

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

by Hani Plumeriastuti

Submission date: 05-Jun-2023 09:37AM (UTC+0800)

Submission ID: 2108969155

File name: Antimicrobial_Resistant_AMR_on_Grouper_fish_Epinepholus_sp..pdf (1.15M)

Word count: 9452

Character count: 53123

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

Azhar Muhammad Helmi¹, Akhmad Taufiq Mukti², Agoes Soegianto³, Ketut Mahardika⁴, Indah Mastuti⁴, Mustofa Helmi Effendi^{5*} and Hani Plumeriastuti⁶

¹Postgraduate Student on Faculty of Fisheries and Marine, Universitas Airlangga.

²Department of Fish Health Management & Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga.

³Department of Biology, Faculty of Sciences and Technology, Universitas Airlangga.

⁴Center for Marine Cultivation Research and Fisheries Extension, Buleleng, Bali

⁵Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga.

⁶Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga.

Jl. Mulyorejo, FKH UNAIR, Kampus C Universitas Airlangga, Surabaya 60115

*Corresponding author Email: mheffendi@yahoo.com

ABSTRACT

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.

Correspondence:

Mustofa Helmi, Effendi

⁵Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga.

*Corresponding author Email: mheffendi@yahoo.com

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 for 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 annual 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 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 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

skin 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 in aquaculture [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 acute 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 cytolytic. [42-47] If not quickly suppressed by the removal of pathogens, infection can worsen rapidly and progress to advanced skin production 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* infection in 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 [58,59].

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 diarrhoea[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 [12] 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 [40]th 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 [27] 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 [34] range of types of secretive systems[90], including the type I secretion mechanism (TISS). The TISS consists of three [12] 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-activity 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* [23] is 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. vul* [25] 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 [26] 2 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 disease [39] especially those affecting the liver, should be mindful of

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.

4 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 [4] 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 [4]

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 (*Epinepholus sp.*) and tilapia. In 1997, the [20] jected annual effect of this bacterial contamination on the US aquaculture sector alone was US\$ 10 million and at [8] 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. [46] [116]. *S. Iniae* impacts several developed types of fish such as rainbow trout [117,118], tilapia [119,120] and grouper (*Epinepholus 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 [43] 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 [36]C 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

etiologic 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 β -lactamases in *Aeromonas* to provide resistance to β -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. Hydrophile, *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*, *Tenualosa toli*, *Anabas testudineus* and red snapper in Malaysia. In addition, multidrug resistance (MDR) has also been shown in *A. hydrophile* 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 strains 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.

REFERENCES

- Fukuda Y, Nguyem HD, Furuhashi M, Nakai T. Mass mortality of cultured seven band grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. *Fish Pathol* 1999;31:167-70.
- Chua FHC, Ng ML, Ng KL, Loo JJ, Wee JY. Investigation of outbreaks of a novel disease, "sleepy grouper disease", affecting the brown-spotted grouper, *Epinephelus tauvina* Forskal. *J Fish Dis* 1994;17:417-27.
- Arthur JR, Ogawa K. A brief overview of disease problems in the culture of marine finfishes in east and southeast Asia. In: Main KL, Rosenfeld C, editors. *Aquaculture health management strategies for marine fishes*. Makapu'u Point, HI, USA: The Oceanic Institute; 1996. p. 9-31.
- Lee KK. Pathogenesis studies on *Vibrio alginolyticus* in the grouper, *Epinephelus malabaricus*, Bloch et Schneider. *Microb Pathog* 1995;19:39-48.
- Yii KC, Yang TI, Lee KK. Isolation and characterization of *Vibrio carchariae*, a causative agent of gastroenteritis in the groupers, *Epinephelus coioides*. *Curr Microbiol* 1997;35:109-15.
- Nash G, Anderson IG, Shariff M, Shamsudin MN. Bacteriosis associated with epizootic in the giant sea perch, *Lates calcarifer*, and the estuarine grouper, *Epinephelus tauvina*, cage cultured in Malaysia. *Aquaculture* 1987;67:105-11.
- Danayadol Y, Krachaiwong V, Ruangpan L, Direkbusarakom S. Causative agents and control measures of red boil disease in cultured grouper (*Epinephelus malabaricus*). *Thai Fish Gazette* 1996;49:29-35.
- Kai YH, Chi SC. Efficacies of inactivated vaccines against betanodavirus in grouper larvae (*Epinephelus coioides*) by bath immunization. *Vaccine* 2008;26:1450-1457.
- Chua FHC, Ng ML, Ng KL, Loo JJ, Wee JY. Investigation of outbreaks of a novel disease, 'Sleepy Grouper Disease', affecting the brown-spotted grouper, *Epinephelus tauvina* Forskal. *J. Fish Dis.* 1994; 17, 417-427.
- Chang SF, Ngho-Lim GH, Kueh SFS, Qin QW, Seng EK, Sin, Y.M., 2002. Initial investigations into two viruses isolated from marine food fish. *Vet. Rec.* 150, 15-16.
- Qin QW, Shi CY, Gin KY, Lam TJ. Antigenic characterization of a marine fish iridovirus from grouper, *Epinephelus* spp. *J. Virol. Methods.* 2002;106, 89-96.
- He JG, Lin L, Huang ZJ. The preliminary research on the pathogenicity of *Vibrio alginolyticus* of marine fishes. *J. South China Normal Univ. (Nat. Sci. Ed.)* . 1998; 53-55.
- Zhu CH, He JG, Huang ZJ. Separation, identification and pathogenicity of ulceration disease of *Epinephelus* in cage. *Acta. Sci. Nat. Univ. Sunyatseni.* 2000;39, 278-282.
- Neely M, Pfeifer J, Caparon MG. *Streptococcus*-zebrafish model of bacterial pathogenesis. *Infect. Immun.* 2002;70:3904-3914.
- Kawamura Y, Hou XG, Sultana F, Miura H, Ezaki T. Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. *Int. J. Syst. Bacteriol.* 1995; 45:406-408.
- Janda JM, Abbott SL. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews.* 2010;23, 35-73.
- Praveen PK, Debnath C, Shekhar S, Dalai N, Ganguly S. Incidence of *Aeromonas* spp. infection in fish and chicken meat and its related public health hazards: A review. *Veterinary World.* 2016;9, 6-11.
- Rahim Z, Aziz KM. Enterotoxigenicity, hemolytic activity and antibiotic resistance of *Aeromonas* spp. isolated from freshwater prawn marketed in Dhaka, Bangladesh. *Microbiology and Immunology.* 1994; 38, 773-778.
- Yano Y, Hamano K, Tsutsui I, Aue-umneoy D, Ban M, Satomi M. Occurrence, molecular characterization, and antimicrobial susceptibility of *Aeromonas* spp. in marine species of shrimps cultured at inland low salinity ponds. *Food Microbiology.* 2015; 47, 21-27.
- Yin ZX, He W, Chen WJ, Yan JH, Yang JN, Chan SM, He JG. Cloning, expression and antimicrobial activity of an antimicrobial peptide, epinecidin1, from the orange-spotted grouper, *Epinephelus coioides*. *Aquaculture.* 2006;253, 204-211.
- Cheng A, Cheng S, Chen Y, Chen J. Effects of temperature change on the innate cellular and humoral immune responses of orange-spotted grouper *Epinephelus coioides* and its susceptibility to *Vibrio alginolyticus*. *Fish Shellfish Immunology.* 2009;26, 768-772.
- Saeed MO. Association of *Vibrio harveyi* with mortalities in cultured marine fish in Kuwait. *Aquaculture.* 1995;136, 21-29.
- Lee KK, Liu PC, Chuang WH. Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. *Marine Biotechnology* 4, 267-277.
- Smith P. (2008) Antimicrobial resistance in aquaculture. *Revue Scientifique et Technique (International Office of Epizootics).* 2002;27, 243-264.
- Samad AP, Santoso U, Lee M, Nan F. Effects of dietary katuk (*Sauropus androgynous* L. Merr. on growth, non-specific immune and disease resistance against *Vibrio alginolyticus* infection in grouper *Epinephelus coioides*. *Fish Shellfish Immunology.* 2014; 36, 582-589.
- Oliver JD. The Biology of *Vibrio vulnificus*. *Microbiol Spectr.* 2015;3(3)
- Amaro C, Sanjuan E, Fouz B, et al. The fish pathogen *Vibrio vulnificus* biotype 2: epidemiology, phylogeny, and virulence factors involved in warm-water *Vibriosis*. *Microbiol Spectr.* 2015;3(3)
- Crim SM, Griffin PM, Tauxe R, et al. Preliminary incidence and trends of infection with pathogens transmitted commonly through food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2006-2014. *MMWR Morb Mortal Wkly Rep.* 2015;64(18):495-9
- Haendiges J, Rock M, Myers RA, Brown EW, Evans P, and GonzalezEscalona N. Pandemic *Vibrio parahaemolyticus*, Maryland, USA, 2012. *Emerging Infectious Diseases.* 2014;20, 718-720.

30. Oliver JD. *Vibrio vulnificus*, In FL. Thompson, B. Austin, and J. Swings (ed). The biology of *Vibrios*. ASM Press, Washington, DC. 2006;349-366.
31. Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the Incidence of infection with pathogens transmitted commonly through food-10 states, 2009. . Morbidity and mortality weekly report. 2010;58, 418-422.
32. Klontz KC, Lieb S, Schreiber M, Janowski HT, Baldy LM, Gunn RA. Syndromes of *Vibrio vulnificus* infections. Clinical and epidemiologic features in Florida cases, 1981-1987. *Annals of Internal Medicine*. 1988;109, 318-323.
33. Liu JW, Lee IK, Tang HJ, Ko WC, Lee HC, Liu YC, Hsueh PR, Chuang YC. Prognostic factors and antibiotics in *Vibrio vulnificus septicemia*. *Archives of Internal Medicine*. 2006;166, 2117-2123.
34. Arthur JR, Ogawa K. A brief overview of disease problems in the culture of marine finfishes in east and southeast Asia. In: Main, K.L., Rosenfeld, C. (Eds.), *Aquaculture Health Management Strategies for Marine Fishes*. The Oceanic Institute, HI, USA, 1996, pp. 9-31.
35. Harikrishnan R, Kim MC, Kim JS, Han YJ, Jang IS, Balasundaram C, Heo MS, Immunomodulatory effect of sodium alginate enriched diet in kelp grouper *Epinephelus bruneus* against *Streptococcus iniae*. *Fish Shellfish Immunology* 2011c;30, 543-549.
36. Palavesam A, Sheeja L, Immanuel G. Antimicrobial properties of medicinal herbal extracts against pathogenic bacteria isolated from the infected grouper *Epinephelus tauvina*. *Journal of Biological Research*. 2006; 6, 167-176.
37. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L. Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture*. 2008;274,1-14.
38. Martínez-Murcia, A, Monera A, Alperi A, Figueras MJ, Saavedra MJ. Phylogenetic evidence suggests that strains of *Aeromonas hydrophila* subsp. *dhakensis* belong to the species *Aeromonas aquariorum* sp. nov. *Curr. Microbiol*. 2008;58, 76-80.
39. Von Graevenitz A. The role of *Aeromonas* in diarrhea: a review. *Infection*. 2007;35, 59-64.
40. Chuang YC, Young CD, Chen CW. *Vibrio vulnificus* infection. *Scand J Infect Dis* 1989; 21: 721-6.
41. Chuang YC, Yuan CY, Liu CY et al. *Vibrio vulnificus* infection in Taiwan: report of 28 cases and review of clinical manifestations and treatment. *Clin Infect Dis* 1992; 15: 271-6.
42. Kim HR, Rho HW, Jeong MH et al. Hemolytic mechanism of cytotoxin produced from *V. vulnificus*. *Life Sci* 1993; 53: 571-7.
43. Biosca EG, Amaro C. Toxic and enzymatic activities of *Vibrio vulnificus* biotype 2 with respect to host specificity. *Appl Environ Microbiol* 1996; 62: 2331-7.
44. Hor LI, Chang TT, Wang ST. Survival of *Vibrio vulnificus* in whole blood from patients with chronic liver diseases: association with phagocytosis by neutrophils and serum ferritin levels. *J Infect Dis* 1999; 179: 275-8.
45. It's 45. Powell JL, Strauss KA, Wiley C, etc. Inflammatory cytokine reaction to *Vibrio vulnificus* triggered by peripheral blood mononuclear cells from chronic alcohol consumers is consistent with cellular oxidative stress biomarkers. *Infection Resistant* 2003; 71: 4212-6.
46. Chiang SR, Chuang YC. *Vibrio vulnificus* infection: clinical manifestations, pathogenesis, and antimicrobial therapy. *J Microbiol Immunol Infect* 2003; 36: 81-8.
47. Hsueh PR, Lin CY, Tang HJ et al. *Vibrio vulnificus* in Taiwan. *Emerg Infect Dis* 2004; 10: 1363-8.
48. Klontz KC, Lieb S, Schreiber M et al. Syndromes of *Vibrio vulnificus* infections. Clinical and epidemiologic features in Florida cases, 1981-1987. *Ann Intern Med* 1988; 109: 318-23.
49. Howard RJ, Bennett NT. Infections caused by halophilic marine *Vibrio* bacteria. *Ann Surg* 1993; 217: 525-30.
50. Liu JW, Lee IK, Tang HJ et al. Prognostic factors and antibiotics in *Vibrio vulnificus septicemia*. *Arch Intern Med* 2006; 166: 2117-23.
51. Kuo Chou TN, Chao WN, Yang C et al. Predictors of mortality in skin and soft-tissue infections caused by *Vibrio vulnificus*. *World J Surg* 2010; 34: 1669-75.
52. Chen SC, Chan KS, Chao WN et al. Clinical outcomes and prognostic factors for patients with *Vibrio vulnificus* infections requiring intensive care: a 10-yr retrospective study. *Crit Care Med* 2010; 38: 1984-90.
53. Howard RJ, Pessa ME, Brennaman BH et al. Necrotizing soft-tissue infections caused by marine *Vibrios*. *Surgery* 1985; 98: 126-30.
54. Tsai YH, Hsu RW, Huang TJ et al. Necrotizing soft-tissue infections and sepsis caused by *Vibrio vulnificus* compared with those caused by *Aeromonas* species. *J Bone Joint Surg Am* 2007; 89: 631-6.
55. Tsai YH, Hsu RW, Huang KC et al. Laboratory indicators for early detection and surgical treatment of *Vibrio* necrotizing fasciitis. *Clin Orthop Relat Res* 2010; 468: 2230-7.
56. Mata AI, Gibello A, Casamayor A, Blanco MM, Domínguez L, Fernández-Garayzábal JF, Applied and Environmental Microbiology 2004;70 (5): 3183-3187,
57. Pier GB, Madin SH. *International Journal of Systematic and Evolutionary Microbiology*. 1976;26 (4): 545-553,.
58. Sun JR, Yan JC, Yeh CY, et al. Invasive infection with *Streptococcus iniae* in Taiwan. *J Med Microbiol*. 2007;56:1246-1249.
59. Weinstein MR, Litt M, Kertesz DA, et al. Invasive infections due to a fish pathogen *Streptococcus iniae*. *N Engl J Med*. 1997; 337(9):589-594.
60. Hisamichi M, Yokoyama T, Yazawa M, Kaneshiro N, Sakurada T, Konno Y, et al. 2015. A rare case of peritoneal dialysis-related peritonitis caused by goldfish water tank-derived *Aeromonas hydrophila*. *Clinical Nephrology*, 84(1), 50-54.
61. Mansour AM, Abd Elkhalek R, Shaheen HI, El Mohammady H, Refaey S, Hassan K, et al. (2012). Burden of *Aeromonas hydrophila*-associated diarrhea among children younger than 2 years in rural Egyptian community. *The Journal of Infection in developing Countries*, 6(12), 842-846.
62. Soltan DMM, Mazaheri NFR, Kavan TM, Aghaiyan L, Salehipour Z. Prevalence, virulence and antimicrobial resistance patterns of *Aeromonas* spp. isolated from children with diarrhea. *Germes*. 2016;6(3), 91-96.
63. de la Morena ML, Van R, Singh K, Brain M, Murray ME, Pickering LK. Diarrhea associated with *Aeromonas* species in children in day care centers. *The Journal of Infectious Diseases*, 1993;168(1), 215-218.

64. Zhang Q, Gao T, Feng X. Acute Diarrhea Outbreak by Drinking water Polluted by *A. Aeromonas*. *Journal of Environmental and Health*, 1993;3(10):99–101.
65. Krovacek K, Dumontet S, Eriksson E, Baloda SB. Isolation, and Virulence Profiles, of *Aeromonas hydrophila* implicated in an outbreak of food poisoning in Sweden. *Microbiology and Immunology*, 1995;39(9), 655–661.
66. Zhang Q, Shi GQ, Tang GP, Zou ZT, Yao GH, Zeng G. A foodborne outbreak of *Aeromonas hydrophila* in a college, Xingyi City, Guizhou, China, 2012. *Western Pacific Surveillance and Response Journal*. 2012;3(4), 39–43.
67. Ventura RJ, Muhi E, de los Reyes VC, Sucaldito MN, Tayag E. A community-based gastroenteritis outbreak after Typhoon Haiyan, Leyte, Philippines, 2013. *Western Pacific Surveillance and Response Journal: WPSAR*. 2015;6(1), 1–6.
68. Linkous DA, Oliver JD. Pathogenesis of *Vibrio vulnificus*, FEMS Microbiol. Lett. 174 1999;207-214.
69. Strom MS, Paranjpye RN. Epidemiology and pathogenesis of *Vibrio vulnificus*, *Microbes Infect*. 2000;2 177-188.
70. Powell JL, Wright AC, Wasserman SS, Hone DM, Morris Jr JG. Release of tumor necrosis factor alpha in response to *Vibrio vulnificus* capsular polysaccharide in vivo and in vitro models, *Infect. Immun*. 1997;65 3713-3718.
71. Wright AC, Powell JL, Kaper JB, Morris Jr JG. Identification of a group 1-like capsular polysaccharide operon for *Vibrio vulnificus*, *Infect. Immun*. 2001;69 6893-6901.
72. Simpson LM, Oliver JD. Siderophore production by *Vibrio vulnificus*, *Infect. Immun*. 1983;41 644-649.
73. L.D. Gray, A.S. Kreger, Purification and characterization of an extracellular cytotoxin produced by *Vibrio vulnificus*, *Infect. Immun*. 1985;48.67-72.
74. P.A. Gulig, K.L. Bourdage, A.M. Starks, Molecular pathogenesis of *Vibrio vulnificus*, *J. Microbiol*. 2005;43 118-131.
75. J.H. Lee, M.W. Kim, B.S. Kim, S.M. Kim, B.C. Lee, T.S. Kim, S.H. Choi, Identification and characterization of the *Vibrio vulnificus* rtxA essential for cytotoxicity in vitro and virulence in mice, *J. Microbiol*. 2007;45 146-152.
76. B.C. Lee, J.H. Lee, M.W. Kim, B.S. Kim, M.H. Oh, K.S. Kim, T.S. Kim, S.H. Choi, *Vibrio vulnificus* rtxE is important for virulence, and its expression is induced by exposure to host cells, *Infect. Immun*. 2008;76 1509-1517.
77. Y.R. Kim, S.E. Lee, H. Kook, J.A. Yeom, H.S. Na, S.Y. Kim, S.S. Chung, H.E. Choy, J.H. Rhee, *Vibrio vulnificus* RTX toxin kills host cells only after contact of the bacteria with host cells, *Cell. Microbiol*. 2008;10 848-862.
78. Tison DL, Nishibuchi M, Greenwood JD, Seidler RJ. *Vibrio vulnificus* biogroup 2: new biogroup pathogenic for eels. *Appl. Environ. Microbiol*. 1982;44:640–646.
79. Bisharat N, Agmon V, Finkelstein R, Raz R, Ben-Dror G, Lerner L, et al. Clinical, epidemiological, and microbiological features of *Vibrio vulnificus* biogroup 3 causing outbreaks of wound infection and bacteraemia in Israel. *Lancet* 1999;354:1421–1424.
80. Rosche TM, Yano Y, Oliver JD. A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. *Microbiol. Immunol*. 2005;49:381–389.
81. Nilsson WB, Paranjpye RN, DePaola A, Strom MS. Sequence polymorphism of the 16S rRNA gene of *Vibrio vulnificus* is a possible indicator of strain virulence. *J. Clin. Microbiol*. 41:442–446.
82. Cohen ALV, Oliver JD, DePaola A, Feil EJ, Fidelma Boyd E. 2007. Emergence of a virulent clade of *Vibrio vulnificus* and correlation with the presence of a 33-kilobase genomic island. *Appl. Environ. Microbiol*. 2003;73: 5553–5565.
83. Morrison SS, Williams T, Cain A, Froelich B, Taylor C, Baker-Austin C, Verner-Jeffreys D, Hartnell R, Oliver JD, Gibas CJ. Pyrosequencing-based comparative genome analysis of *Vibrio vulnificus* environmental isolates. *PLoS One* 2012;7: 37553.
84. Warner E, Oliver JD. Population structures of two genotypes of *Vibrio vulnificus* in oysters (*Crassostrea virginica*) and seawater. *Appl. Environ. Microbiol*. 2008;74:80–85.
85. Bogard RW, Oliver JD. Role of iron in human serum resistance of the clinical and environmental *Vibrio vulnificus* genotypes. *Appl. Environ. Microbiol*. 2007;73:7501–7505.
86. Rosche TM, Binder EA, Oliver JD. *Vibrio vulnificus* genome suggests two distinct ecotypes. *Environ. Microbiol. Rep*. 2010;2:128–132.
87. Sanjuan E, Fouz B, Oliver JD, Amaro C. 2009. Evaluation of genotypic and phenotypic methods to distinguish clinical from environmental *Vibrio vulnificus* strains. *Appl. Environ. Microbiol*. 75:1604–1613.
88. Gulig PA, de Crecy-Lagard V, Wright AC, Walts B, Telonis-Scott M, McIntyre LM. SOLiD sequencing of four *Vibrio vulnificus* genomes enables comparative genomic analysis and identification of candidate clade-specific virulence genes. *BMC Genomics* 2010;11:512.
89. Finlay BB, Falkow S. Common themes in microbial pathogenicity revisited, *Microbiol. Mol. Biol. Rev*. 1997;61 136-169.
90. Thanassi DG, Hultgren SJ, Multiple path way sallow protein secretion across the bacterial outer membrane, *Curr. Opin. Cell. Biol*. 2000;12 420-430.
91. Delepelaire P. Type I secretion in gram-negative bacteria, *Biochim. Biophys. Acta* 2004;1694 149-161.
92. Boardman BK, Satchell KJ. *Vibrio cholerae* strains with mutations in an atypical type I secretion system accumulate RTX toxin intracellularly, *J. Bacteriol*. 2004;186 8137-8143.
93. Garmory HS, Titball RW. ATP-binding cassette transporters are targets for the development of antibacterial vaccines and therapies, *Infect. Immun*. 2004;72 6757-6763.
94. Cook DW, Bowers JC, DePaola A. Density of total and pathogenic (tdh1) *Vibrio parahaemolyticus* in Atlantic and Gulf coast molluscan shellfish at harvest. *J Food Prot* 2002;65:187380.
95. DePaola A, Kaysner CA, Bowers J, Cook DW. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Appl Environ Microbiol* 2000;66:464954.
96. Hara-Kudo Y, Sugiyama K, Nishibuchi M, Chowdhury A, Yatsuyanagi J, Ohtomo Y, et al. Prevalence of pandemic thermostable direct hemolysin-producing *Vibrio parahaemolyticus* O3:K6 in seafood and the coastal environment in Japan. *Appl Environ Microbiol* 2003;69:388-391.

97. Zhang W, Meng DM, Pan JC, Zhu FY, Chen K. Characteristics of virulence gene in *Vibrio parahaemolyticus* strains isolated from clinical patients and environment in Hangzhou, China. *Zhonghua Yu Fang Yi Xue Za Zhi* 2004;38:2003.
98. DePaola A, Nordstrom JL, Bowers JC, Wells JG, Cook DW. Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Appl Environ Microbiol* 2003;69:1521-6.
99. Miwa N, Kashiwagi M, Kawamori F, Masuda T, Sano Y, Hiroi M, et al. Levels of *Vibrio parahaemolyticus* and thermostable direct hemolysin gene-positive organisms in retail seafood determined by the most probable number-polymerase chain reaction (MPN-PCR) method. *Shokuhin Eiseigaku Zasshi* 2006;47:415.
100. DePaola A, Jones JL, Woods J, Burkhardt W 3rd, Calci KR, Krantz JA, et al. Bacterial and viral pathogens in live oysters: 2007 United States market survey. *Appl Environ Microbiol* 2010;76:275-468.
101. Nordstrom JL, Kaysner CA, Blackstone GM, Vickery MC, Bowers JC, DePaola A. Effect of intertidal exposure on *Vibrio parahaemolyticus* levels in Pacific Northwest oysters. *J Food Prot* 2004;67:217-882.
102. Honda T, Ni Y, Miwatani T, Adachi T, Kim J. The thermostable direct hemolysin of *Vibrio parahaemolyticus* is a pore-forming toxin. *Can J Microbiol* 1992;38:117-580.
103. Yanagihara I, Nakahira K, Yamane T, Kaieda S, Mayanagi K, Hamada D, et al. Structure and functional characterization of *Vibrio parahaemolyticus* thermostable direct hemolysin. *J Biol Chem* 2010;285:1626774.
104. Matsuda S, Kodama T, Okada N, Okayama K, Honda T, Iida T. Association of *Vibrio parahaemolyticus* thermostable direct hemolysin with lipid rafts is essential for cytotoxicity but not hemolytic activity. *Infect Immun* 2010;78:60-310.
105. Hiyoshi H, Kodama T, Iida T, Honda T. Contribution of *Vibrio parahaemolyticus* virulence factors to cytotoxicity, enterotoxicity, and lethality in mice. *Infect Immun* 2010;78:177-280.
106. Hoashi K, Ogata K, Taniguchi H, Yamashita H, Tsuji K, Mizuguchi Y, et al. Pathogenesis of *Vibrio parahaemolyticus*: intraperitoneal and orogastric challenge experiments in mice. *Microbiol Immunol* 1990;34:35-566.
107. Park KS, Ono T, Rokuda M, Jang MH, Iida T, Honda T. Cytotoxicity and enterotoxicity of the thermostable direct hemolysin-deletion mutants of *Vibrio parahaemolyticus*. *Microbiol Immunol* 2004;48:31-38.
108. Ottaviani D, Leoni F, Serra R, Serracca L, Decastelli L, Rocchegiani E, et al. Nontoxicogenic *Vibrio parahaemolyticus* strains causing acute gastroenteritis. *J Clin Microbiol* 2012;50:41-413.
109. Acha PN, Szyfres B. Zoonoses and communicable diseases common to man and animals. Vol. I. Bacterioses and mycoses. 3rd ed. Scientific and Technical Publication No. 580, Pan American Health Organization, Regional Office of the WHO, Washington, USA, ISBN 92 75 31580 9, 2003:384pp.
110. Kern W, Vanek E, Jungbluth H. Fish breeder granuloma: infection caused by *Mycobacterium marinum* and other atypical mycobacteria in the human. Analysis of 8 cases and review of the literature (in German). *Med. Klin.* 1989;84, 578-583.
111. Harth M, Ralph ED, Faraawi R. Septic arthritis due to *Mycobacterium marinum*. *J. Rheumatol.* 21, 1994:957-960.
112. Ryan JM, Bryant GD. Fish tank granuloma - a frequently misdiagnosed infection of the upper limb. *J. Accid. Emerg. Med.* 1997;14, 398-400.
113. Ang P, Raana-Apiromyakij N, Goh CL. Retrospective study of *Mycobacterium marinum* skin infections. *Int. J. Dermatol.* 2000;39,343-347.
114. Pier GB, Madin SH. *Streptococcus iniae* sp. nov., a beta hemolytic *Streptococcus* isolated from an Amazon freshwater dolphin, *Inia geoffrensis*. *Int J Syst Bacteriol* 1976;26:545-53.
115. Austin D, Austin B. Characteristics of the diseases. Bacterial fish pathogens: disease of farmed and wild fish. Berlin: Springer Press; 2008. p. 18.
116. Low DE, Liu E, Fuller J, McGeer A. *Streptococcus iniae*: an emerging pathogen in the aquaculture industry. In: Scheld WM, Craig WA, Armstrong D, Hughes JM, editors. *Emerging infections*, 3. Washington, DC: ASM Press; 1999. p. 53-65.
117. Eldar A, Bejerano Y, Bercovier H. *Streptococcus shiloi* and *Streptococcus difficile*: two new streptococcal species causing a meningoencephalitis in fish. *Curr Microbiol* 1994;28:139-43.
118. Lahav D, Eyngor M, Hurvitz A, Ghittino C, Lublin A, Eldar A. *Streptococcus iniae* type II infections in rainbow trout *Oncorhynchus mykiss*. *Dis Aquat Organ* 2004;62:177-80.
119. Kvitt H, Colorni A. Strain variation and geographic endemism in *Streptococcus iniae*. *Dis Aquat Organ* 2004;61:67-73.
120. Shoemaker CA, Evans JJ, Klesius PH. Density and dose: factors affecting mortality of *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). *Aquaculture* 2000;188:229-35.
121. Arthur JR, Ogawa K. A brief overview of disease problems in the culture of marine finfishes in east and southeast Asia. In: Main KL, Rosenfeld C, editors. *Aquaculture health management strategies for marine fishes*. Makapu'u point, HI, USA: The oceanic institute; 1996. p. 9-31.
122. Koziriska A. Dominant pathogenic species of mesophilic aeromonads isolated from diseased and healthy fish cultured in Poland. *Journal of Fish Diseases*, 2007;30, 293-301.
123. Candan A, Kucuker M, Karatas S. Motile aeromonad septicemia in *Salmo salar* cultured in the Black Sea in Turkey. *Bulletin of the European Association of Fish Pathologists*. 1995;15, 195-196.
124. Ashraf A, Ahmed A, Fatma I, Amany O, Emad EE. Molecular studies on antibiotic resistant genes of *Aeromonas* species isolated from fish. *Nature and Science*, 2017;15, 90-97.
125. Figueras MJ, Alperi A, Beaz-Hidalgo R, Stackebrandt E, Brambilla E, Monera A, Martínez-Murcia, A. J. et al. *Aeromonas rivuli* sp. nov., isolated from the upstream region of a karst water rivulet. *International Journal of Systematic and Evolutionary Microbiology*, 2011;61, 242-248.
126. Tison DL, Nishibuchi M, Greenwood JD, Seidler RJ. *Vibrio vulnificus* biogroup 2: new biogroup pathogenic for eels. *Appl Environ Microbiol* 1982;44:6406.
127. Oliver JD. *Vibrio vulnificus*. In: Thompson FL, Austin B, Swing J, editors. *Biology of Vibrios*. Washington, DC: American Society for Microbiology; 2006. p. 34966.

128. Oliver JD. *Vibrio vulnificus*. In: Belkin S, Colwell RR, editors. *Oceans and Health: Pathogens in the Marine Environment*. New York: Springer Science; 2006. p. 25376.
129. Amaro C, Biosca EG, Fouz B, Alcaide E, Esteve C. Evidence that water transmits *Vibrio vulnificus* biotype 2 infections to eels. *Appl Environ Microbiol* 1995;61:11337.
130. Amaro C, Biosca EG. *Vibrio vulnificus* biotype 2, pathogenic for eels, is also an opportunistic pathogen for humans. *Appl Environ Microbiol* 1996;62:14547.
131. Amaro C, Hor LI, Marco-Noales E, Bosque T, Fouz B, Alcaide E. Isolation of *Vibrio vulnificus* serovar E from aquatic habitats in Taiwan. *Appl Environ Microbiol* 1999;65:13525.
132. Biosca EG, Oliver JD, Amaro C. Phenotypic characterization of *Vibrio vulnificus* biotype 2, a lipopolysaccharide-based homogeneous O serogroup within *Vibrio vulnificus*. *Appl Environ Microbiol* 1996;62:91827.
133. Bisharat N, Agmon V, Finkelstein R, Raz R, Ben-Dror G, Lerner L, et al. Clinical, epidemiological, and microbiological features of *Vibrio vulnificus* biogroup 3 causing outbreaks of wound infection and bacteraemia in Israel. *Lancet* 1999;354:14214.
134. Broza YY, Danin-Poleg Y, Lerner L, Valinsky L, Broza M, Kashi Y. Epidemiologic study of *Vibrio vulnificus* infections by using variable number tandem repeats. *Emerg Infect Dis* 2009;15:12825.
135. Bisharat N, Amaro C, Fouz B, Llorens A, Cohen DI. Serological and molecular characteristics of *Vibrio vulnificus* biotype 3: evidence for high clonality. *Microbiology* 2007;153:84756.
136. Bisharat N, Cohen DI, Harding RM, Falush D, Crook DW, Peto T, et al. Hybrid *Vibrio vulnificus*. *Emerg Infect Dis* 2005;11:305.
137. Yanestria, S.M., Rahmaniar, R.P., Wibisono, F.J., Effendi, M.H. Detection of *invA* gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Veterinary World*, 2019; 12(1):170-175.
138. Srinivasan P, Ramasamy P. Occurrence, distribution and antibiotic resistance patterns of *Vibrio* species associated with viral diseased shrimp of south Indian Aquaculture environment. *Int. J. Agric. Sci.* 2009; 1, 1-10.
139. Putra, A. R. S., Effendi, M.H., Koesdarto, S., and Tyasningsih, W. Molecular Identification of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* Isolated from Dairy Cows in East Java Province, Indonesia. *Indian Vet. J.* 2019; 96 (10): 26-30.
140. Putra, A.R. Effendi, M.H. Koesdarto, S. Suwarno, S. Tyasningsih, W. and Estoe pangestie, A.T. Detection of the extended spectrum β -lactamase produced by *Escherichia coli* from dairy cows by using the Vitek-2 method in Tulungagung regency, Indonesia. *Iraqi Journal of Veterinary Sciences*, 2020; 34 (1):203-207.
141. Wibisono, F.J. Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A., Witaningrum. A.M.. The Presence of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* On Layer Chicken Farms In Blitar Area, Indonesia. *Biodiversitas*, 2020; 21 (6): 2667-2671
142. Lechumanan V, Yin WF, Lee LH, Chan KG. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* o isolated from retail shrimps in Malaysia. *Front. Microbiol.* 2015; 6, 33.
143. Lechumanan V, Pusparajah P, Tan LTH, Yin WF, Lee LH, Chan KG. Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. *Front. Microbiol.* 2015; 6, 14-17.
144. Zulkifli Y, Alitheen NB, Son R, Raha AR, Samuel L, Yeap SK, Nishibuchi M. Random amplified polymorphic DNA-PCR and ERIC PCR analysis on *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia. *Int. Food Res. J.* 2009, 16, 141-150.
145. Roig FJ, Amaro C. Plasmid diversity in *Vibrio vulnificus* biotypes. *Microbiology* 2009, 155, 489-497.
146. Elhadi N. Antibiotic Resistance and Plasmid Profiling of Clinically Significant *Vibrio vulnificus* Isolated from Coastal Water in Eastern Province of Saudi Arabia. *Br. J. Pharm. Toxicol.* 2012, 3, 93-97.
147. Stevens DL. The flesh-eating bacterium: what's next? *J. Infect. Dis.* 1999; 179:S366-S374.
148. Thornsberry C, Ogilvie P, Kahn J, Mauriz Y. Surveillance of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the United States in 1996-1997 respiratory season. The Laboratory Investigator Group. *Diagn. Microbiol. Infect. Dis.* 1997;29:249-257.
149. Effendi, M. H., Oktavianto, A and Hastutiek, P. Tetracycline Resistance Gene In *Streptococcus Agalactiae* Isolated From Bovine Subclinical Mastitis In Surabaya, Indonesia. *Philipp. Journal of Veterinary Medicine.* 2018; 55 (S1): 115-120.
150. Puah SM, Puthuchearu SD, Liew FY, Chua KH. *Aeromonas aquariorum* clinical isolates: Antimicrobial profiles, plasmids and genetic determinants. *International Journal of Antimicrobial Agents*, 2013.41.
151. Yucel N, Aslim B, Beyatli Y. Prevalence and resistance to antibiotics for *Aeromonas* species isolated from retail fish in Turkey. *Journal of Food Quality*, 2005.28, 313-324.
152. Saavedra M J, Guedes-Novais S, Alves A, Rema P, Tacão M, Correia A, et al. Resistance to β -lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (*Onchorhynchus mykiss*). *International Microbiology*, 2004.7, 207-211.
153. Guz L, Kozinska A. Antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp (*Cyprinus carpio* L.). *Bulletin of the Veterinary Institute in Pulawy*, 2004.48, 391-395.
154. Vivekanandhan G, Savithamani K, Hatha AA, Lakshmanaperumalsamy P. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. *International Journal of Food Microbiology*, . 2002. 76, 165-168.
155. Yi SW, Chung TH, Joh SJ, Park C, Park BY, Shin GW. 2014. High prevalence of blaCTX-M group genes in *Aeromonas dhakensis* isolated from aquaculture fish species in South Korea. *The Journal of Veterinary Medical Science*, 76, 1589-1593.
156. Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, 2006; 8, 1137-1144.
157. Helmi, AM, Mukti, AT, Soegianto, A and Effendi, MH. A Review of Vibriosis in Fisheries: Public Health Importance. *Sys Rev Pharm*, 2020;11(8):51-58

158. Effendi, M. H., Bintari, I. G., Aksoro, E. B. and Hermawan. I. P. Detection of blaTEM Gene of Klebsiella pneumoniae Isolated from swab of food-producing animals in East Java. *Trop. Anim. Sci. J.* 2018; 41:174–178.
159. Oliver JD. The biology of *Vibrio vulnificus*. *Microbiology Spectrum*, 2015.3, 1–10.
160. Roig FJ, González-Candelas F, Sanjuán E, Fouz B, Feil EJ, Llorens C, et al. (2018). Phylogeny of *Vibrio vulnificus* from the analysis of the core-genome: Implications for intra-species taxonomy. *Frontiers in Microbiology*, 5(8), 2613.
161. Drake SL, Whitney B, Levine JF, De Paola A, Jaykus LA. Correlation of mannitol fermentation with virulence-associated genotypic characteristics in *Vibrio vulnificus* isolates from oysters and water samples in the Gulf of Mexico. *Foodborne Pathogen Disease*, 2010.7, 97–101.
162. Sanjuán E, González-Candelas F, Amaro C. Polyphyletic origin of *Vibrio vulnificus* biotype 2 as revealed by sequence-based analysis. *Applied and Environmental Microbiology*, 2011.77, 688–695.
163. Baker-Austin C, Oliver, JD. *Vibrio vulnificus*: New insights into a deadly opportunistic pathogen. *Environmental Microbiology*, 2018.20, 423–430.
164. Centers for Disease Control and Prevention (CDC). *Vibrio parahaemolyticus* Centers for Disease Control and Prevention, Atlanta: 2013
165. Centers for Disease Control and Prevention (CDC). *Vibrio vulnificus* . Centers for Disease Control and Prevention, Atlanta: 2013
166. Oliver JD. *Vibrio vulnificus*, In FL. Thompson, B. Austin, and J. Swings (ed). *The biology of Vibrios*. ASM Press, Washington, DC. 2006;349-366.
167. Zanetti S, Spanu T, Deriu A, Romano L, Sechi LA, Fadda G. In vitro susceptibility of *Vibrio* spp. isolated from the environment. *International Journal of Antimicrobial Agents*. 2001;17, 407-409.
168. Widodo, A., Effendi, M.H., Khairullah, A.R. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. *Sys Rev Pharm*, 2020;11(7): 382-392.
169. Ramandinianto, S.C., Khairullah, A.R., Effendi, M.H., Tyasningsih, W. and Rahmahani, J. Detection of Enterotoxin type B gene on Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from raw milk in East Java, Indonesia. *Sys Rev Pharm*, 2020;11(7):290-298.
170. Khairullah, AR, Sudjarwo, SA, Effendi, MH, Harijani, N, Tyasningsih, W, Rahmahani, J, Permatasari, DA, Ramandinianto, SC, Widodo, A, Riwu, KHP.. A Review of Methicillin-Resistant *Staphylococcus aureus* (MRSA) on Milk and Milk Products: Public Health Importance. *Sys Rev Pharm* 2020;11(8): 59-69.
171. Ramandinianto, S.C., Khairullah, A.R., Effendi, M.H. *MecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dairy farms in East Java, Indonesia. *Biodiversitas*, 2020; 21(8): 3562-3568.

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

ORIGINALITY REPORT

19%

SIMILARITY INDEX

14%

INTERNET SOURCES

16%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

- | | | |
|---|--|----|
| 1 | academic.oup.com
Internet Source | 2% |
| 2 | www.tandfonline.com
Internet Source | 2% |
| 3 | apps.who.int
Internet Source | 1% |
| 4 | www.agriculturejournals.cz
Internet Source | 1% |
| 5 | Sara Elmahdi, Ligia V. DaSilva, Salina Parveen. "Antibiotic resistance of <i>Vibrio parahaemolyticus</i> and <i>Vibrio vulnificus</i> in various countries: A review", <i>Food Microbiology</i> , 2016
Publication | 1% |
| 6 | Abigail Pérez-Valdespino. "Detection and characterization of class 1 integrons in <i>Aeromonas</i> spp. isolated from human diarrheic stool in Mexico", <i>Journal of Basic Microbiology</i> , 10/06/2009
Publication | 1% |

7	Bunlipatanon, Paiboon, and Kittichon U-taynapun. "Growth performance and disease resistance against <i>Vibrio vulnificus</i> infection of novel hybrid grouper (<i>Epinephelus lanceolatus</i> × <i>Epinephelus fuscoguttatus</i>)", <i>Aquaculture Research</i> , 2016.	1 %
8	docksci.com Internet Source	1 %
9	Junqi Liu, Lingfeng Xie, Dun Zhao, Taotao Yang, Yunfei Hu, Zhiliang Sun, Xinglong Yu. " A fatal diarrhoea outbreak in farm-raised s in China is newly linked to potentially zoonotic ", <i>Transboundary and Emerging Diseases</i> , 2018	1 %
10	acikbilim.yok.gov.tr Internet Source	1 %
11	repository.uhamka.ac.id Internet Source	1 %
12	link.springer.com Internet Source	1 %
13	J.J. Guo, C.M. Kuo, Y.C. Chuang, J.W. Hong, R.L. Chou, T.I. Chen. "The effects of garlic-supplemented diets on antibacterial activity against <i>Streptococcus iniae</i> and on growth in	<1 %

orange-spotted grouper, *Epinephelus coioides*", *Aquaculture*, 2012

Publication

14

libres.uncg.edu

Internet Source

<1 %

15

Junqi Liu, Lingfeng Xie, Dun Zhao, Taotao Yang, Yunfei Hu, Zhiliang Sun, Xinglong Yu. " A fatal diarrhea outbreak in farm-raised s in China is newly linked to potentially zoonotic ", *Transboundary and Emerging Diseases*, 2018

Publication

<1 %

16

Keirstead, N. D., J. W. Brake, M. J. Griffin, I. Halliday-Simmonds, M. A. Thrall, and E. Soto. "Fatal Septicemia Caused by the Zoonotic Bacterium *Streptococcus iniae* During an Outbreak in Caribbean Reef Fish", *Veterinary Pathology*, 2013.

Publication

<1 %

17

Sara Elmahdi, Salina Parveen, Sylvia Ossai, Ligia V. DaSilva, Michael Jahncke, John Bowers, John Jacobs. " and Recovered from Oysters during an Oyster Relay Study ", *Applied and Environmental Microbiology*, 2018

Publication

<1 %

18

J Triastuti, D Kintani, E M Luqman, D Y Pujiastuti. " The motility and motion duration of jatimbunan tilapia () spermatozoa in

<1 %

different salinity ", IOP Conference Series:
Earth and Environmental Science, 2018

Publication

19

Ramasamy Harikrishnan, Ju-Sang Kim, Man-Chul Kim, Chellam Balasundaram, Moon-Soo Heo. "Lactuca indica extract as feed additive enhances immunological parameters and disease resistance in Epinephelus bruneus to Streptococcus iniae", Aquaculture, 2011

Publication

<1 %

20

etd.auburn.edu

Internet Source

<1 %

21

tessera.spandidos-publications.com

Internet Source

<1 %

22

Freshinta Jellia Wibisono, Mustofa Helmi Effendi, Freshindy Marissa Wibisono. "Occurrence, antimicrobial resistance, and potential zoonosis risk of avian pathogenic Escherichia coli in Indonesia: A review", International Journal of One Health, 2022

Publication

<1 %

23

interesjournals.org

Internet Source

<1 %

24

myweb.ncku.edu.tw

Internet Source

<1 %

25

H. Hasegawa, E. J. Lind, M. A. Boin, C. C. Hase. "The Extracellular Metalloprotease of Vibrio

<1 %

tubiashii Is a Major Virulence Factor for Pacific Oyster (*Crassostrea gigas*) Larvae", Applied and Environmental Microbiology, 2008

Publication

26

Judith A. Johnson. "Vibrio", Topley & Wilson's Microbiology and Microbial Infections, 03/15/2010

Publication

<1 %

27

Williams, Tiffany C., Mesrop Ayrapetyan, and James D. Oliver. "Identifying the molecular and physical factors that influence *Vibrio vulnificus* chitin attachment", Applied and Environmental Microbiology, 2015.

Publication

<1 %

28

commons.lib.jmu.edu

Internet Source

<1 %

29

spandidos-publications.com

Internet Source

<1 %

30

www.researchgate.net

Internet Source

<1 %

31

www.vetmedmosul.com

Internet Source

<1 %

32

"Infections of Leisure", Springer Nature, 1994

Publication

<1 %

33

Alicia Gibello, Ana Isabel Vela, Eva Martínez - Nevado, Antonio Rodríguez - Bertos et al. "

<1 %

Potentially human - virulent isolates from diseased great pompano () ", Transboundary and Emerging Diseases, 2019

Publication

34

B. C. Lee, J. H. Lee, M. W. Kim, B. S. Kim, M. H. Oh, K.-S. Kim, T. S. Kim, S. H. Choi. "Vibrio vulnificus rtxE Is Important for Virulence, and Its Expression Is Induced by Exposure to Host Cells", Infection and Immunity, 2008

Publication

35

K.P. Koutsoumanis, A. Lianou, J.N. Sofos. "Food Safety: Emerging Pathogens", Elsevier BV, 2014

Publication

36

Myoung Sug Kim, Hyun Do Jeong. "Development of 16S rRNA targeted PCR methods for the detection and differentiation of Vibrio vulnificus in marine environments", Aquaculture, 2001

Publication

37

Ozlem Ulusarac. "Varied Clinical Presentations of Vibrio vulnificus Infections: A Report of Four Unusual Cases and Review of the Literature", Southern Medical Journal, 02/2004

Publication

38

YUTAKA YANO, MASAHITO YOKOYAMA, MASATAKA SATOMI, HIROSHI OIKAWA, SHUNSHENG CHEN. "Occurrence of Vibrio vulnificus

<1 %

<1 %

<1 %

<1 %

<1 %

in Fish and Shellfish Available from Markets in China", Journal of Food Protection, 2004

Publication

39	archive.org Internet Source	<1 %
40	doctiktak.com Internet Source	<1 %
41	dyuthi.cusat.ac.in Internet Source	<1 %
42	mind-in-the.com Internet Source	<1 %
43	scien.net Internet Source	<1 %
44	vdoc.pub Internet Source	<1 %
45	www.yumpu.com Internet Source	<1 %
46	Hsing-Yen Huang, Yan-Chun Chen, Pei-Chi Wang, Ming-An Tsai, Shih-Chun Yeh, Hong-Jen Liang, Shih-Chu Chen. "Efficacy of a formalin-inactivated vaccine against Streptococcus iniae infection in the farmed grouper Epinephelus coioides by intraperitoneal immunization", Vaccine, 2014 Publication	<1 %

47

Koton, Yael, Michal Gordon, Vered Chalifa-Caspi, and Naiel Bisharat. "Comparative genomic analysis of clinical and environmental *Vibrio vulnificus* isolates revealed biotype 3 evolutionary relationships", *Frontiers in Microbiology*, 2015.

Publication

<1 %

48

Lusida, Maria Inge. "Preface", *Procedia Chemistry*, 2016.

Publication

<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

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

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10
