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# A Review of Bacterial Zoonoses and Antimicrobial Resistant (AMR) on Grouper fish (*Epinepholus sp.*)

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## ABSTRACT

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Grouper fish (*Epinepholus sp.*) is the most important commercial marine culture fish species with high market value and good protein. Although it has high economic value, grouper fish also has the potential to be the target of several zoonotic bacterial agents, including *Streptococcus iniae*, *A. hydrophila* and *Vibrio vulnificus*. Zoonosis is a disease that can be transmitted naturally between vertebrates and humans. Indirect transmission can occur through contact with the environment around the fish. Grouper fish can also be a source of antimicrobial resistant (AMR) transmission. It is necessary to think about controlling infectious diseases in fish without using antibiotics. High antibiotic use can lead to increased antibiotic resistance. It is hoped that this review will raise our awareness of the potential bacterial zoonoses and AMR of high value grouper fish. Therefore, it is hoped that the consumption of grouper fish will not cause public health problems.

**Keywords:** Grouper fish, Bacterial zoonoses, AMR, Public health.

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## INTRODUCTION

Grouper fish (*Epinepholus sp.*) is the most common commercial marine fish species with strong consumer value and high protein content. Given its strong economic importance, groupers often have the ability to become disease targets [22]; many bacterial agents [1, 2] such as *Streptococcus iniae* [3], *Vibrio alginolyticus* [4], *Vibrio carchariae* [5], *Pseudomonas sp.* [6] *Flexibacter sp.* [7], *sp. Aeromonas*. Infection of groupers of fish in Southeast Asia has also been confirmed.

The grouper's approximate [27] value is more than USD 300 million [8]. Disease has been a serious problem in the breeding and processing of groupers and has a severe influence on the reduction of their output potential. A variety of grouper diseases have been identified and the major pathogens are bacteria and viruses [9,10,11]. However, the cultivation of brackish water from coastal areas reveals grouping fish, in particular *Vibrio sp.* Even this triggers severe medical complications. *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio vulnificus*, and other *Vibrio sp.* They have been described as pathogenic bacteria from fish clusters [12,13].

*Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae* are streptococcal bacteria that cause bloodstream infection and can be spread to humans. In order to determine the virulence mechanism used by systemic pathogens, an infectious disease model has been developed using streptococcal pathogens such as *Streptococcus iniae* and its natural host, zebrafish (*Danio rerio*), to investigate the phase of systemic infection in the natural host pathogenic system [14]. *S. Iniae* is a major pathogen in both marine and human organisms, contributing to systemic infection of both hosts. Signs of

infection are very identical to those triggered by a variety of human streptococcal bacteria, such as *S. Pyogenes*, *S. Agalactiae*, *S. Pneumoniae* [14]. *S. Iniae* and *S. Pyogenes* may cause cellulitis in humans, particularly after skin abrasion, which gives bacterial access to the dermal layer. *S. Iniae*, *S. Pneumoniae*, *S. Agalactiae* are all capable of producing bloodstream infections that lead to meningitis and bacterial diseases. In contrast, 16S rRNA of the streptococcal community phylogenetic tree indicates that it is a rather close genetic ancestor to the special human pathogen *S. Agalactiae* [15] that is capable of transmitting it to humans.

Another disease that may transmit through groupers to humans is caused by the *Aeromonas* gene and can cause [6] intestinal and extra-intestinal infections in humans, such as gastroenteritis, skin and soft tissue infections, and bacteria [16]. *Aeromonas* infection is gained by consumption of infected food and water and open wounds in contact with *Aeromonas*-contaminated areas. The isolation of aeromonas from many aquatic organisms has [49] shown that food sources of marine fish can be a vector for the transmission of this pathogen to food suppliers and food users [17,18,19].

Vibriosis [20] is another significant disease that causes severe economic losses and is considered a major problem in community farming. This condition is triggered by bacteria with the *Vibrio* genome, including *V. vulnificus*, *V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi* and *V. anguillarum*. Overcrowding at water temperatures of more than 15 °C can increase the susceptibility of fish to vibriosis, as the fish are subject to stress and compromised immunity [21]. This is *Vibrio spp.* It can infect fish via the

in or through oral ingestion. The usual symptoms of vibriosis in fish are lethargy and ulceration of the skin and muscles[22]. In addition, low discharge (gastroenteritis) has been recorded in the intestines of *Vibrio*-infected fish[23]. Vibriosis management centres primarily on chemotherapy and prevention steps. Fish farmers also use pesticides and disinfectants to cure infected fish. In reality, this activity is not compatible with the ideal of sustainable cultivation. Improper use of drugs has developed unsafe residues of antibiotics in fish and poses a risk of developing antibiotic-resistant pathogens in aquaculture systems[24]. Many useful methods have recently been introduced to avoid or monitor diseases aimed at improving the immune response of fish to pathogens, including vaccinations and natural products with immunostimulating properties[25]. It is anticipated that the development of new hybrid fish with powerful innate defensive mechanisms would also be a successful solution to disease reduction.

*Vibrio vulnificus* is a water gram-negative bacterium capable of inducing different pathologies in fish or human host infections [26, 27]. *V. Vulnificus* fish infection exists mostly in aquaculture, where outbreaks of hemorrhagic septicemia that are perpetuated by *V. Vulnificus* are spread by water or by direct interaction with animals. In humans, two distinct forms of diseases-severe skin lesions and septicemia-are typically triggered by infection with *V. vulnificus*. Skin lesions form after exposure to wounds in seawater or aquatic organisms colonised by *V. vulnificus*, whereas septicemia occurs from ingestion of aquatic food infected by pathogens [27]. Wound infection can also contribute to septicemia, especially in immunocompromised individuals and those with elevated blood iron levels associated with chronic liver disease, suggesting an 80-fold increase in the risk of *V. vulnificus* septicemia relative to healthy individuals [26,28]. *Vibrio vulnificus* is a naturally occurring estuarine bacterium, the primary source of aquatic mortality and disease in the United States [29,30]. *V. vulnificus* is responsible for more than 95% of marine-related deaths in the United States [31], particularly among individuals who are immunocompromised or have liver disease [32,33].

The purpose of writing this review is to explain the general definition of bacteria that often cause disease in grouper fish which are also zoonotic which can attack human health, a general description of pathogenic bacteria and zoonoses in humans, modes of transmission of disease, and also discuss antimicrobial resistance (AMR) bacteria. It is hoped that we will get a complete picture of the potential of Groupers fish and how to reduce the dangers they cause.

#### GENERAL DESCRIPTION OF ZOOSES BACTERIA ON GROUPER FISH

The Gram-positive *S. Iniae*, which develops naturally in aquatic and estuarine habitats and is one of the big opportunistic pathogens of wounded or unsanitary grouped fish, induces systemic inflammation, a red ulcer disease known as streptococcosis. *Streptococcus* is an infectious illness triggered by a bacterial infection by *Streptococcus*. This disease produces multiple deaths owing to the high death rates owing to the assault on the bacteria. A great deal has been achieved to eliminate streptococcal disease, including by way of antibiotics and protective vaccine strategies [34,35,36].

Gram-negative bacteria *Aeromonas hydrophila* is a widespread and heterogeneous organism that induces a disease known as motile aeromonad septicemia that

induces severe economic losses in aquatic and freshwater aquaculture[37]. *Aeromonas* bacteria are widespread in marine settings, including mineral water, drinking water and hot water. *Aeromonads* can also be separated from foods such as beef, fish, fish, and vegetables. The gene comprises of 19 distinct animals. Any recently described organisms are not included in the majority of classifications[38]. Members of the genus trigger disease in a large range of invertebrates and vertebrates, including frogs, fish, birds and domestic animals. Several species, including *A. Hydrophila* is concerned with human intestinal and extra-intestinal diseases[39].

*Vibrio vulnificus* is a motile, halophilic, rod-shaped Gram-negative pathogen generally identified in warm estuarine ecosystems. Human illness is uncommon and intermittent, but life-threatening. *V. Vulnificus* infection, mainly expressed as skin or soft tissue infection and/or septicemia,[40,41] can progress to fulminants associated with bacterial expression of toxins and enzymes, including capsular polysaccharides, metalloproteases, lipopolysaccharides and cytolysine. [42-47] If not quickly suppressed by the removal of pathogens, infection can worsen rapidly and progress to advanced soft tissue infection or soft tissue involvement. Extreme process of *V. vulnificus* soft tissue infection, necrotizing fasciitis (NF), sometimes contributes to adverse effects or even death within 24 hours of admission,[41, 48-51] particularly when combined with sepsis or septic shock with a mortality risk. The recorded cases ranged from 26% to 71 percent [52-56].

#### ZOOSES IN HUMAN

*Streptococcus iniae* is one of the main species responsible in relation to Streptococcal disease. It is a well-known bacteria for both humans and fish, which is a Gram-positive coccus, [57]. *S. iniae* infects humans is thought to be complicating and has been documented primarily in North America, the Middle East, and the Asia-Pacific region. These bacteria can be found in the mouth, intestines of humans, animals and fish. There are several types that are pathogenic. Pathogenic *Streptococcus* bacteria can cause diseases such as pneumonia, meningitis, necrotizing fasciitis, erysipelas, laryngitis, and endocarditis in human [17,59].

Among human beings *A. Hydrophila* has been involved in diet or waterborne gastroenteritis, diarrhoea, septicemia, peritonitis, septicemia and soft tissue wound infection [60-62]. *A. Hydrophila* outbreaks have been recorded in humans since 1992, when 382 children in two child care centres experienced symptoms of diarrhea[63]. Subsequent cases were all linked to polluted drinking water or food: 83 cases were recorded in China[64] in 1993; 27 cases were reported in Sweden[65] in 1995; more than 200 cases were reported in China[66] in 2012; and 60 cases were reported in the Philippines[67] in 2013. In addition, there is circumstantial proof that this is *A. Hydrophila* could be zoonotic; bacteria have been isolated from peritonitis and diarrhoea in patients whose pet goldfish have been treated with polluted tank water[60]. *Vibrio vulnificus*, a Gram-negative bacterium, induces septicemia in humans with liver cirrhosis, hemochromatosis, immunocompromised diseases, and diabetes [68,69]. Deaths attributed to *V. vulnificus* infection surpass 50 per cent and escalate to more than 90 per cent of patients who are on shock immediately after admission. The majority of fatal cases are triggered by septic shock due to numerous virulence factors generated by *V. vulnificus*, including capsular polysaccharides



[70,71], siderophores [72], hemolysin [73], matrix metalloproteinases, flagella [74] and toxins RtxA [75-77]. The *V. vulnificus* strain has genetic and phenotypic diversity and is grouped into Biotypes and genotypes on the basis of their respective biochemical and genetic features. Biotype 1 strains are responsible for the bulk of human infection (78, 79). Genetic polymorphisms in virulence-related genes function as a crucial function in the separation of clinical genotype (C) strains from environmental factors (E) that were historically more frequently correlated with disease (80). Similarly, polymorphisms in the 16S rRNA gene can be used to discriminate between biologically and environmentally associated genotypes referred to as forms B and A, respectively (81). The usage of multi-locus sequence typing and phylogenetic study of the sequenced genome further delineated genotypes C and E into distinct evolutionary lines (82, 83). Previous studies have shown that the C-and E-genotype strains exhibit different ecologies, where the E-genotype strains tend to have a distinct advantage in inhabiting oysters, while the C-genotype strains are more effective in infecting human hosts (80, 84-86). Furthermore, genomic comparisons have permitted the identification of several potential virulence factors (such as genome XI).

Bacterial pathogenicity relies on the secretion of virulence factors [89]. Gram-negative bacteria produce a range of types of secretive systems[90], including the type I secretion mechanism (TISS). The TISS consists of three plasmic membrane elements, a particular external membrane protein (OMP), an ATP binding cassette (ABC) and a membrane fusion protein (MFP)[91]. *V. cholerae* toxin RtxA is the strongest cytotoxic toxin with actin cross-reactivity and is excreted from cells through TISS consisting of RtxB (ABC), RtxD (MFP), RtxE (ABC), and TolC (OMP)[92]. Thus, TISS performs a direct and/or indirect role in the degradation of bacterial toxins [91,93]. The mutant gene of *V. vulnificus* rtxA moderately weakened by cytotoxicity and is lethal, *in vitro* and *in vivo*[77]. The results indicate that the RtxA toxin released by the RtxE transporter from *V. vulnificus* contributes to the cytotoxic behaviour and cell death of *V. vulnificus* disease.

*V. vulnificus* was isolated from the Atlantic and Pacific coasts of the United States, but much of the infections occurred during the ingestion of fresh oysters obtained from the Gulf of Mexico[94,95]. *V. vulnificus* infection has been documented from water across the world and different climates, including Denmark, Sweden, Germany, the Netherlands, Belgium, Israel, Italy, Japan, Taiwan, Australia and Brazil. [94-106] As a result. A research conducted over 30 years in Florida recorded that of all *Vibrio* species, *V. vulnificus* was the most frequent cause of primary septicemia, resulting in 75 (64 per cent) of a total of 118 cases, with a mortality rate of 56 per cent [107]. A more detailed epidemiological analysis of *V. vulnificus* infection[108], 23 countries recorded a total of 422 *V. vulnificus* infections from 1988 to 1996 to the CDC. In this analysis, 86 percent of all patients were male. Wound infection (45 per cent), main septicemia (43%), gastroenteritis (5 per cent) and undetermined infection (7 per cent) is both triggered by *V. vulnificus*. Data from the analysis showed that patients with primary septicemia had underlying liver disorder and 96 per cent developed infection after the ingestion of fresh oysters obtained from the Gulf of Mexico. 61% of instances of septicemia culminated in the death of a patient[108]. All these studies agree that individuals with underlying chronic diseases, especially those affecting the liver, should be mindful of

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the risks associated with the ingestion of raw shellfish, especially when obtained from the Gulf of Mexico. Controls targeted at teaching immunocompromised people and the excessive vaccine, warning them about the dangers involved with proximity to seawater and the ingestion about fresh shellfish.

## 2. TRANSMISSION OF BACTERIA FROM FISHERIES

Human infections caused by bacteria spread from the fish or the aquatic ecosystem are very frequent depending on the season, the patient's interaction with the fish and the climate, the eating patterns and the state of the person exposed to the immune system. These are also bacterial organisms that are optionally pathogenic to fish and humans and may be removed from fish without strong signs of disease. The cause of infection can be fish raised either for food or as a hobby [109]. A detailed background and microbiological analysis are important for the right diagnosis.

It is very difficult to detect certain slow-growing disease-causing agents *in vitro* such as Mycobacterial contamination or contamination triggered by anaerobic pathogens. Mycobacterial diseases are frequently misdiagnosed by subsequent ineffective therapy [110-122]. As a consequence, the disease will last for many years [113]. Streptococcal infection triggered by *Streptococcus iniae* was first recorded in rainbow trout Japan in 1958. Later pathogens have been identified in snapper yellowtail, grouper (*Epinephelus sp.*) and tilapia. In 1997, the projected annual effect of this bacterial contamination on the US aquaculture sector alone was US\$ 10 million and an approximate US\$ 100 million worldwide. [35]. Yes. *Streptococcus iniae* (*S. iniae*) [114], a Gram-positive bacterium, causes streptococcosis, a disorder defined by meningoencephalitis, systemic septicemia, and skin lesions [115] which can contribute to serious mortality [116]. *S. iniae* impacts several developed types of fish such as rainbow trout [117,118], tilapia [119,120] and grouper (*Epinephelus sp.*) [121]. *Aeromonas caviae* is a less popular inhabitant of healthy fish but a significant opportunistic pathogen that infects fish under physiological and environmental stress [122]. *Aeromonas caviae* can cause motile aeromonas septicemia and significant mortality in salmon farms in the Black Sea, Turkey [123]. Insulation of *A. Caviae* from a broad range of diseased fish including tilapia, catfish and goldfish have been recorded worldwide [124]. The data indicate that the possible danger of *Aeromonas caviae* in Tiger Grouper and Goby Marble Fish is not to be overlooked. *Aeromonas rivuli*, a recently described and isolated species in Germany, has also been extracted from different sections of Goby Marble and strongly connected to *Aeromonas molluscorum* and *Aeromonas bivalvium* [125].

The strain *V. vulnificus* from diseased fish developed a distinctive physiological profile from the previously isolated strain *V. vulnificus* [126]. The DNA hybridization experiments found that this strain was *V. vulnificus*, was unable to develop at 42°C and did not have indole and ornithine decarboxylase operation. The strain *V. vulnificus* was divided into two biotypes. Biotype 1 comprises of human pathogenic strains and strains are being examined for highest human virulence [127,128]. Biotype 2 provided the strain *V. vulnificus* derived from the eels. Biotype 2 strain *V. vulnificus* has been well researched for more knowledge on this eel pathogen, which is very significant and may kill animals if raised in aquaculture ponds [129-132].

In Israel, a strain of *V. vulnificus* isolated from humans became infected after eating fresh fish on *Tilapia* spp. [133]. This isolate was confirmed as *V. vulnificus* by PCR amplification of the *vvhA* gene, but the pattern obtained after restriction of DNA endonuclease digestion did not match biotype 1 or 2. This new strain of *V. vulnificus* is biotype 3, and is now recognized as being responsible for several cases of dead in humans [134]. Biotype 3 strains are homogeneous clones which are genetically different compared to strains of biotype 1 and 2 [135]. Further evidence was presented by researchers [136] which showed that the biotype 3 strains were the result of genomic hybridization of biotypes 1 and 2.

#### ANTIMICROBIAL RESISTANT (AMR) BACTERIA

The marine ecosystem may be a source of resistant bacteria that can be spread directly to, and induce infection in humans, and can result in failure of care due to the existence of the resistance. The direct transmission of resistance to humans from the aquatic setting can include human pathogens such as *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Shigella* spp. And this is *Salmonella* spp. Opportunistic pathogens such as *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Edwardsiella tarda*, *Streptococcus iniae*, and *E. Coli* [137]. The existence of resistant *Salmonella* spp and *E. Coli* in the marine ecosystem is a product of pollution from the human, animal or agriculture ecosystem. Resistant human pathogenic or opportunistic bacteria can be spread by close interaction with water or marine species, by drinking water or by the processing or ingestion of fish products [138]. In general, these diseases are quite uncommon. Infection of opportunistic bacteria occurs more common in people with weakened immune systems. Antibiotic resistance is used as an epidemiological method to monitor foodborne diseases; it also offers knowledge on antibiotics that may aid in the management of this bacterial disease. Several antibiotic resistant or antimicrobial resistant bacteria (AMRs) can pose a threat to human health [138–141]. The growth of AMR bacteria is attributed to the usage of antibiotics in the clinical medicine, agriculture and aquaculture industries without discrimination [138]. *Vibrio* sp has been stated to be extremely susceptible to most widely used antibiotics [142]. However, the rise in the amount of *Vibrio* sp is based on the annual data. Become more resistant to antibiotics throughout therapeutic use [143]. Antibiotics can contribute to the survival of bacterial strains that may produce resistant plasmids (R). Transferring plasmid R from immune to non-resistant species is of considerable medical significance since it decreases the usage of antibiotics. Previous experiments found a link between the tolerance to antibiotics and the existence to plasmids in *Vibrio* spp. [144]. Biotype 2 strains of *V. vulnificus* had one or more plasmid virulence [145] varying between 68 and 70 kb. *V. vulnificus* strain has also been shown to bear more than one plasmid of different sizes [146]. Streptococcal pathogens can trigger serious disease and are life-threatening systemic infections in immunocompromised and stable individuals from birth to old age. The infection is evolving so quickly that surgical treatments have had limited effectiveness. Streptococcal bacteria are now re-emerging in invasive human infections, partially due to the growth of antibiotic resistance and the creation of modern infectious serotypes [147,148,149]. *S. Iniae* has a genetic affinity with *Streptococcus agalactiae* [15], which is found in *Streptococcus* Group B (GBS). GBS is a significant

etiological agent in a wide spectrum of human infections. GBS is mostly borne asymptotically by safe adults varying from 20 to 40 per cent in developed countries. The prevalence of GBS reported in pregnant women varies from 6% to 26%.

Resistance evidence for amoxicillin and cephalotin were present in both strains of *Aeromonas*. Other investigators have also found a strong incidence of resistance to amoxicillin owing to the development of different  $\beta$ -lactamases in *Aeromonas* to provide resistance to  $\beta$ -lactams [150]. Large levels of resistance to cephalotin have also been recorded in *A. caviae* separated from safe market fish in Ankara, Turkey [151], and *A. Rainbow* trout hydrophilic and renal lesions in Portugal [152]. Tetracycline resistance is focused on data of 80 percent of the isolated A standard. It's *A. caviae*. Other studies have shown that tetracycline-resistant *Aeromonas* have also been documented in diseased goldfish raised in Poland [153] and in stable retail fish (53 percent) and shrimp in India [154].

*A. hydrophila*, *A. veronii* Biovar *Sobria* and *A. caviae* with antimicrobial resistance (AMR) has been extracted from five species of safe retail fish in *Tilapia* moss ambica, *Clarias batrachus*, *Tenuulosa toli*, *Anabas testudineus* and red snapper in Malaysia. In addition, multidrug resistance (MDR) has also been shown in *A. hydrophila* is differentiated from seven distinct types of stable and diseased fish, including *Anabas testudineus*, *Aristichthys nobilis*, *Clarias* spp., *Cyprinus* spp., *Ophiocephalus striatus*, *Oreochromis* spp. And *Puntius binotatus*, *guy*. A number of AMR accidents amongst *Aeromonas* spp. Water species from other parts of the planet have also been reported [17,155]. These multidrug-tolerant bacteria in fish may allow the spread of antibiotic-resistant determinants to different regions around the world via the export of fish. This can pose a significant hazard to human health and, thus, antimicrobial therapy must be used with proper caution in the treatment of aquatic pathogens [156–158].

Not all strains of *V. vulnificus* can cause human disease. In reality, the existence of a capsule is important for the virulence of *V. vulnificus*, such that the non-encapsulated strains are non-virulent [159, 160]. In addition, both these species ferment mannitol, which is considered a simple way to predict the virulence of *V. vulnificus* [161]. Wound inflammation is the primary source of infection with *V. vulnificus* [162, 163]. Doxycycline, cephalosporins, fluoroquinolone and trimethoprim sulfamethoxazole plus aminoglycosides are the antibiotics prescribed for the care of *V. vulnificus* infection [164]. Tetracycline or ciprofloxacin can also be used in serious or persistent diseases of *V. parahaemolyticus* [165]. *Vibrio* spp, *guy*. Usually prone to certain antibiotics of value to animals and humans [166]. *V. vulnificus* is, however, immune to ampicillin [167]. The overuse of antibiotics in humans, animals, and aquaculture processes has culminated in the creation of antibiotic tolerance in several pathogenic bacteria [156, 168–171].

#### CONCLUSION

Zoonoses bacteria have been identified in groupers fish, mostly found in the form of *Streptococcus iniae*, *A. hydrophila* and *Vibrio vulnificus* because these strains can be transmitted to other fish species and humans. However, this grouper also has antimicrobial resistant (AMR) bacteria. It has been reported that humans who have had direct contact with groupers that contain zoonoses bacteria have a risk of being infected by these bacteria.



Monitoring cases of zoonoses bacterial infection that often occurs in groupers and humans needs to be done to observe changes in epidemiology and to determine effective zoonoses bacterial infection control measures, without the need to increase the incidence of antimicrobial resistance obtained from groupers fish.

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