urease operon have been determined also as the virulence factors of this pathogen. However, the distribution of virulent factors varied between species.[214] The screening of six virulence genes from *E. ictaluri* isolated from tilapia outbreaks revealed that the presence of *esrC*, *evpC* and *ureA-C* genes were in all strains, but they did not have *virD*, *eseI* and *escD* genes which were present in strains of channel catfish.[208,225] The completed pathogenicity test conducted by the latest study from outbreaks in southeast Asia showed that the lethal dose LD50 of the Asian strain is very low, $<10^2$ CFU/fish, to kill 50% of the tilapia population. The results suggested that new, hyper virulent *E. ictaluri* strains are circulating and spreading in this region.[208] Thus, the mechanism and virulent gen distribution of *E. ictaluri* strains infecting tilapia need to be clarified in further studies.

The pathogen could be transferred horizontally between fish and spreads by the water flow. The disease outbreak has existed in both freshwater ponds[203,208] and in floating cage farms on the rivers and reservoirs.[207,208] However, the data from the survey showed that the open tilapia culture system has a higher risk for the disease than culture ponds do.[208] Disease outbreaks have occurred from fingerling fish to marketable size,[203,207] but fish less than 350 g were more sensitive to this pathogen. The mortality rate from the outbreaks ranged from 30% to 65%.[207,208] *E. ictaluri* can attach and penetrate host mucosal membranes rapidly and establish a systemic infection. It is also a facultative intracellular pathogen, which may survive inside phagocytic cells, which could be a mechanism of dissemination. This characteristic plays a vital role in the rapid spread of the disease. The disease appeared in the temperature range of 23–29°C. The detection of *E. ictaluri* associated with disease outbreaks from two different continents (America and Asia) highlights the risk of transboundary spread and potential impact on the tilapia industry.

Although the serious fish disease caused by *E. ictaluri* was first detected in farmed tilapia in Asia only 5 years ago, the isolated *E. ictaluri* show already high levels of antibiotic resistance.[208] Nevertheless, alternatives to antibiotics should be further explored to tackle this emerging, highly pathogenic bacterium. Current studies investigate the presence of homologous strains from outbreaks. Thus, an autogenous vaccine might be the best option to combat this emerging disease in the present time before a better vaccine candidate for a wider region is discovered.[207,208]

2.2.3 | *Edwardsiella tarda*

Edwardsiella tarda is a Gram-negative, motile, short, rod-shaped bacterium (1 μm × 2–3 μm) of the family Enterobacteriaceae. It is a severe pathogen for a variety of fish.[168,227] Principally, *E. tarda* have been isolated from different aquatic water environments and affected fish are common intestinal carriers of this pathogen, thereby resulting in possible contamination of fish carcasses during fish processing. They have been found in the intestines of infected humans, after consumption of contaminated fish. This pathogen is often responsible for septicaemic fish disease, causing mass mortalities (up to 70%) and high economic losses in fish farms of freshwater and marine fish in many countries.[228,229] Tilapia is one of the susceptible fish to *E. tarda* and disease cases have been reported in several countries[201,230] in Nile tilapia (*O. niloticus)* and red tilapia.[231,232]

The clinical, gross and microscopic changes caused by *E. tarda* have been relatively well characterized for a range of different fish species, especially catfish. For tilapia, gross disease signs include corneal opacity and loss of the eyes, reddening of the anal papilla and marked pallor of the gills (Figure 8). Internally, the kidney and liver may be pale and seeded with white nodules. The swim bladder and kidney existed of flocculent material, with congestion and

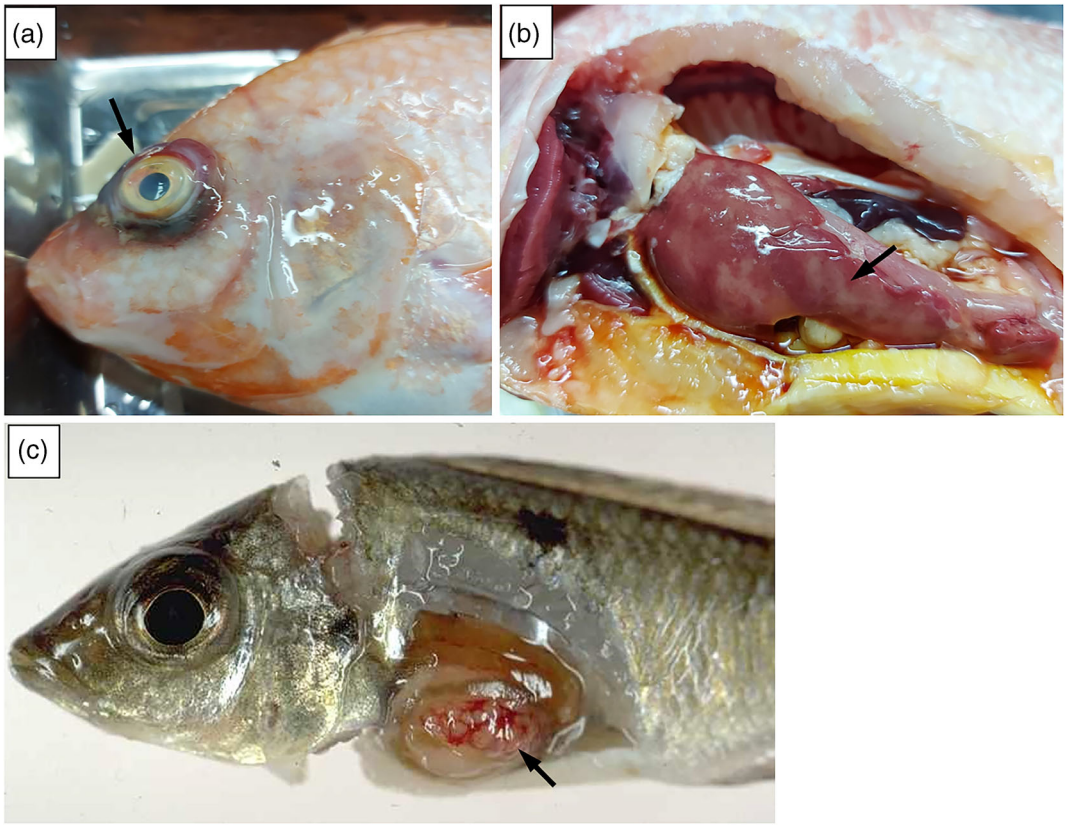

FIGURE 8 Edwardsiellosis by *Edwardsiella tarda* in tilapia or cichlids. (a) Corneal opacity, inflammation and loss of the eyes. (b) Pale organs with white nodules. (c) Cichlid from a zoo with a systemic *E. tarda* infection: anorexia and bacterial nodules (arrow) can be seen. Pictures courtesy (a, b): Dr H. T. Dong (co-author); (c) Dr O. Haenen (leading author)

haemorrhage on the intestine.[233] Microscopic lesions in the brain and lymphoid organs of tilapia were also demonstrated.[233,234]

Edwardsiella tarda is usually identified based on its unique biochemical characteristics after isolation on brain-heart infusion agar or tryptone soya agar from infected fish. PCR-based detection with *gyrB* gene was developed for *E. tarda* from fish species and successfully modified to nested PCR and applied to detect affected tilapia using tissues samples.[233,235] Since 2013, *E. tarda* has been subdivided into three genetically distinct species regarding infecting fish, *E. tarda*, *E. piscicida* from various fish,[236] and *E. anguillarum* (from eel),[237] based on several identification techniques including sequencing analysis of *gyrB* and *sodB* genes, nested PCR, rep-PCR and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF), proven effective for *E. tarda* identification.[238,239] However, the above techniques have almost not yet been used for tilapia isolates of *E. tarda*, and further assessment needs to be done. Also, we should realize, that published casus with identifications of *E. tarda* from tilapia from before 2013 may have represented causes of *E. anguillarum*, or perhaps of *E. piscicida*.

Virulent factors of *E. tarda* were well characterized in fish species including type III secretion systems (TTSS apparatus protein *Esa*B-V, TTSS chaperone protein *Ecs*A-C, TTSS effector protein *Ese*B-G and TTSS regulator protein *Esr*A-C), type IV secretion systems (*Evp*A-P) and other proteins including autotransporter protein (AidA), α-hemolysin-modulator like protein (HhaEt), hemolysin A, B (EthA, EthB), DNA-binding transcriptional regulator and sensor protein QseC (QseB, QseC), component regulator and sensor proteins (PhoP and PhoQ).[229] In tilapia, the role of regulator FucP regulation of the T3SS in *E. tarda* has been demonstrated to contribute to pathogenesis.[240] *E. tarda* isolated from diseased Southern flounder (*Paralichthys lethostigma*) has been demonstrated to be virulent to Nile tilapia.[241]

Edwardsiella tarda could be transferred horizontally between fish via the faecal–oral route. The wide range of hosts such as invertebrates, amphibians, reptiles, birds, a variety of fish, mammals and humans indicated that it has a wide geographical distribution and is an important pathogen in terms of public health as an epizootic and zoonotic bacterium. In aquaculture, this pathogen commonly exists in the environment, pond water and sediment. High temperature, poor water quality and high organic load increase the risks of infection.[242] In addition, cross-contamination may occur during manipulation of fish skin, handling and preparing fish seed, or in integrated farming where tilapia are reared in conjunction with other animals, or the cross-infection between other fish species and tilapia in the polyculture system.[242,243]

A variety of chemicals have been tested and demonstrated to be effective disinfectants against this pathogen, including ethyl alcohol (30%, 50% or 70%), benzyl-4-chlorophenol/phenylphenol (1%), sodium hypochlorite (50, 100, 200 or 50,000 mg/L), n-alkyl dimethyl benzyl

ammonium chloride (1:256), povidone–iodine (50 or 100 mg/L), glutaraldehyde (2%) and potassium peroxy–monosulphate/sodium chloride (1%). However, using chemicals may raise concerns about toxicity to the environment, costs and human health risks, and is impractical in a large volume of water or cage culture in rivers or lakes.[242]

Antibiotics have been used popularly for the treatment of the disease. However, overuse of antibiotics has accounted for a major antibiotic resistance of *E. tarda* in tilapia.[232,244,245] Alternatives to chemical and antibiotic use have been investigated against *E. tarda* in tilapia, including use of natural compounds (carvacrol and cymene),[246] glucose, polysaccharides, yeast oligosaccharide,[247–249] essential oils,[250] ascorbic acid, α-tocopheryl acetate and selenium,[251] kugija *Lycium chinense*[252] and probiotics.[253–255] Another affordable alternative to antibiotics is the use of vaccines. Several developed vaccine candidates were investigated, including the vaccines *E. tarda* ghost,[256] live cells of *E. tarda*[257] and a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) vaccine from *E. ictaluri* against *E. tarda*.[258]

2.2.4 | *Edwardsiella anguillarum*

Edwardsiella anguillarum shares similar characteristics to other *Edwardsiella* isolates, such as the growth capability under anaerobic conditions; however, its non-motile nature differentiated it from other groups.[259] *E. anguillarum* was the last group to be distinguished from the *E. tarda* group and demonstrated virulence to a variety of fish species, including tilapia in Costa Rica and Korea.[202,260]

2.2.5 | *Aeromonas schubertii*

Aeromonas schubertii is a Gram-negative, short rod-shaped bacterium with a single polar flagellum required for its motility.[261] *A. schubertii* infection-causing multi-organs necrosis is considered an emerging tilapia disease.[261,262] Diseased fish usually showed haemorrhages in the caudal, pectoral and dorsal fins. Internally, affected fish exhibited visceral white spots in internal organs (i.e. liver, kidney and kidney),[261] similar to clinical signs caused by *F. orientalis* or *E. ictaluri* infection.

Natural disease outbreaks in both farmed and wild Nile tilapia were reported in China,[261,262] after its emergence in snakehead fish in 2012.[263–265] Although there is no evidence of disease outbreak in tilapia in other countries, active transferring live tilapia for aquaculture highlights a potential risk of its transboundary spread and broader distribution. Increased awareness and active surveillance are required to gain a better understanding of disease prevalence and impact on tilapia farming countries that have relied on imported tilapia stocks.

Presumptive diagnosis is based on observing visceral white necrotic foci and the presence of short rod-shaped bacteria in smeared tissue stained with Diff-Quick.[261] Previous studies employed trypticase soy agar supplemented with 5% sheep blood[261] or Luria–Bertani (LB) agar[262] for bacterial isolation. An approached combination of phenotypic tests, sequencing of 16S rRNA and several housekeeping genes (e.g. *gyrB*, *rpoB*, *ela* and *dnaJ*) has been used for bacterial identification.[261,262] Recently, Liu et al.[40] reported a highly sensitive TaqMan MGB probe fluorescence real-time quantitative PCR for detecting and quantifying *A. schubertii* from snakehead fish. This method might be helpful for early screening of an infection in tilapia.

Experimental infection revealed that *A. schubertii* was capable to induce disease and acute fish mortalities by both intraperitoneal and intramuscular injection. In contrast, immersion and oral challenges have resulted in no or low mortalities.[262] Zebrafish is a susceptible model fish to study the disease pathogenesis of this bacterium.[261] Histopathological changes described in diseased fish include vacuolization in the liver, haemorrhage in the spleen, and swelling capillaries in the brain. Necrotic lesions filled with a large number of short rod-shaped bacteria were also found in the liver, spleen and kidney.[261,262]

Little is known about the transmission of *A. schubertii* in tilapia. Ren et al.[262] suggested that the damages on the body surface and/or digestive tract might be natural routes of *A. schubertii* infection.

2.3 | Other bacterial diseases

2.3.1 | Lactococcosis (*Lactococcus garvieae*)

Lactococcus garvieae is a facultatively anaerobic, non-motile, non-spore-forming, Gram-positive, ovoid cocci bacteria belonging to the family Streptococcaceae. *L. garvieae* is a significant pathogen of both freshwater and marine aquaculture species, such as rainbow trout (*Oncorhynchus mykiss*), yellowtail (*Seriola quinqueradiata*)[266–268] and tilapia (*Oreochromis* spp.).[269]

In tilapia, *L. garvieae* infections were reported as an emerging disease during the last decade in several countries such as Egypt, Zambia, Brazil, and Singapore.[269–272] The experimental challenge of tilapia showed that the infected fish exhibited ocular opacity, exophthalmia, haemorrhages and cataract, skin erosion and scale detachment.[270,271,273] Lamellar congestion with necrosis of respiratory epithelium of primary and secondary gill lamellae, mild fatty degeneration of hepatocytes with multiple cell necrosis, sinusoidal congestion and necrosis in the spleen has been reported.[270]

To date, the studies on *L. garvieae* infection in tilapia focused on isolation, identification and confirmation of suspicion of the disease.[269,271,273] Further studies should investigate the prevalence of this pathogen in tilapia, the risk factors and geographical distribution of this pathogen, as well as its pathogenesis. On the other hand, comparative analysis of *L. garvieae* strains from different fish hosts may shed light on the evolution of this bacterium in tilapia.

2.3.2 | Aerococcosis (*Aerococcus viridans*)

Aerococcus viridans is a Gram-positive coccoid, order Lactobacillales, phylum Firmicutes. It is facultatively anaerobic and forms tetrads and pairs. The bacterium does not grow well on agar. *A. viridans* causes greening (alpha haemolysis) on rabbit or horse blood agar. The Gram-positive tetrads (four bacteria together) are visible by microscopy.

Also, the co-agglutination technique of Saxegaard and Håstein[274] or the API-Zym may be used for diagnosis. For better understanding of this disease, further investigation on its prevalence and disease pathogenesis in tilapia are recommended.

In aquaculture, *A. viridans* var. *homari* is known to cause gaffkemia in farmed European lobster (*Homarus gammarus*) and American lobster (*H. americanus*).[275,276] Ke et al.[277] described for the first time a tilapia disease outbreak in 2010 caused by *A. viridans* in China, with a loss of 30%–40%. The diseased fish showed congested gills and abdomen, a swollen gall bladder and a severe diffusion in the liver. *A. viridans* infections have been subsequently reported in Indonesian,[278] and in Egyptian tilapia farms,[279,280] always in combination with other bacteria. In Indonesia, the bacterium was isolated in a screening of water from a tilapia pond and in faeces of tilapia, and was identified by biochemistry.[278] In Egypt, the bacterium was isolated as one of 17 in a multibacterial infection of wild caught tilapia from the Nile River,[279] and it was isolated from diseased tilapia from two tilapia farms, in combination with *Enterococcus faecalis*,[280] and the *A. viridans* were identified by molecular methods, like 16SrRNA typing.

2.3.3 | Pseudomonasis

Pseudomonas spp. are aerobic motile Gram-negative rods and are representatives of the order Pseudomonadales.[281] Most *Pseudomonas* spp. are non-pathogenic, but some cause diseases in fish. *Ps. anguilliseptica* is the most pathogenic species, especially to Japanese and European eel, in which it may cause red spot disease or 'Sekiten byo'.[282–284] It has also been isolated from diseased tilapia with *Ps. fluorescens*,[7] and together with *Ps. fluorescens*, *Ps. putida* and *Ps. aeruginosa*.[285] The diseased tilapia showed clinical signs of pseudomonas septicaemia, including reddening of the whole body, abdominal swelling, cloudiness of eyes, loosening scales and congested gills.[285] In another study, *Ps. anguilliseptica* caused disease in Nile tilapia, showing anorexia, darkening, petechial haemorrhage on the body and at the base of fins, loose scales, eroded and erected fins, with some fish showing slight abdominal distension, exophthalmia and pale gills. At post-mortem enlarged kidneys and spleen were seen.[286]

Pseudomonas fluorescens is more often described as an opportunistic pathogen of tilapia (*Oreochromis* spp.) especially under stressful environmental circumstances.[7,230,287–290] Miyazaki et al.[291] described an outbreak of *Ps. fluorescens* in Nile tilapia in Japan. The systemically infected fish showed exophthalmia, darkening, spotty or nodular lesions in the liver, spleen, kidney and gills, and an inflamed swim-bladder. By histopathology, abscess formation in eyes, spleen and swim-bladder and focal necrosis in the liver, gills and kidney were seen in some of the diseased fish. Some other fish showed granuloma formation in all infected lesions.

Several disease cases in cultured tilapia (*O. niloticus)* associated with other *Pseudomonas* spp. were also reported, including on *Ps. aeruginosa*. The tilapia showed darkening of the body, loss of scales, tail rot and congestion of all internal organs.[292] *Pseudomonas aeruginosa* is however not considered to be a primary pathogen for tilapia.

Pseudomonas spp. are found in the aquatic and terrestrial environment at a global level. Although *Pseudomonas* infections occur globally, the *Ps. fluorescens* cases were described in Japan,[230] Philippines,[287] Kingdom of Saudi Arabia,[288] Egypt[289] and Guatemala.[290] *Pseudomonas mosselii* was described as a fish pathogen of Mozambique tilapia (*O. mossambicus*) in Mexico.[57] The disease is transmitted horizontally, via water, gear and by direct fish-to-fish contact.

Pseudomonas fluorescens produces fluorescein. After inoculation of blood or TSA agar, or *Pseudomonas* F agar at 22–28°C, the cream/white fluorescent colonies will appear. Apart from biochemical identification, API 20E or API 20NE may be used,[281] or molecular- or protein-based diagnostic methods. More research is needed, like screenings and artificially induced infections studies, to estimate the real impact of *Pseudomonas* infections in tilapia culture.

Regarding therapy of pseudomonasis, in general, an antibiogram is best to test the susceptibility of the isolate. *Ps. anguilliseptica* from Nile tilapia was found susceptible to ciprofloxacin, erythromycin, gentamycin, oxytetracycline, streptomycin and trimethoprim and sulphamethoxazole.[286] Additionally, they found the bacterium sensitive to methanolic extracts of *Anabaena wisconsinense* and *Oscillatoria curviceps* (blue-green algae or cyanobacteria), and ciprofloxacin and a methanolic extract of *Anabaena wisconsinense* were highly effective in the experimental treatment of pseudomonas septicemia at a dose of 10 mg per kg body weight, after i.p. injection.[286] In another study, lime oil nano-emulsion was tested *in vitro* and *in vivo* against *Ps. aeruginosa* infection in *O. mossambicus*, with good results.[293]

2.3.4 | Mycobacteriosis (*Mycobacterium marinum*)

Mycobacterium marinum is one of the fish mycobacteria, Gram-positive, acid–alcohol-fast, non-motile, non-spore forming rods which may cause stress-induced chronic and lethal 'fish tuberculosis' in warmwater fish, including in tilapia all over the world, in warmwater fish from freshwater, brackish and marine waters.[294,295] Sonda-Santos & Lara-Flores[296] and Lara-Flores et al.[297] reported disease and significant mortality of tilapia (*O. niloticus*) in Mexico through *M. marinum*. Skin discoloration, non-appetite, lethargy, abnormal swimming, cutaneous ulcerations or erosions, ascites, reduced growth, exophthalmia, grey or white nodules (granuloma) in internal organs, and hypertrophy of spleen, kidney and liver are signs of the disease by *M. marinum* in warmwater fish.[295] Also in indoor warm recirculation systems of fish culture *M. marinum* may occur, and clinical signs may only be noted after weeks, whereas internal disease already caused granuloma in organs.[298] As a consequence, fish may show mortality, morbidity and, also in case of subclinical infection, decreased feed uptake and growth rates, and is subsequently less marketable.[299] Granulomatous melano-macrophage centres have been described in Nile tilapia in its spleen.[300]

Diagnosis of mycobacteriosis starts with making a fresh smear of the inside of fish organs like liver, preferably taken at the site of nodules or granuloma, fixing the smear 3× through a flame, and staining the smears Ziehl–Neelsen, after which the smear is read by light

microscopy with a 100× (oil immersion) objective lens for presence of pink, rod-form bacteria, a sign of the acid-fast mycobacteria.

Identification of mycobacteria in fish was traditionally done based on time-consuming isolation (weeks, to max 2 months of incubation to declare a mycobacterial isolation negative) and on biochemical methods. Dong[301] however isolated the *M. marinum* within days from betta fish, *Betta splendens*. Currently, fast and accurate molecular methods are used for identification of the disease and phylogenetic studies.[297,302] Therapy of infected fish requires months of costly antibiotic treatments, and therefore this is not applied for edible fish, also, because high concentrations of residues of antibiotic will accumulate in the fish, which is then not marketable for consumption.[295] There is no vaccine available for *M. marinum*. The transmission of *M. marinum* from fish to fish is not yet clear, and is at least horizontal, via oral uptake of infected dead fish, contact with infected fish skin or through gills.[303]

Mycobacterium marinum is known as a potential contact-zoonotic bacterium, causing 'swimming pool granuloma', 'fish tank granuloma', 'fish handlers/fanciers disease' or 'fish TB' after entry in the skin of humans through injuries for instance.[304] It is not a food zoonosis, as the bacterium often does not grow at 37°C or above, although there are exceptions.[305] As hospitals incubate at 37°C or above, the diagnosis may be missed.[298]

2.3.5 | Epitheliocystis (*Chlamydia* spp.)

Epitheliocystis is a fish disease caused by obligate intracellular bacteria (most of them *Chlamydia*).[306] The disease is characterized by enlarged infected epithelial cells of mostly the gills and skin, which can be seen as tiny white cysts in the gill or skin epithelium. The disease has been reported in over 90 fish species, freshwater, marine and in cold to tropical areas. Characteristic is the presence of a basophilic inclusion in the cytoplasm of an enlarged cell. Severe infection of the gills results in inflammation and respiratory distress.

Although the disease epitheliocystis is widespread, the causative agents in most species of fish so far found are unique, and therefore isolates appear to be very host species specific. *Chlamydia*-like organisms (CLOs) have been the main agents related to this disease.[306] Epitheliocystis has been diagnosed in most regions worldwide in saltwater and freshwater fish. The specific agents causing epitheliocystis, however, appear more regionally restricted.[307] In Brazil, histologically epitheliocystis was found in rare cases in cultured Nile tilapia.[308,309]

Individual cysts from skin and gills up to 400 μm can be seen in wet mounts of gill clippings. Histologically cysts are seen as basophilic inclusions in infected epithelial cells, with a thickened membrane. Sometimes a host response is seen, as a cell proliferation, which even worsens the respiratory inefficiency of the affected gills. The pleomorphic development cycle of epitheliocystis in organisms obtained from *Tilapia mossambica* and *T. aurea* × *T. nilotica*, and the connection between epitheliocystis organisms and known chlamydial organisms of (in)vertebrates are discussed.[310] Epitheliocystis may be confirmed by molecular methods, like amplification of the 16S rRNA gene and sequencing.[311]

More research is needed, like screenings, to judge the real impact of *Chlamydia* infections in tilapia culture. Because there is no established way to culture *Chlamydia* in most fish disease labs, there are hardly data on host range or ways of transmission. At least there is horizontal transmission, from fish to fish, or via water, fish gear and so forth.[312] For this pathogen however also vertical transmission via eggs may be the case, since genomic presence of the pathogen in pre-hatched eggs, and in subsequent generations of barramundi suggested this.[313] Treatment of epitheliocystis with antibiotics is not possible, since it is caused by an intercellular bacterium. Prevention is through good farming management, at least by keeping the environmental factors optimal.[100]

2.3.6 | Nocardiosis (*Nocardia* spp.)

Nocardia is a genus of Gram-positive rod-shaped bacteria of the Order Mycobacteriales, Family *Nocardiaceae*, which show a weak Gram-staining, and are catalase-positive.

Labrie et al.[314] described cases of nocardiosis in freshwater tilapia (*Oreochromis* spp.). In general, fish with nocardiosis may show lethargy, multiple skin ulcers, and red spots. Brownish or haemorrhagic gills, abscess inside the operculum, a greyish or haemorrhagic liver with white nodules, fibromatosis in the abdominal cavity, spleen necrosis associated with the presence of macroscopic white nodules, ascites, haemorrhagic brain and swollen kidney often associated with the presence of white nodules may be seen. On-farm mortality is mostly chronic and may in cases reach 30%.[314]

Nocardiosis in fish is caused by *N. asteroides* and *N. seriolae*, and results in septicaemia in many marine species with serious mortality in some.[315] *Nocardia* in tilapia has been reported in large (>100–600 g) freshwater tilapia in Indonesia,[314] were it could be isolated from the skin and gills, brain, spleen and liver.

Isolation of the pathogen can be accomplished by taking samples from fresh lesions and culture them on nutrient-rich media, like Eugon agar, for *N. seriolae*. Colonies may appear matt to velvety and dry, with a granular surface, irregularly shaped edges, and are light brown. Impression prints represent a fast and reliable method to demonstrate the presence of *Nocardia* sp.[314] Histopathology may also be used, showing typical granuloma.[314] PCR can be used to confirm the identity up to species,[314,316,317] while LAMP (loop-mediated isothermal amplification) can be used as well for detection of *N. seriolae*.[318]

Nocardia asteroides can be found in soil, but can also be found in lake and marine sediments, like scum-activated sludge.[319] It can be transmitted via fresh fish feeds to a fish population, and has a horizontal transmission.

As nocardiosis is a chronic disease, which is often discovered in a late stage only, months of antibiotic treatment would be needed. This is costly and non-effective, and implies risk of AMR-development. Therefore, prevention through good husbandry and good management practices is the best approach for nocardial infections.[100,320] One of the aspects is to avoid the use of uncooked fish feeds (live, raw or frozen) when rearing fish, as these may transmit the pathogen.

Diagnosis of nocardiosis is not easy, as special media are necessary, and more research should focus on artificially induced infections, to estimate the real impact of *Nocardia* on tilapia culture. Thereby, the possibility, that nocardiosis may be zoonotic should be considered, and therefore prevented for, through good hygiene.

2.4 | Zoonotic potential of tilapia bacterial pathogens

Tilapia is cultured in relatively warm water.[100] Some of the pathogenic bacteria of tilapia grow well at these temperatures of 20–30°C, and may be contact-zoonotic, that is, also harmful to humans, after direct skin contact with the infected fish or fish-water, especially when humans have an injured skin, and are immunocompromised.[298] Although this risk is present in open tilapia (pond) culture, in infected warm water recirculation aquaculture systems, including aquaponics systems this may be even a bigger risk, as infected water is recirculated and bacteria may accumulate, being a risk to the fish culture professionals.

Some of the tilapia pathogenic bacteria described in paragraphs above may cause bacterial contact–zoonotic infections in humans, as a few of these bacteria have been isolated from wounds, superficial soft tissue, or even from invasive systemic infections in humans. Often those diseases were connected to a spine, puncture or exposure event, or after humans ingested the bacterium, the latter being a food zoonosis. A choice of potential contact- or food-zoonotic bacteria are *S. agalactiae* ST283, *S. dysgalactiae* subsp. *equisimilis*, *S. iniae*, *A. hydrophila*, *E. tarda*, *M. marinum* and *V. vulnificus*.[298,304,321–323]

2.4.1 | *Streptococcus agalactiae* ST283

Early evidence for association between fish consumption and *S. agalactiae* colonisation came from a prospective longitudinal cohort study among college students living in a dormitory in United States.[25] This study showed that fish consumption increased the risk of *S. agalactiae* colonisation with capsular types 1a and 1b combined 7.3-fold.[25] Group B *Streptococcus* (GBS) has been associated with superficial and invasive infections in immunocompromised non-pregnant adults, and is the main cause of neonatal sepsis. Invasive infections in non-pregnant adults without comorbidities came to light after the 2015 fish-associated outbreak in Singapore involving at least 146 people manifesting as bacteraemia, septic arthritis and meningitis. Through various researches and official investigations, it was revealed that this 2015 GBS foodborne outbreak in Singapore was caused by Sequence Type 283 (ST283) belonging to serogroup III-4, as explained below, and case-control studies found the outbreak to be associated with the consumption of raw freshwater fish.[324,325]

While there are different methods that classify GBS types in different ways, Capsular typing (serotyping) and multi-locus sequence typing (MLST) are the major typing systems.[22] Serotyping, which is based on the capsular type of the organism and can be conducted using antibodies or primers targeting the capsular operon, recognizes 10 types (Ia, Ib, II–IX). In fish, three major serotypes of *S. agalactiae* are recognized, that is, type Ia, Ib and III.[32] MLST, which is a standardized method based on the DNA sequence of seven conserved housekeeping genes,[326] recognizes some 2000 Sequence Types (STs) and hence provides more discriminatory identification of *S. agalactiae* strains across host species and countries. The major serotypes of *S. agalactiae* found in fish largely correspond with three STs: isolates of serotype Ia belong to ST7 or closely related ST, isolates of serotype Ib belong to ST260 or closely related ST, and isolates of serotype III belong to ST283 or closely related ST.[32] The fish-specific serotype Ib/ST260 clade has never been detected in humans, whereas the serotype Ia/ST7 clade has been detected in fish, dolphins and humans.[32,327] There is no evidence, however, of direct fish-to-human transmission. Such evidence only exists or serotype III (subtype 4)/ST283: Molecular epidemiological studies revealed that GBS ST283 isolated from freshwater fish (food) samples and infected patients were identical, supporting the hypothesis of foodborne transmission of GBS ST283.[328–330]

Barkham et al.[331] showed that GBS ST283 had been present in human blood cultures in Singapore since 1998. Data and collections of GBS associated with invasive infections were retrieved from other South-East Asian countries. Taken together, 29% of human GBS from Hong Kong, Thailand, Lao PDR, Vietnam and Singapore turned out to be ST283: the earliest known isolate was from Hong Kong in 1995. 97% of patients with ST283 were adults and 36%–80% did not have comorbidities. The prevalence of ST283 in invasive GBS infections varied from 11% in Hong Kong to 73% in Thailand and 76% in Lao PDR.[329] However, none of 18 isolates from Malaysia and only 5/4198 (0.1%) of GBS isolates from mainland China, Africa, Europe, North and South America belonged to ST283.[22] FAO convened an expert group which found insufficient data for a full risk analysis, but published a risk profile detailing gaps in knowledge that would benefit from more research.[22]

Identification of GBS ST283 in freshwater fish has been reported from a number of species such as grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), Nile tilapia (*Oreochromis niloticus*), red and black tilapia (*Oreochromis* sp.), Mekong giant catfish (*Pangasianodon gigas*), freshwater frogs (*Hoplobatrachus rugulosus* and *H. chinensis*) and marine species, Asian seabass (*Lates calcarifer*).[37,332–334] The outbreak in Singapore was controlled after advising the public against consumption of raw freshwater fish. It is well known that consumption of raw fish is associated with risk of infection with bacterial, viral and parasitic infections. Data indicates that *S. agalactiae* can be inactivated by pasteurization and therefore adequately cooked tilapia and other fish would be safe for consumption.[22]

Remarkable to add, in exceptional cases, fish may get infected from humans as well, so, in an anthroponosis: Experimental induced infection of Nile tilapia *(O. niloticus)* with a human isolate of GBS (serotype Ia, ST7) was able to cause disease and mortality in the tilapia.[335]

2.4.2 | *Streptococcus dysgalactiae*

Only incidental reports have been published on seafood as source of *S. dysgalactiae* subspp. zoonosis in humans, especially percutaneous

injuries, like upper limb cellulitis in humans after skin spine or puncture while cleaning seafood.[336,337] Based on genomic sequencing, *S. dysgalactiae*, subsp. *dysgalactiae* (SDSD) is associated with ruminants, whereas *S. dysgalactiae* subsp. *equisimilis* has been found in humans, companion animals (e.g. dogs and horses) and fish. Subspecies identification based on data from individual genes may not be accurate, resulting in some inaccurate reporting of species identity.[323]

Streptococcus dysgalactiae has been isolated from diseased farmed Nile tilapia in Brazil showing septicaemia and subcutaneous abscesses in the caudal peduncle region[338,339] and from tilapia in Egypt.[279] In Brazil, induced infection experiments with the isolated strain of *S. dysgalactiae* were performed, causing reproduction of disease in adult Nile tilapia, showing anorexia, lethargy, tachypnoea and darkened skin, rapidly leading to mortality rates up to 100% and 83% after intramuscular and intraperitoneal injection, respectively, with re-isolation of bacteria from diseased tilapia.[338]

2.4.3 | *Streptococcus iniae*

Streptococcus iniae has not been assigned to any Lancefield group, but 16S rRNA sequencing indicates that these are closely related to GBS. Human infections have been reported in elderly people and individuals with underlying conditions like diabetes mellitus, rheumatic heart disease or cirrhosis handling fresh fish. Infections following fish consumption have not been reported so far. The disease may manifest as cellulitis following soft tissue injuries while handling fresh tilapia (*Sarotherodon galilaeus*), also known as St. Peter's fish or Hawaiian sunfish.[24] But complications such as arthritis, meningitis, endocarditis and osteomyelitis may also develop.[340] Most infections have been associated with people of Asian origin, possibly due to the habit of handling whole tilapia. Studies in Canada using pulse field gel electrophoresis (PFGE) showed that strains causing fish infections and human infections belong to same clone.[341] *S. iniae* infections in humans may be under-reported since identification of this pathogen in clinical laboratories is hampered by the limitations of the commercial identification systems.[342]

2.4.4 | *Aeromonas* spp.

Aeromonas spp. are Gram-negative rods. The motile *Aeromonas* spp., like *A. hydrophila* and *A. sobria*, are opportunistic bacteria and can be found everywhere, in- and outdoor, in soil and in fresh to brackish water, as aquatic commensals and secondary pathogens.[343,344] In humans, *Aeromonas* spp. originating from various fish species may cause acute haemorrhagic diarrhoea. It may also cause invasive skin and soft tissue infections, after aquatic injuries through spines, punctures and bites of animals. Within 24 h after infection, infected wounds may show erythema, oedema and purulent discharge, which may develop into fever in untreated or improperly treated cases, which may progress into invasive infections, especially in the immunocompromised patients, with necrotizing fasciitis, necrotizing myositis and osteomyelitis.[304,322,343]

Aeromonas isolates isolated from human infections were found to be susceptible to various antibiotics, of which sulphonamids were less effective.[48] In serious cases, besides wound drainage and debridement, *Aeromonas* wound infections should be treated initially with either a fluoroquinolone or a third-generation cephalosporin, possibly plus an aminoglycoside until culture and antibiotic sensitivity results are known, and rule out *Pseudomonas* coinfections.[48,345]

2.4.5 | *Edwardsiella tarda*

Edwardsiella tarda is a Gram-negative rod of the family Enterobacteriaceae. It is known as pathogen of various fish, like eel, tilapia and it causes emphysematous putrefactive disease of catfish.[322,346] It may cause 'fish gangrene', 'emphysematous putrefactive disease of catfish' or 'red disease of eels', referred to as *Edwardsiella* septicaemia (ES), a systemic disease of fish.[298]

Edwardsiella tarda from cold-blooded animals like marine, brackish and freshwater fish, reptiles and amphibians may also cause disease in humans.[347] Slaven et al.[348] described various zoonosis cases in the 1990s in humans in Louisiana by *E. tarda*: 11 extraintestinal infections, with five wound infections (three with exposures to marine fish or fish bones), five abscesses requiring surgical drainage and one case of bacteraemia. In severe and scarce cases, extensive myonecrosis and fatal septic shock in immunocompromised patients, especially in patients with chronic liver disease was seen. Therapy recommended consisted of antibiotics, like ampicillin, cepahalosporins, such as cefazolin and ceftazidime, aminoglycosides and fluoroquinolones.[348]

2.4.6 | *Vibrio vulnificus*

Vibrio vulnificus is a multi-host fish pathogen that inhabits coastal ecosystems in temperate, subtropical and tropical areas (>18°C) and likes low to moderate salinities.[179,349] It is a zoonotic agent as vibriosis can be transmitted directly from diseased fish to humans by contact.[184,322,350] In humans, *V. vulnificus* may cause a range of diseases with variable clinical manifestations, like acute gastroenteritis from eating undercooked shellfish, progressing into acute sepsis, or, in rare cases, primary sepsis and severe wound infections from marine injuries and water exposures, which may develop into life-threatening necrotizing fasciitis.[179,184,298,322,350–353] Historically, the species was divided into three biotypes (Bt), all of which contained human pathogenic strains. Pathovar *piscis* (pv. *piscis*; formerly Bt 2), is considered as primary fish pathogen and is subdivided into several clades/serovars, from which Ser E and Ser T have proven zoonotic potential and thus represent a risk to also aquaculture professionals.[184,185]

Regarding zoonosis through *V. vulnificus* infected tilapia, several clinical cases have been reported. Chan et al.[354] described a case of a septicaemia that progressed into necrotizing fasciitis after the patient experienced a puncture by the dorsal fin of an infected tilapia. Nudelman et al.[355] and Bisharat et al.[356] described wound infections after

injuries in extremities by the sharp spines of infected tilapia in Israel. Vinh et al.[357] also reported a fatal case of *V. vulnificus* sepsis developed in a patient with chronic hepatitis B and chronic renal failure after handling and ingesting tilapia.

Other authors experimentally challenged Nile tilapia with the zoonotic pv. *piscis* Ser E and fish developed a haemorrhagic septicaemia similar to eel vibriosis, warning that this bacterium could constitute a serious health hazard for tilapia and, indirectly for humans.[191,193] Interestingly, there have been reports of the isolation of *V. vulnificus* from diseased tilapia cultured in Indian and eastern Mediterranean farms, all of them potentially dangerous for humans.[185,189,190] Moreover, it has been demonstrated that human clinical isolates which had not been linked to fish vibriosis or to zoonosis cases, also belong to pv. *piscis*, demonstrating their zoonotic nature.[185] Thus, apart from the risk for tilapia, these facts might also imply a risk to humans and, thus, the species should be higher estimated as a zoonotic pathogen.

Therefore, tilapia farm environments, with high nutrient concentrations and host densities, may clearly contribute to an increase in *V. vulnificus* populations and provide advantageous conditions for the emergence of genetically more diverse and more virulent strains and/or the expansion of particular lineages/clades, including the zoonotic ones.[185,188] Moreover, under the climate change scenario, the increased water temperatures may favour these events.[179,188,358]

Regarding therapy of diseased humans, prompt intervention with antibiotics should be performed, as sepsis and fasciitis necroticans may be fatal within 48–72 h. The U.S. Centers for Disease Control and Prevention[359] recommended a third-generation cephalosporin, especially ceftazidime, plus doxycycline, as initial empiric antibiotic combinations for suspected *V. vulnificus* infections; see their website. Other cephalosporins can be used as well, as well as fluoroquinolones like ciprofloxacin, see CDC.[359] The treatment may include early surgery for wound debridement and monitoring for compartment syndromes, as these increase the survival rate when a systemic human infection is the case.

Development of effective control and preventive measures in fish farms against *V. vulnificus*, the most infectious of all zoonotic *Vibrio* spp., is considered highly necessary, including development of effective vaccines.

2.4.7 | *Mycobacterium marinum*

Mycobacterium marinum is one of the fish mycobacteria, Gram-positive, acid–alcohol-fast, non-motile, non-spore forming rods that may cause chronic and lethal fish tuberculosis in warmwater fish, including tilapia.[294,303]

In humans, *M. marinum* may cause 'swimmer granuloma', 'fish tank granuloma' or 'fish handler's disease',[294,298,303,304,360–362] which may be chronic infections of hands and feet, but not easily lethal (Figure 9). *M. marinum* has an optimum temperature of 30°C (Haenen, own findings), and is inhibited at 37°C. This means, in humans, almost exclusively, skin infections occur in extremities, which are cooler. The incubation time for mycobacteriosis in the skin is 7–21 days after skin injury.[322]

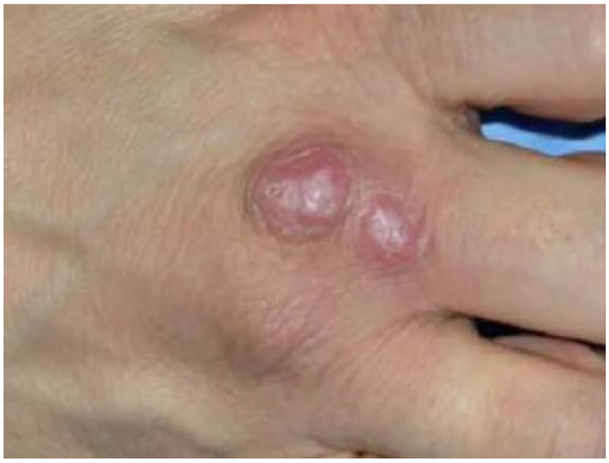

FIGURE 9 Swimmer granuloma on the right hand, after infection by *Mycobacterium marinum* through skin contact with warmwater fish and fish water. Picture courtesy Dr Cassetty and Dr Sanchez, 2004; details in Dermatology Online Journal[361]

In a later phase, granulomatous nodules will develop on the skin, which may become secondary infected. Also deeper, invasive infections may develop, like septic arthritis, bursitis, tenosynovitis and osteoarthritis.[363] Yacisin et al.[364] monitored *M. marinum* skin or soft tissue infections cases at Chinese markets in New York City, and concluded, the highest risk of acquiring the zoonosis was through skin injury of the finger or hand during fish handling.

Fast preliminary diagnostics is done by acid-fast staining smears of nodules and lesions, and through culture from nodules. PCR identification *M. marinum* is confusing[322] and requires more than one PCR. Only chronic treatments are considered effective.[365] According to Aubry et al.[366] clarithromycin, cyclines and rifampin were the most commonly prescribed antibiotics, with an effective cure of 87% of the 63 patients. *M. marinum* is susceptible to macrolides like clarithromycin, sulfonamides/trimethoprim-sulfamethoxazole, ethambutol and rifampin/rifabutin.[367] A typical treatment consists of a combination of two of these drugs (i.e. clarithromycin plus ethambutol, or clarithromycin plus rifampin) for approximately 3–4 months, to be ended only 4–8 weeks after symptoms have vanished.

2.5 | Status of antimicrobial resistance in fish culture through imprudent antibiotic use, and its future

In semi-intensive and intensive aquaculture, access to safe and effective veterinary medicines or drugs is essential to a successful operation. However, if used imprudently, antibiotics used to treat bacterial diseases may be ineffective and may lead to unacceptable residue levels in aquaculture products that can result in bans on importation, import rejections and detentions.[2] Misuse of veterinary medicines may lead to the development of antibacterial-resistant genes in bacteria, and this may therefore cause antimicrobial resistance (AMR). This

consequence happens across all food-producing sectors, including aquaculture. There are many examples, like a joint 97% antibiotic resistance to ampicillin, erythromycin, and oxytetracycline in 173 bacterial isolates from apparently healthy tilapia in Trinidad.[6] Therefore, if antibiotics are to be used, the choice of antibiotic must always be based on the results of an antibiogram, to be sure, the therapy is effective.

There is increased global attention through various assemblies, meetings and conferences where AMR has been specifically mentioned as a vital and growing problem. The Global Action Plan (GAP) on AMR (with contributions from FAO and OIE) was adopted during the 68th World Health Assembly in 2015.[368] In the same year, the World Assembly of the OIE delegates adopted the strategy, and the 39th FAO Conference adopted Resolution 4/2015. A political declaration was made during a high-level meeting on AMR at the 71st United Nations General Assembly (UNGA, September 2016). The UNGA called upon the Tripartite (i.e. FAO as global leader for food and agriculture, the OIE as global leader for animal health and welfare and the World Health Organization [WHO] as global leader for human health) and other intergovernmental organizations to support the development and implementation of National Action Plans (NAPs) and AMR activities at the national, regional and global levels under the One Health platform. The FAO, OIE and WHO agreed to step up a joint action to combat health threats associated with interactions between humans, animals and the environment.

A memorandum of understanding was signed in May 2018 to strengthen their long-standing partnership, with a strong focus on tackling AMR. In addition, the United Nations Secretary-General convened the Interagency Coordination Group (IACG) on AMR in May 2017 in consultation with Tripartite members to provide guidance on approaches for ensuring sustained global action on AMR, and reported back to the Secretary-General during the 73rd General Assembly in 2019. This mandate included making recommendations on enhancing coordinated action across sectors and countries, building political momentum, future governance and mobilizing stakeholders.[2]

Countries are now encouraged to develop National Action Plans (NAP) on AMR. In the development of the aquaculture component of a country's NAP on AMR, understanding and increasing knowledge of bacterial diseases affecting the sector, how they are being managed, complexities associated with AMR in the aquatic environment and how to achieve One Health goals are essential.[369]

These developments should now serve as a signal of the urgent need for aquaculture countries, especially those with substantial aquaculture production and food security objectives through aquaculture, to pay high attention to the emergence of antimicrobial-resistant organisms that can result from antimicrobial (specifically antibiotics) imprudent and irresponsible use in the aquaculture sector.

Hanson[370] provided practical management measures to minimize AMR from bacterial diseases of finfish by reducing the use of antibiotics and ensuring its prudent use when it is needed. Good husbandry (good seed, adequate nutrition, good water quality and environment, minimizing stress, etc.) and biosecurity practices (e.g. health monitoring, rapid action on first signs of abnormal observations or clinical signs of disease, vaccination, breaking disease transmission pathways) through all phases of production should be part of normal practice. Disease prevention can be achieved by managing the environment and host, by pathogen avoidance and by having a biosecurity plan, as parts of Good Aquaculture Practice.[2,100]

FAO[2] listed several biosecurity measures that may reduce or eliminate AMR. These include avoidance, using clean facilities, use of immunostimulants to enhance innate immunity, inclusion of probiotics in feeds, vaccination, phage therapy via feeds and the use of plant extracts. Of these, vaccines have been widely used against fish infections. Avoidance of AMR can also be achieved by farming high-value SPF (Specific Pathogen Free) fish species in a controlled way.

3 | CONCLUSIONS

There are many microbial agents in the aquatic environment, some of which are potential pathogens to tilapia, depending on a variety of factors specific to the host, pathogen and environment.

Since decades, some bacterial species, belonging to at least four genera, are considered important pathogens for tilapia: *S. agalactiae*, *S. dysgalactiae* and *S. iniae*, motile *Aeromonas* species, *F. orientalis*, *F. columnare* (new name: *F. oreochromis*) and *V. vulnificus* pv. *piscis* and some other *Vibrio* species. Additionally, at least two bacterial tilapia diseases are emerging, edwardsiellosis through *E. ictaluri* and *E. tarda* as well as disease by *A. schubertii*. Furthermore, bacteria with zoonotic potential, like *S. agalactiae* ST283, *S. dysgalactiae* subsp. *equisimilis*, *S. iniae*, *Aeromonas* sp., *E. tarda*, *V. vulnificus* pv. *piscis* and *M. marinum* are included in the review, to provide altogether the current overview of the disease risks affecting production and post-harvest stages.

Various other bacteria may be opportunistic and pathogenic to tilapia as well, especially under favourable conditions of the environment (water at a high temperature, with high loads of organic material, low oxygen and other stress factors), and vulnerable fish (low in immune status, in too high stocking densities, too variable in size, etc.), like *L. garvieae*, *A. viridans*, *Pseudomonas* spp. and *Chlamydia* spp.

The important role played by aquaculture in providing high-quality nutrition, improving livelihoods, stimulating and creating decent work and economic growth and alleviating poverty, particularly in low-income food-deficit countries will be only possible, if disease challenges (including bacterial diseases) affecting production can be addressed in a decent way. It is of utmost importance to train the tilapia farmers in good aquaculture practices (GAP), including hygiene at the fish farm, to avoid spread of fish bacterial disease and fish mortality. For this, it is very important to educate fish health professionals for field work, to be able to control bacterial diseases in tilapia farming and avoid spread.

Regarding bacterial zoonosis, cases from tilapia culture are mostly not recorded on a global scale. For sure they occur, from mild (mycobacteriosis, swimmer granuloma, i.e. chronic skin infections by *M. marinum*) to serious (necrotic fasciitis through systemic infection by *V. vulnificus*), depending on the patient's immune status, and they can be

prevented for through good hygiene. Awareness of *One Health* and Good Hygiene Practice should be in place in aquaculture, including in the whole tilapia production chain up to the consumer. This means avoiding direct contact of potential zoonotic pathogens with the human skin, and avoid inhalation and ingestion of those pathogens. At tilapia farms, slaughter facilities and packing sites this means special clothing, wearing gloves and face masks and regularly wash hands and skin with soap after any contact with fish and fish water. It also means, that when professionals would develop signs of a contact or food safety zoonosis they should mention to the medics, that they work with warmwater fish, and may have acquired a zoonotic infection from the fish.

Regarding antimicrobial resistance (AMR), responsible use of antimicrobial agents is an important part of farm biosecurity to ensure that pathogen challenges are minimized, that the natural defence mechanisms of the cultured stocks are maximized, and that disease and mortality, including costs of containing, treating and/or eradicating diseases, are reduced.[2] Therefore, the use of antimicrobial agents should be minimized, and be consistent with established principles of prudent use, to safeguard public and animal health.[2] Furthermore, apart from Good Aquaculture Practice (GAP), development and use of effective and economically favourable vaccines is recommended.

AUTHOR CONTRIBUTIONS

Olga Haenen: Conceptualization; data curation; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Ha Thanh Dong:** Conceptualization; data curation; investigation; resources; writing – original draft; writing – review and editing. **Truong Dinh Hoai:** Resources; writing – original draft; writing – review and editing. **Margaret Crumlish:** Conceptualization; data curation; investigation; methodology; writing – original draft; writing – review and editing. **Iddya Karunasagar:** Conceptualization; data curation; investigation; methodology; writing – original draft; writing – review and editing. **Timothy Barkham:** Writing – original draft; writing – review and editing. **Swaine L. Chen:** Conceptualization; investigation; writing – original draft; writing – review and editing. **Ruth Zadoks:** Conceptualization; investigation; writing – original draft; writing – review and editing. **Andreas Kiermeier:** Conceptualization; investigation; methodology; writing – original draft. **Bing Wang:** Writing – original draft; writing – review and editing. **Esther Garrido Gamarro:** Conceptualization; funding acquisition; investigation; project administration; resources; writing – original draft; writing – review and editing. **Masami Takeuchi:** Conceptualization; funding acquisition; investigation; project administration; resources; writing – original draft; writing – review and editing. **Mohammad Noor Amal Azmai:** Data curation; formal analysis; investigation; methodology; supervision; validation; writing – original draft; writing – review and editing. **Belén Fouz:** Conceptualization; data curation; investigation; resources; validation; visualization; writing – original draft; writing – review and editing. **Rolando Pakingking Jr.:** Conceptualization; data curation; investigation; writing – original draft; writing – review and editing. **Zeng Wei Wei:** Data curation; investigation; writing – original draft; writing – review and editing. **Melba G. Bondad-Reantaso:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

This study was supported by two FAO projects namely: GCP/GLO/979/NOR: Improving Biosecurity Governance and Legal Framework for Efficient and Sustainable Aquaculture Production and GCP/GLO/352/NOR: Responsible use of fisheries and aquaculture resources for sustainable development, both funded by the Norwegian Agency for Development Cooperation (Norad). We also acknowledge the support from Regular Programme funds under FAO's strategic framework on better production and three relevant programme priority areas, that is Blue Transformation, One Health and Safe Food, and the support from National Governments. We thank Dr Kevin Fitzsimmons for helping in the review process.

CONFLICT OF INTEREST

The views expressed in this publication are those of the author(s) and do not necessarily reflect the views or policies of the Food and Agriculture Organization of the United Nations.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study. Existing literature was used, Open and Restricted Access papers, peer review journals, book chapters, health organisms reports and so forth.

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How to cite this article: Haenen OLM, Dong HT, Hoai TD, et al. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. *Rev Aquac*. 2023;15(Suppl. 1):154-185. doi:10.1111/raq.12743