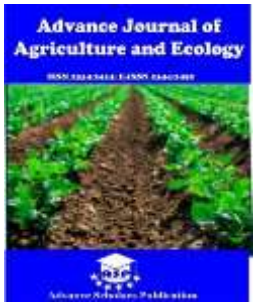
Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



REVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF VIBRIO SPP. AND AEROMONAS SPP. IN PANGASIUS FISH FROM MARKETS IN SIEM REAP, CAMBODIA

Sokhom Dara Vicheka, and Sreyneang Chansovanna Roth
Faculty of Agro-Industry, Royal University of Agriculture, Phnom Penh, Cambodia

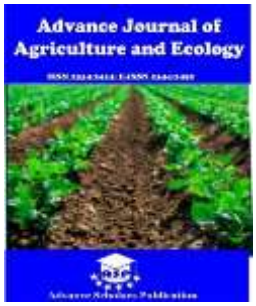
Key words: *Pangasius fish, Vibrio spp., Aeromonas spp., antimicrobial resistance, antibiotic susceptibility*

Abstract: *Pangasius fish, widely consumed in Cambodia, can harbor pathogenic bacteria such as Vibrio spp. and Aeromonas spp., posing risks to both fish production and public health. The increased use of antibiotics in aquaculture has led to the emergence of antibiotic-resistant bacteria, which are more difficult to manage and control. This study investigated the occurrence and antibiotic resistance profiles of Vibrio parahaemolyticus and Aeromonas hydrophila in 30 Pangasius fish samples collected from three markets in Siem Reap, Cambodia. Samples were handled under strict cold-chain conditions before being analyzed in the Microbiology Laboratory at the Royal University of Agriculture (RUA). Bacterial isolation was performed using selective media (TCBS Agar and MacConkey Agar), followed by purification and identification using Gram staining, biochemical tests (catalase, oxidase, TSI, and motility tests), and confirmation with the API 20E kit. Susceptibility testing was conducted against seven antibiotics using the disk diffusion method. Of the samples tested, V. parahaemolyticus (n = 12, 40%) and A. hydrophila (n = 14, 46.67%) were prevalent. Both isolates exhibited high resistance to Ampicillin and Colistin sulphate but remained highly sensitive to Florfenicol, Sulfamethoxazole/Trimethoprim, and Oxytetracycline. These findings highlight the significant presence of antibiotic-resistant bacteria in Pangasius fish, underlining the need for prudent antibiotic use to reduce the risk of antimicrobial resistance that endangers both aquaculture sustainability and public health.*

INTRODUCTION

Antimicrobial resistance (AMR) is an escalating global threat to public health, food security, and economic stability. The emergence and rapid proliferation of drug-resistant pathogens have severely diminished the effectiveness of available antimicrobials for treating infections in humans and animals (WHO, 2014). AMR occurs when bacteria and other microorganisms acquire

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



genetic traits that enable them to survive the lethal effects of antimicrobial agents that would normally inhibit or kill them. These genetic adaptations can occur spontaneously through mutations or be acquired horizontally through mobile genetic elements like plasmids and transposons. Once established, AMR spreads rapidly through ecosystems and food chains, especially under selective pressure driven by inappropriate or excessive antibiotic use in both human medicine and food-animal production (McMillan et al., 2019).

Historical Overview of Antibiotic Discovery and Use

The discovery of penicillin on September 3, 1928, by Alexander Fleming was a transformative milestone in modern medicine (Fleming, 1929). Prior to this breakthrough, minor infections often resulted in severe illness or death. Penicillin's introduction revolutionized healthcare and ushered in the antibiotic era. In the early 1930s, German chemist Gerhard Domagk developed Prontosil, a sulfonamide compound that was effective against streptococcal infections, leading to the first clinical use of a synthetic antimicrobial agent (Sneader, 2001). These advances were followed by large-scale production of penicillin in 1940 by Howard Florey and Ernst Chain, who successfully purified penicillin and initiated its distribution, especially during World War II, saving countless lives (Chain et al., 2005).

By 1947, Waksman formally defined antibiotics as substances produced by microorganisms that inhibit or destroy other microbes, catalyzing an era of rapid drug discovery and commercialization (Waksman, 1947). However, the indiscriminate and sometimes excessive use of these agents over subsequent decades created evolutionary pressure that led to AMR. Resistance to multiple classes of antibiotics, known as multidrug resistance (MDR), has now been detected across a broad range of bacterial pathogens, rendering many frontline treatments ineffective.

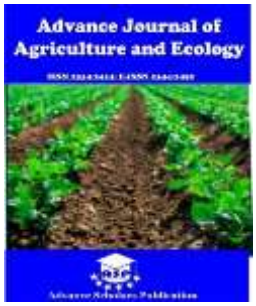
AMR and its Impact on Human and Animal Health

The World Health Organization (WHO) warns that AMR threatens to return us to a "pre-antibiotic era," where infections considered minor today could once again become fatal. The impact of AMR is profound and multidimensional — extending beyond direct clinical challenges to economic burdens associated with long hospital stays, increased treatment costs, and loss of productivity. Moreover, resistant infections in food-producing animals pose serious public health risks. Many zoonotic pathogens originate in food animals, and AMR can transfer from these animals to humans along the food chain, a process well-documented in aquaculture and fishery sectors where antibiotics have been intensively used.

Aquaculture, Food Safety, and Antimicrobial Resistance

Aquaculture, one of the fastest-growing food sectors worldwide, has become a major focus of attention for AMR management. Fish farming

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



often requires the use of prophylactic and therapeutic antibiotics to prevent and control infections in dense farming conditions. However, indiscriminate antibiotic use, especially in countries with lax regulation, can encourage the proliferation of resistant bacteria and contamination of aquatic environments.

Fish are an important protein source across the globe, and their safety and quality are of paramount concern. Pangasius, a freshwater fish species widely farmed in Southeast Asia, including Cambodia, is economically important due to its affordability, nutritional value, and adaptability to different farming systems. Pangasius fish products are consumed by millions and processed into diverse forms such as fillets, fish paste, and traditional dishes like Prahok. However, like other aquatic animals, pangasius can serve as a reservoir for antibiotic-resistant pathogens with implications for food safety and public health.

Pathogens of Concern: Vibrio spp. and Aeromonas spp.

Among the myriad bacteria associated with fish, Vibrio spp. and Aeromonas spp. are notable pathogens. These bacteria are Gram-negative, facultatively anaerobic rods that inhabit aquatic environments and cause infections in fish, humans, and other animals.

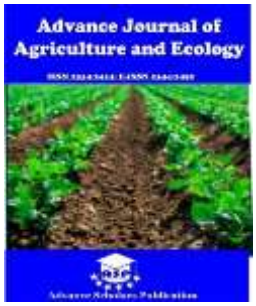
Vibrio spp. include more than 148 recognized species (Wright et al., 1996), several of which — most notably V. parahaemolyticus, V. cholerae, and V. vulnificus — cause foodborne infections in humans, often linked to consumption of raw or undercooked seafood. Infections may present as gastroenteritis, with symptoms including diarrhea, nausea, vomiting, and abdominal pain; some infections can escalate into severe and potentially fatal systemic illnesses (Farmer et al., 2015). V. parahaemolyticus in particular is a leading cause of seafood-associated gastroenteritis worldwide, and its ability to produce thermostable direct hemolysin (TDH) contributes to its virulence. The presence of Vibrio spp. in pangasius fish indicates potential contamination of fish during harvest, transport, or handling, especially if cold-chain management is inadequate.

Aeromonas spp., especially A. hydrophila, are commonly present in freshwater and brackish water, as well as on fish surfaces, gills, and intestines. These bacteria can produce a range of extracellular toxins and enzymes that facilitate infection. In humans, A. hydrophila infections may cause gastroenteritis and, in some cases, more severe infections, including wound infections, sepsis, and necrotizing fasciitis (Swann, 2007). Aeromonads have also been implicated in causing hemorrhagic septicemia in fish, leading to significant economic losses in aquaculture.

Aquaculture Practices and Selective Pressure for Resistance

Poor husbandry, crowded rearing conditions, and inadequate water quality exacerbate infections in farmed fish, prompting increased antibiotic use. Under these conditions, antibiotic-resistant strains gain selective

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



advantages, rapidly outcompete susceptible populations, and disseminate across production systems. Beyond the farm, resistant bacteria may also contaminate processing equipment, transport containers, and retail market surfaces, increasing consumer exposure.

Studies have shown that Vibrio spp. and Aeromonas spp. can harbor a range of resistance genes and often exhibit resistance to commonly administered aquaculture antibiotics, including tetracyclines, ampicillin, and colistin (Rasul & Majumdar, 2017). Some strains possess Shiga toxin-encoding genes (stx1, stx2), which can provoke severe gastrointestinal illness, further raising public health concerns (Alperi & Figueras, 2010).

Public Health and Regulatory Implications

Consumers often do not appreciate the microbial risks present in seafood products, especially when fish is purchased at open-air markets under minimal hygienic control. Resistance of pathogens to first-line antibiotics complicates the clinical management of infections acquired through consumption of contaminated fish. Moreover, transfer of resistance genes from fish-associated bacteria to human gut flora could establish reservoirs of AMR in the human population.

The growing crisis necessitates a multifaceted response. Strengthening veterinary oversight and implementing prudent antibiotic use guidelines in aquaculture, coupled with improving fish handling and cold chain logistics, can reduce the spread of AMR. Enhanced surveillance of retail fish markets, molecular characterization of resistant isolates, and ongoing public awareness campaigns about seafood safety and AMR are also vital. Finally, adopting an integrated "One Health" approach that acknowledges the connections between aquatic ecosystems, agricultural practices, food safety, and public health is essential for sustainable control of AMR.

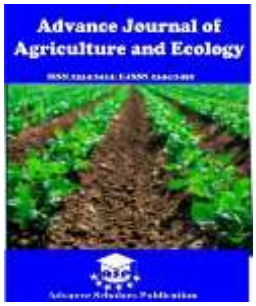
Conclusion

Given the global threat of AMR and its emergence at the aquaculture–human interface, this study aims to detect and identify Vibrio spp. and Aeromonas spp. present in pangasius fish from three markets in Siem Reap, Cambodia, and to assess their antibiotic susceptibility patterns. Understanding these microbial profiles will support evidence-based interventions to reduce antibiotic misuse, improve fishery practices, and protect public health.

LITERATURE REVIEW

Pathogens Threatening Pangasius Fish Survival Pangasius fish are known to survive in polluted environments that undergo seasonal changes, as well as in high-density populations. However, these fish are prone to infection problems (Nam, 2009). Aeromonas spp., particularly Aeromonas hydrophila, have been identified as major pathogens for fish transmission (Ferguson et al., 2001). Other pathogens such as Vibrio spp., Mycobacterium spp., Listonella anguillarum, Vibrio salmonicida, and Photobacterium damsela (Lafferty et al.,

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



2015) can also significantly affect the health and survival of fish.

The Case Study of Foodborne Illness in Cambodia Attention to tightening food safety in Cambodia is still limited. Foodborne illness caused by microorganisms is a matter of concern because the health of many people who eat it is affected, so food quality factors need to be taken into account. Precautions for food safety reduce the risk of contamination and protect consumers from foodrelated diseases and injuries (Shahen, 2024). Pangasius is a type of fish that is popularly eaten and processed into a variety of food products. The popularity of eating this type of fish requires careful caution regarding to the safety of harmful microorganisms present in the body of the fish. Due to an incident that occurred on April 10, 2012, the administration of Kampong Speu Provincial Hall informed the Department of Infectious Diseases Management that 49 cases of diarrhea and severe vomiting were found two days after most patients attended the wedding in Tbong Boeng village on April 8. The next conclusion is that these cases are food poisoning caused by Vibrio spp. which V. parahaemolyticus species were present in wedding dishes (Vandy et al., 2012).

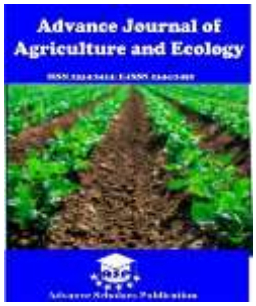
Environmental Resilience and Health Risks of Vibrio **spp.** Vibrio spp. is a gram-negative bacteria that contain lipopolysaccharide outside the cell membrane. It belongs to the Vibrionaceae family which can survive in water that is resistant to salinity, including hot water, clear water, sewage and seawater. Vibrio is a multi-genetic group with genetic evolution and rod shape (Thompson et al., 2005). It is important to note that three of Vibrio spp. are most commonly found in seawater and freshwater known as V. cholerae, V. parahaemolyticus, and V. vulnificus. Risks caused by Vibrio spp. to the environment, animals, and human health are believed to be spread by factors of climate change, especially global warming, which also affects seawater (Vezzulli et al., 2013). Rising temperatures may directly or indirectly contribute to mutations in the genes of viruses and the recent geographical adaptation of Vibrio spp (Greenfield et al., 2017). Significant factors of cholera transmission caused by Vibrio spp. occur through water and food. Conditions that favor the occurrence of cholera are due to poor quality sanitation of water sources (EFSA, 2021).

Aeromonas

spp. as a Threat to Fish Health and Public Health

Aeromonas spp. is a gram negative, rod-shaped, 1 to 3.5 μm long, belonging to the family Enterobacteriaceae (Martin et al., 2005). On the other hand, the presence of Aeromonas spp. is also a problem that contributes to foodborne illness. Experiments have shown that fish in poor environments due to improper water quality, such as high nitrate levels, low dissolved oxygen levels or high carbon dioxide levels, are vulnerable to Aeromonas hydrophila infections. The presence of this bacterium not only causes disease but also affects fish production, causing

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



damage and economic loss (Amber, 2022). The risk of transmitting A. hydrophila to humans is through the consumption of infected fish, which can cause gastrointestinal diseases, pneumonia, diarrhea and meningitis, so attention should be paid to the presence of this bacterium crucially for public health (Kirov, 1993).

Antimicrobial Resistance in Cambodia as a Part of Growing Global Concern

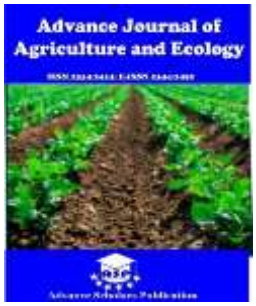
Meanwhile, in response to the antimicrobial resistance problem, which is a major global issue, as well as part of the vision of a multi-sectoral action plan on antimicrobial resistance in Cambodia, the use of non-compliance with technical principles and standards recommended by livestock experts has led to bacterial resistance to antimicrobials and natural immunity. Antimicrobial residues such as tetracycline, ciprofloxacin, enrofloxacin and amoxicillin are found in meat and eggs sold for human consumption (Islam et al., 2016). Antimicrobial resistance occurs when a type of microbe becomes resistant to antibiotics due to overuse. These immunocompromised microbes can infect humans or animals and are more difficult to treat than diseases caused by non-immune bacteria (O'Neill, 2015). Antibiotics have been used in human treatment, animal production and in preventing disease. It has been mixed with feed or water for animals or if the animal has a serious disease, the injection with antibiotics has been applied (Rahman et al., 2021). Hence, food systems are delicate, and current farming methods have a big impact on food security. (Chapagai et al., 2023).

Treatment Approaches for Vibrio **spp. Infections** Most infections that caused by Vibrio spp. does not require much clinical treatment, but is often treated with antibiotics in case of severe Vibriosis (Loo et al., 2020). Currently, in some cholera treatments, the antibiotic known as Azithromycin, Doxycycline (Tetracycline) or Ciprofloxacin (Aquinolone) are used (Das et al., 2020). Doxycycline and third-generation cephalosporins are currently recommended for V. vulnificus as a primary treatment, while Doxycycline or Quinolone are for V. parahaemolyticus. V. vulnificus infections often have serious consequences, even when prescribed antibiotic therapy is applied at an early stage (Hendren et al., 2017). Increased immunity to Vibrio spp. is due to the association or transfer of genes between immune and non-immune strains, including the multifaceted environmental factors that people use (Pérez et al., 2021).

Antibiotic Resistance in Aeromonas **spp.**

Ciprofloxacin was effective against A. hydrophila as well as effective against bacteria A. caviae and A. veronii bv sobria (Ko et al., 2003). Susceptibility of Aeromonas spp. that resistance to the antibiotics such as Cefotaxime, Ciprofloxacin, Trimethoprim, Chloramphenicol, Tetracycline and Cotrimoxazole has also been reported in a separate study (Vila et al., 2003). The presence of immunity in the food and water in which Aeromonas spp. bacteria are present

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



has been observed to increase their ability to survive and grow even under antimicrobial conditions. This suggests that the immunity plays a crucial role in the persistence of these bacteria in different environments (Alcaid et al., 2010). The widespread use of antimicrobials for the prevention and treatment of humans and fish actually contributes to the increasing number of A. hydrophila species that can develop its immunity.

MATERIALS AND METHODS

Study Area and Sampling Size

The study was conducted to isolate Vibrio spp. and Aeromonas spp. from three markets in Siem Reap province, in each market, 10 samples were randomly caught and done by the sellers who usually used to collect or sell to the buyers as usual. Each stall caught a total of 30 samples which were tightly packed in cooler bags during transport. The sample bag is tightly closed and does not sink or seep into the sample during transport to the laboratory at Royal University of Agriculture.

Sampling Methodology

10 grams of each fresh pangasius fish were cut into a sterile bag. Each 90 ml of Buffered peptone water was prepared to mix with the sample in a sterile bag. The sample bags were placed to be separated by the Stomacher machine at 1200 rpm for 2 minutes, equivalent to the first dispersal (10^{-1}). Then, 1 ml was taken from the sealed sample bag and separated with each prepared tip containing 9 ml of saline solution to be diluted. The samples were ready to be detected and identified for Vibrio spp. and Aeromonas spp.

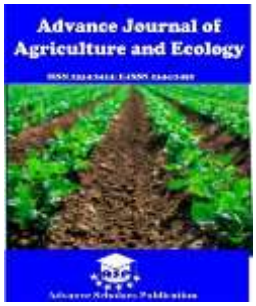
Detection of Vibrio spp. and Aeromonas spp. Methodology

After the sample was prepared and diluted, each diluted tip was filtered by 0.1 ml spreading on TCBS Agar for detecting Vibrio spp. and the same for detecting Aeromonas spp. but on MacConkey Agar prepared in Petri dishes. After placing the bacteria in the incubator for 24 hours, if Vibrio spp., the colonies that grew on the surface of the seedlings would be yellow or green. If Aeromonas spp., the colonies would be gray. The suspected colonies were then purified on the nutrient agar for 24h incubation before the chemical test to confirm the strains. After the purification, the confirmed colonies were detected and ready to be identified.

Identification of Vibrio spp. and Aeromonas spp. Methodology

The identification process consisted of various steps. Starting from the pure culture colonies were tested for morphology and gram type of bacteria. Vibrio spp. is identically a gram-negative, in the form of curved rodshaped bacteria rods, that can be clustered or solitary and move by flagellin and hairs. Aeromonas spp. is also identically a negative gram type that has a single round shape or a pair. After the gram staining, a catalase test was performed to test if the bacteria had the presence of the enzyme Catalase. This test used hydrogen peroxide (H_2O_2) because the enzyme Catalase has the ability to break down hydrogen peroxide into

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



oxygen and water. The test performed by the colony must not last more than 24 hours, as enzyme activity may decrease. Both Vibrio spp. and Aeromonas spp. are identically catalase-positive. The catalase-positive colonies were then continued to oxidase test. The test was performed to confirm if the bacteria had the ability to produce the enzyme using Oxidase strips (OXIOD). Both Vibrio spp. and Aeromonas spp. are identically oxidase-positive. If the color on the strip changes to purple, it can be confirmed that it is an oxidasepositive. Next, TSI test was performed to demonstrate the ability of bacteria to use sugars to ferment and produce hydrogen sulfide which both bacteria identically have. Using a sterile needle, the pure colony was scraped into the slant surface, poured into the center of the bottom of the test tube of TSI agar, and placed it in the incubator at 35 °C for 24 hours to read the results. A motility test was also performed simultaneously to test the ability of the bacteria to move on their own through a tail called flagella or by moving through a fiber called a fibril. This step is one of the necessary identification processes because both bacteria detected identically can move. A Sterile needle was used to extract the pure colony and inject it into the center of the TSA agar without reaching the bottom of the tube then incubated in an incubator at 35 °C for 24 hours. If the stain is still visible, it means that the bacteria are not moving (Non-Motile) and if there is movement, the bacteria will grow all over the surface of the tube. Lastly, to accurately confirm the identification of the bacteria strains, this experiment in the study included the confirmation process using the API 20E System Version 5.0. First, the refined colonies were mixed

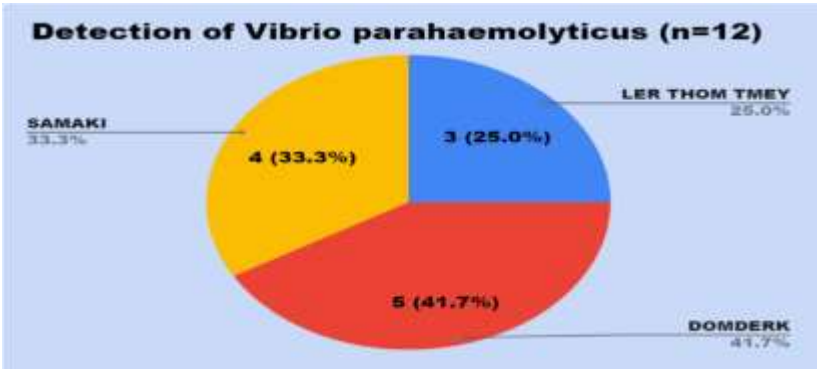
Table 1: Susceptibility to antibiotic resistance

into a tube containing 7 ml of API AUX Medium using a loop. The lid of the API 20E Test kit was opened to drip distilled water through the small holes in the cover to maintain the level of evaporation during insertion into the incubator. Then the solution of 1 ml was pumped into each of the 20 holes of the chemical. Carefully, the lid of the API 20E Test kit closed and placed in the incubator at 35 °C for 24 hours. Then the results were recorded, interpreted, and analyzed the identification in the API System to confirm which strain of the bacteria in what species.

Disk Diffusion Method for Antibiogram

First, the purified colonies were transferred to a tube containing 4 ml of saline solution (0.9% NaCl) and mixed thoroughly. 0.1 ml of solution was transferred onto the Mueller Hinton Agar (MHA) using a spreader to cover the surface of the MHA. Sterile braces were carefully used to apply antibiotics to the measured surface of the agar. Then placed in an incubator at 35 °C for 24 hours. After the incubation, the immunity of the antibiotic was measured using diameter.

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



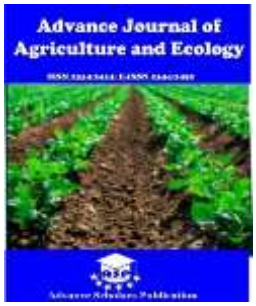
No.	Antibiotics	Abbreviation	Amount	Critical concentration (mg/l)		Critical Diameters (mm)	
				S	R	S	R
1	Ampicillin	AMP	10µg	≤4	>16	≥19	<14
2	Florfenicol	FFC	30µg	≤8	>16	≥23	<19
3	Oxytetracycline	OT	30µg	≤4	>8	≥13	<10
4	Erythromycin	E	15µg	≤1	>4	≥22	<17
5	Colistin Sulphate	CL	10µg	≤2	>4	≥16	<10
6	Ciprofloxacin	CIP	5µg	≤1	>1	≥22	<22
7	Sulfamethoxazole/ Trimethoprim	SXT	1.25/23.75µg	≤2/38	>8/152	≥16	<10

Note: S: Sensitive, R: Resistant, I: Intermediate
Adapted from: CLSI Guideline (2006, 2016, & 2021)

RESULTS AND DISCUSSION

Detection and Identification of Vibrio parahaemolyticus According to the result, out of the 30 samples collected from three markets in Siem Reap province, 12 were found to be positive for Vibrio spp, which represents 40% of the total samples tested. The API 20E System Version 5.0 tests have conclusively identified the presence of the Vibrio parahaemolyticus strain in all of the 12 samples. Furthermore, Vibrio parahaemolyticus was detected in 5 samples from the Domdek market, accounting for 41.7% of the samples. The Samaki market had 4 samples, making up 33.3%, while 3 samples from the Ler Thom Tmey

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



market, making up 25%, were also identified as having the same strain.

Figure 1: Detection of Vibrio parahaemolyticus from the three markets

Detection and Identification of Aeromonas hydrophila

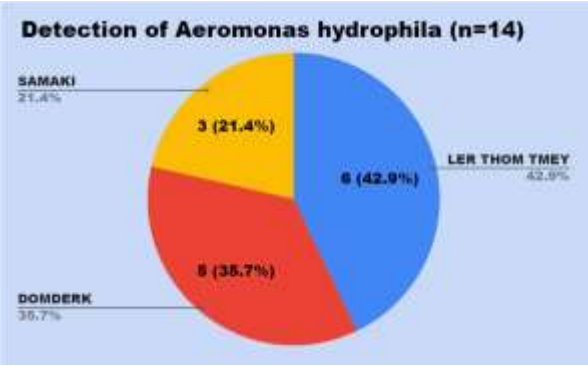
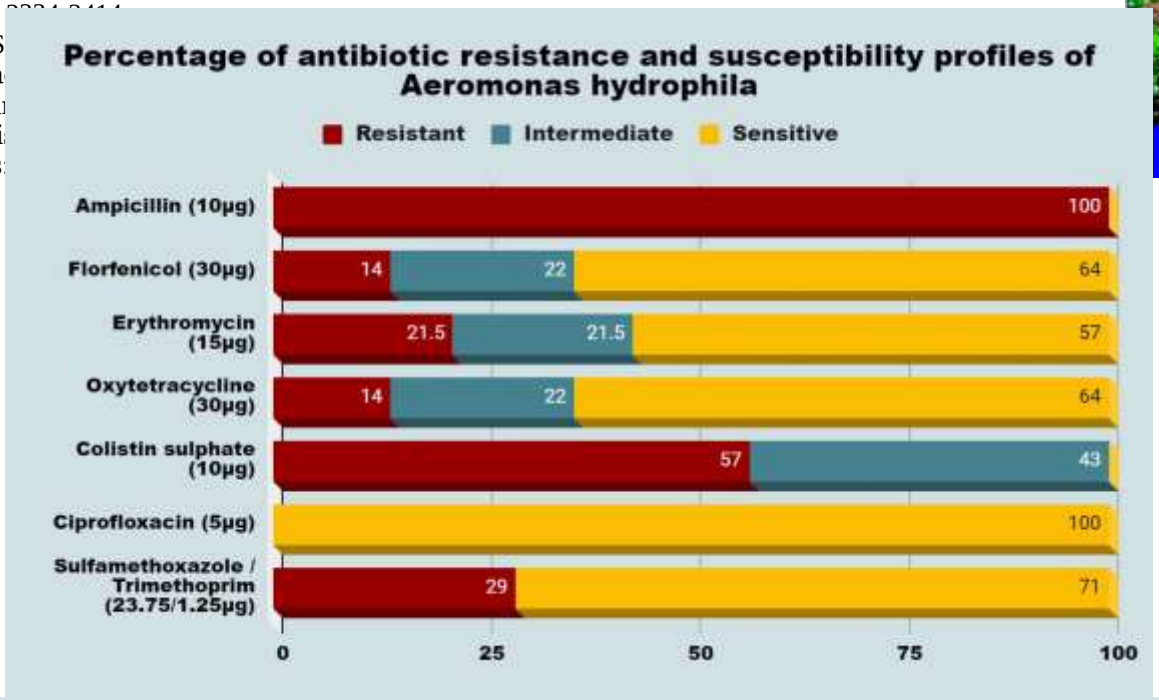
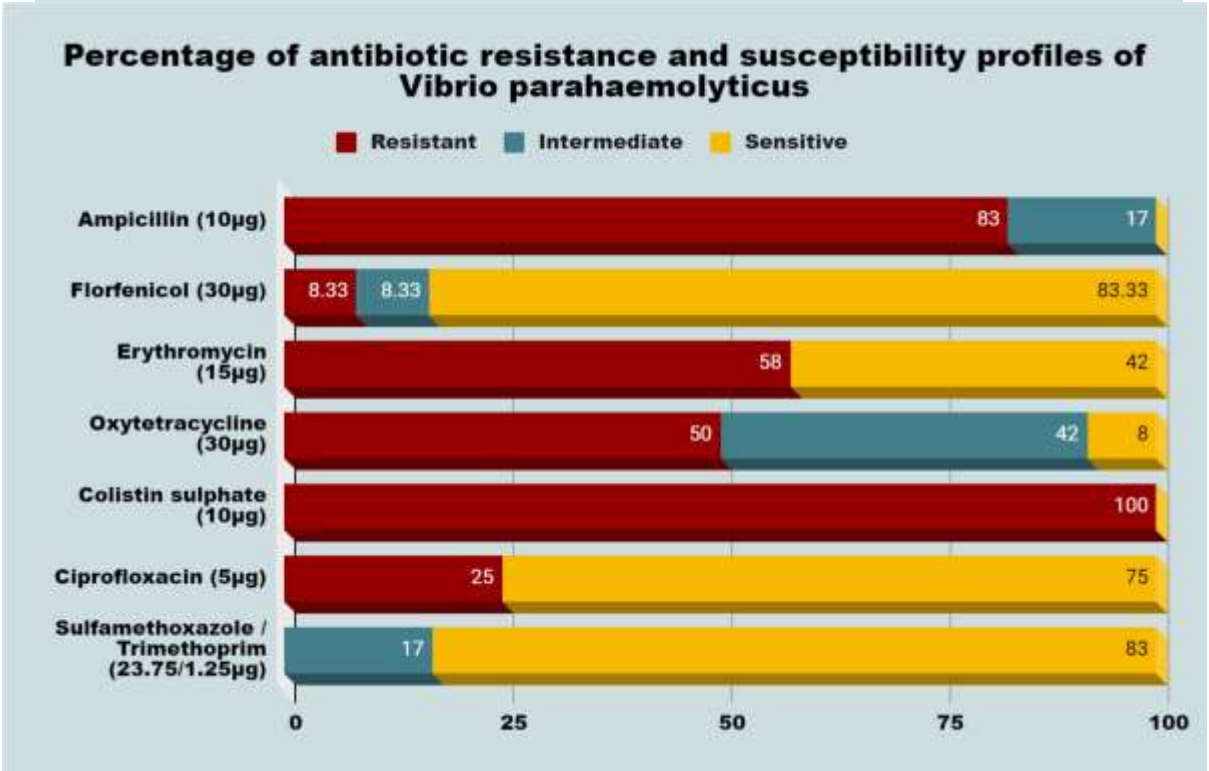


Figure 2: Detection of Aeromonas hydrophila from the three markets

According to the result, out of the 30 samples collected from three markets in Siem Reap province, 14 were found to be positive for Aeromonas spp., which represents
46.67% of the total samples tested. The API 20E System Version 5.0 tests have conclusively identified the presence of the Aeromonas hydrophila strain in all of the 14 samples. Furthermore, Aeromonas hydrophila was detected in 6 samples from the Ler Thom Tmey market, accounting for 42.9% of the samples. The the Domdek market had 5 samples, making up 35.7%, while 3 samples from the Samaki market, making up 21.5%, were also identified as having the same strain.

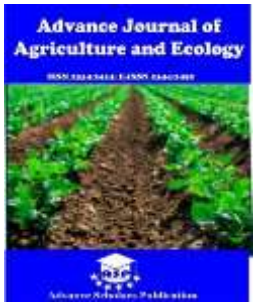
Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN
E-ISS
Impa
Adva
Publi
https





A

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



ntibiotic Resistance and Susceptibility Profiles of Vibrio parahaemolyticus

The results showed that Vibrio parahaemolyticus was resistant to Ampicillin at 83%, Florfenicol at 8.33%, erythromycin at 58%, and oxytetracycline at 50%, Colistin sulphate was 100%, ciprofloxacin was 25% and sulfamethoxazole/ Trimethoprim was 0%. Hence, Vibrio parahaemolyticus was 0% sensitive to Ampicillin, 83,33% to Florfenicol, 42% to Erythromycin, 8% to Oxytetracycline, 0% to colistin sulphate, 75% to ciprofloxacin and sulfamethoxazole/ Trimethoprim was 83%.

Figure 3: Antibiotic resistance and susceptibility profiles of Vibrio parahaemolyticus as percentage

Figure 4: Antibiotic resistance and susceptibility profiles of Aeromonas hydrophila as percentage

Multidrug resistance profiles of Vibrio parahaemolyticus resistance profiles that are resistant to more than two Based on their susceptibility number by group of antimicrobials were grouped into 6 profiles. antimicrobials, Vibrio parahaemolyticus with multidrug

Table 1: Multidrug resistance profiles of Vibrio parahaemolyticus

Profile of Multidrug Resistance	Sample Code	Group of antimicrobials	Number of Drugs
Profile 1	SR-DD-R14 and SR-SK-R26	AMP, E, OT, CL, CIP, SXT	6
Profile 2	SR-DD-R15	AMP, E, OT, CL, CIP	5
Profile 3	SR-DD-R16	AMP, E, OT, CL	4
Profile 4	SR-SK-R28	AMP, FFC, E, CL	4
Profile 5	SR-LT-R6	AMP, OT, CL	3
Profile 6	SR-LT-R10	E, OT, CL	3

Note: Confirmed strains resistant to more than two antimicrobials are considered multidrug resistance profiles.
Ampicillin (AMP), Florfenicol (FFC), Erythromycin (E), Oxytetracycline (OT), Colistin Sulphate (CL), Ciprofloxacin (CIP) and Sulfamethoxazole/Trimethoprim (SXT)

Multidrug resistance profiles of Aeromonas hydrophila resistance profiles that are resistant to more than two Based on their susceptibility number by group of antimicrobials were grouped into 4 profiles. antimicrobials, Aeromonas hydrophila with multidrug

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index

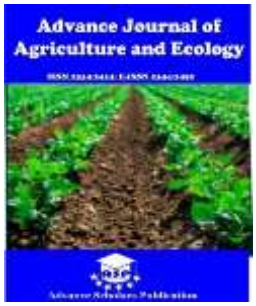


Table 2: Multidrug resistance profiles of Aeromonas hydrophila

Profile of Multidrug Resistance	Sample Code	Group of antimicrobials	Number of Drugs
Profile 1	SR-LT-R2	AMP, FFC, E, SXT	4
Profile 2	SR-LT-R8	AMP, OT, CL, SXT	4
Profile 3	SR-LT-R1	AMP, FFC, E	3
Profile 4	SR-LT-R4	AMP, E, CL	3
Profile 5	SR-LT-R7	AMP, OT, SXT	3

Note: Confirmed strains resistant to more than two antimicrobials are considered multidrug resistance profiles.

Ampicillin (AMP), Florfenicol (FFC), Erythromycin (E), Oxytetracycline (OT), Colistin Sulphate (CL), Ciprofloxacin (CIP) and Sulfamethoxazole/Trimethoprim (SXT)

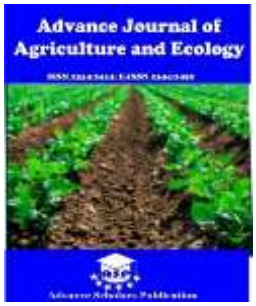
CONCLUSION

In conclusion, the results of the above experimental study can be concluded that the total presence of Vibrio parahaemolyticus bacteria (n=12) was about 40% and Aeromonas hydrophila (n=14) was about 46.67% of the total sample taken from pangasius fish in the three markets at Siem Reap province. This shows that Aeromonas hydrophila was 6.66% more than Vibrio parahaemolyticus. The presence of both bacteria is often a cause for concern for fish infections and food poisoning or disease in people who eat fish containing these toxins and bacteria. Antibiotics with the highest resistance to these two bacteria are Ampicillin and Colistin Sulphate, and the two most sensitive to Florfenicol, Sulfamethoxazole/ Trimethoprim and Oxytetracycline. The high doses of Ampicillin and Colistin Sulphate have been shown to induce bacterial immunity, both of which are not feared, and these drugs are not effective in killing or treating fish that contain both bacteria. Based on the result, the study suggests that farmers should follow the antibiotics guidelines recommended by veterinarians to avoid the misuse and overuse of Antibiotics which could lead to AMR. Moreover, next academic researchers should identify the gap between AMU and AMR including the alternative options. Significantly, Policy researchers and lawmakers should strengthen the effectiveness of AMU policy following the One Health guideline by WHO.

REFERENCES

Alcaide, E., Blasco, M. D., & Esteve, C. (2010). Mechanisms of quinolone resistance in *Aeromonas* species isolated from humans, water, and eels. *Research in Microbiology*, 161(1), 40–45.

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



https://doi.org/10.1016/j.resmic.2009.10.006

Alperi, A., & Figueras, M. J. (2010). Human isolates of *Aeromonas* possess Shiga toxin genes (stx1 and stx2) highly similar to the most virulent gene variants of *Escherichia coli*. *Clinical Microbiology and Infection*, 16(7), 631–637. https://doi.org/10.1111/j.1469-0691.2009.02904.x

Amber, C. A. (2022). Foodborne illness: Symptoms, causes, treatment. *Healthline*. https://www.healthline.com/health/food-borne-illness

Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., & Sanders, A. G. (2005). The classic: Penicillin as a chemotherapeutic agent. 1940. *Clinical Orthopaedics and Related Research*, 439, 23–26. https://doi.org/10.1097/01.blo.0000184737.38947.e8

Chapagai, P. P., Katel, O., & Penjor, P. (2023). Relationships between food systems, agricultural practices, and food security amidst climate change in Western Bhutan. *American Journal of Food Science and Technology*, 3(1), 1–12. https://doi.org/10.54536/ajfst.v3i1.2295

Clinical and Laboratory Standards Institute. (2006). *Performance standards for antimicrobial disk susceptibility tests: Approved standard* (9th ed., M2-A9). Wayne, PA: Author.

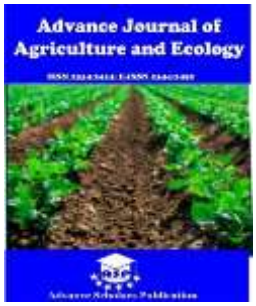
Clinical and Laboratory Standards Institute. (2016). *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria* (3rd ed., M45). Wayne, PA: Author.

Clinical and Laboratory Standards Institute. (2021). *Performance standards for antimicrobial susceptibility testing* (31st ed., M100). Wayne, PA: Author.

Das, B., Verma, J., Kumar, P., Ghosh, A., & Ramamurthy, T. (2020). Antibiotic resistance in *Vibrio cholerae*: Understanding the ecology of resistance genes and mechanisms. *Vaccine*, 38(A), 83–92. https://doi.org/10.1016/j.vaccine.2019.06.031

European Food Safety Authority, & European Centre for Disease Prevention and Control. (2021). The European Union One Health 2019 zoonoses report. *EFSA Journal*, 19(1), e05926. https://doi.org/10.2903/j.efsa.2021.5926

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



FAO. (2021). *Advances in science and risk assessment tools for Vibrio parahaemolyticus and V. vulnificus associated with seafood*. Food and Agriculture Organization. https://doi.org/10.4060/cb5834en

Farmer, J. J., Michael, J. G., Brenner, F. W., Cameron, D. N., & Birkhead, K. M. (2015). Vibrio. In The Editorial Board; Association with Bergey's Manual Trust (Eds.), *Bergey's manual of systematics of Archaea and bacteria* (pp. 1–79). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118960608.gbm00284

Ferguson, F. W., Turnbull, J. F., Shinn, A., Thompson, K., Dung, T. T., & Crumlish, M. (2001). Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish Diseases*, 24(8), 509–513. https://doi.org/10.1046/j.1365-2761.2001.00320.x

Fleming, A. (1929). On antibacterial action of culture of *Penicillium*, with special reference to their use in isolation of *Bacillus influenzae*. *British Journal of Experimental Pathology*, 10(3), 226–236.

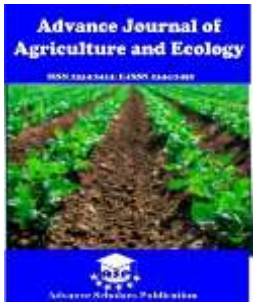
Greenfield, D., Moore, J., Stewart, J. R., Hilborn, E., George, B., Li, Q., Dickerson, J., Keppler, C., & Sandifer, P. (2017). Temporal and environmental factors driving *Vibrio vulnificus* and *V. parahaemolyticus* populations and their associations with harmful algal blooms in South Carolina detention ponds and receiving tidal creeks. *GeoHealth*, 1(9), 306–317. https://doi.org/10.1002/2017GH000105

Hendren, N., Sukumar, S., & Glazer, C. (2017). *Vibrio vulnificus* septic shock due to a contaminated tattoo. *BMJ Case Reports*, bcr2017220199. https://doi.org/10.1136/bcr-2017-220199

Islam, A., Saifuddin, A., Al Faruq, A., Islam, S., Shano, S., Alam, M., & Hassan, M. (2016). Antimicrobial residues in tissues and eggs of laying hens at Chittagong, Bangladesh. *International Journal of One Health*, 2(2), 75–80. https://doi.org/10.14202/IJOH.2016.75-80

Kirov, S. M. (1993). The public health significance of *Aeromonas* spp. in foods. *International Journal of Food Microbiology*, 20(3–4), 179–198. https://doi.org/10.1016/0168-1605(93)90164-C

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



Ko, W. C., Chiang, S. R., Lee, H. C., Tang, H. J., Wang, Y. C., & Chuang, Y. C. (2003). In vitro and in vivo activities of fluoroquinolones against *Aeromonas hydrophila*. *Antimicrobial Agents and Chemotherapy*, 47(7), 2217–2222. https://doi.org/10.1128/AAC.47.7.2217-2222.2003

Lafferty, K. D. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual Review of Marine Science*, 7, 471–496. https://doi.org/10.1146/annurev-marine-010814-015646

Lamy, B., Kodjo, A., & Laurent, F. (2009). Prospective nationwide study of *Aeromonas* infections in France. *Journal of Clinical Microbiology*, 47(4), 1234–1237. https://doi.org/10.1128/JCM.02091-08

Loo, K. Y., Letchumanan, V., Law, J. W., Pusparajah, P., Goh, B. H., Mutalib, N. S. A., & Lee, L. H. (2020). Incidence of antibiotic resistance in *Vibrio* spp. *Reviews in Aquaculture*, 12(4), 2590–2608. https://doi.org/10.1111/raq.12460

Martin, A., Joseph, S., & Genus, I. (2005). *Aeromonas*. In D. J. Brenner & N. R. Krieg (Eds.), *Bergey's manual of systematic bacteriology* (2nd ed., Vol. 2, pp. 556–578). Springer.

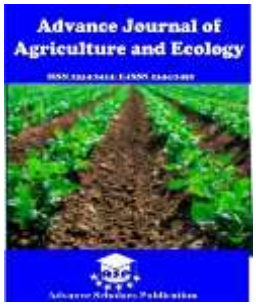
McMillan, E. A., Gupta, S. K., Williams, L. E., Jové, T., Hiott, L. M., Woodley, T. A., Barrett, J. B., Jackson, C. R., Wasilenko, J. L., & Simmons, M. (2019). Antimicrobial resistance genes, cassettes, and plasmids present in *Salmonella enterica* associated with United States food animals. *Frontiers in Microbiology*, 10, 832. https://doi.org/10.3389/fmicb.2019.00832

Nam, S. (2009). *Fisheries resources in Cambodia: Current status, key issues, directions*. Fisheries Administration.

O'Neill, J. (2015). *Securing new drugs for future generations: The pipeline of antibiotics*. Review on Antimicrobial Resistance. Retrieved from https://amr-review.org/sites/default/files/SECURING%20NEW%20DRUGS%20FOR%20FUTURE%20GENERATIONS%20FINAL%20WEB_0.pdf

Rahman, M., Rahaman, M., Islam, M. S., Hossain, M., Mithi, F., Ahmed, M., Saldías, M., Akkol, E. K., & Sobarzo, E. (2021). Multifunctional therapeutic potential of phytocomplexes and natural extracts for antimicrobial properties.

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



Antibiotics, 10(10), 1076. https://doi.org/10.3390/antibiotics10091076

Rasul, M., & Majumdar, B. (2017). Abuse of antibiotics in aquaculture and its effects on human, aquatic animals, and the environment. *Haya: The Saudi Journal of Life Sciences*. https://doi.org/10.21276/haya

Reed, T. (2019). Antimicrobial resistance in Cambodia: A review. *International Journal of Infectious Diseases*, 85, 98–107. https://doi.org/10.1016/j.ijid.2019.05.036

Shahen, M. A. (2024). The roles of NGOs in skill development of street food vendors for hygienic food preparation and serving in Bangladesh. *Journal of Innovative Research*, 2(2), 17–24. https://doi.org/10.54536/jir.v2i2.2793

Sneader, W. (2001). History of sulfonamides. *Encyclopedia of Life Sciences*.

Sudha, S., Mridula, C., Silvester, R., & Hatha, A. (2014). Prevalence and antibiotic resistance of pathogenic Vibrios in shellfish from Cochin market. *Indian Journal of Marine Sciences*, 43(5), 815–824.

Swann, L. (2007). *Diagnosis and treatment of Aeromonas hydrophila infection of fish*.

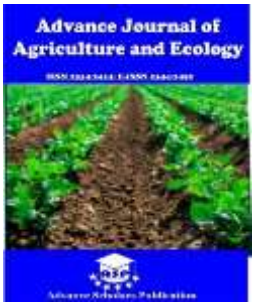
Thompson, J., Marcelino, L., & Polz, M. (2005). Diversity, sources, and detection of human bacterial pathogens in the marine environment. In S. Belkin & R. R. Colwell (Eds.), *Oceans and health: Pathogens in the marine environment* (pp. 29–68). Springer. https://doi.org/10.1007/0-387-23709-7_2

Vandy, S., Leakhann, S., Phalmony, H., Denny, J., & Marie, C. (2012). *Vibrio parahaemolyticus* enteritis outbreak following a wedding banquet in a rural village – Kampong Speu, Cambodia, April 2012. *Western Pacific Surveillance and Response Journal*, 3(4), 25–28. https://doi.org/10.5365/wpsar.2012.3.4.004

Vezzulli, L., Colwell, R., & Pruzzo, C. (2013). Ocean warming and spread of pathogenic Vibrios in the aquatic environment. *Microbial Ecology*, 65, 817–825.

Vila, J., Ruiz, J., Gallardo, F., Vargas, M., Soler, L., & Figueras, M. (2003). *Aeromonas spp.* traveler's diarrhea: Clinical features and antimicrobial resistance. *Emerging Infectious Diseases*, 9(5), 552–555. https://doi.org/10.3201/eid0905.020462

Advance Journal of Agriculture and Ecology
Adv. J. Agric. & Eco.
Volume: 10; Issue: 8
August -2025
ISSN 2334-2414
E-ISSN 2344-2492
Impact Factor: 7.12
Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/ajae/index



Waksman, S. (1947). What is an antibiotic or an antibiotic substance? *Mycologia*, 39, 565–569.World Health Organization. (2014). *Antimicrobial resistance: Global report on surveillance*. WHO.Wright, A. C., Hill, R. T., Johnson, J. A., Roghman, M