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Risk profile of antimicrobial resistance in

Vibrio parahaemolyticus

Information on AMR microorganism(s) and/or determinant(s)

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Transforming lives through translational research

Risk profile of antimicrobial resistance in *Vibrio parahaemolyticus*

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1.1 Characteristics of *V. parahaemolyticus*

- *V. parahaemolyticus* is a gram-negative, curved-rod-shaped halophilic bacterium that naturally inhabits marine, estuarine, and coastal environments. Discovered in the 1950s in Japan from clinical samples and dried sardines, this pathogen is well-recognized for its ability to cause gastroenteritis in humans. It thrives in environments with high salt concentrations, such as coastal and estuarine waters. This characteristic is essential for its natural habitat in marine environments.
- Although *V. parahaemolyticus* is a commonly found isolate in seafood, the majority of these isolates are non-pathogenic to humans wherein only 0.6% of these bacterial strains harbouring thermostable direct hemolysin *(tdh)* and/or TDH-related hemolysin *(trh)* genes are responsible for infection.
- *V. parahaemolyticus* is motile, possessing a single polar flagellum that allows it to move in aqueous environments. *V. parahaemolyticus* is a motile bacterium that is found in free-swimming form and possesses a single polar flagellum, which helps in its attachment to zooplankton, shellfish, fishes, or any underwater inanimate surfaces.
- Like other *Vibrio* species, *V. parahaemolyticus* is oxidase-positive, meaning it produces the enzyme oxidase. *V. parahaemolyticus* can ferment various sugars, and its biochemical profile includes the ability to ferment mannitol and sucrose.
- It can be isolated and identified using selective media, such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar, which is commonly used for the isolation of *Vibrio* species.

1.2 Sources and transmission routes of *V. parahaemolyticus*

- The most common way one could get exposed to this pathogen is by consuming infected half-cooked/undercooked seafood.
- Exposing open wounds to contaminated salt water could also potentially cause an infection.
- *V. parahaemolyticus*- related illnesses have increased, particularly in countries with high levels of seafood consumption where *V. parahaemolyticus* causes over half of all food-poisoning outbreaks.
- Exposure to this pathogen results in various ailments ranging from nausea, and gastroenteritis conditions like watery diarrhea and abdominal cramps. Rarely, *V. parahaemolyticus* can cause ear infections, wound infections and septicemia that can be fatal to immune-compromised individuals.

As per CDC reports, most cases of vibriosis are part of smaller outbreaks rather than larger ones. However, a larger vibriosis outbreak involving multiple countries could occur. The following table summarises vibriosis outbreaks in various states published by CDC (Table 1)

Table 1: Outbreaks associated with *V. parahaemolyticus* (Source: CDC & NCEZID)

Sl No	Year	Outbreak data	statistics
1	2019	Multistate Outbreak of Gastrointestinal Illnesses Linked to Oysters Imported from Mexico	• Reported Cases: 16 • States: 5 • Hospitalizations: 2 • Deaths: 0 • Recall: Yes
2	2018	*Vibrio parahaemolyticus* Infections Linked to Fresh Crab Meat Imported from Venezuela	• Case Count: 26 • Jurisdictions: 8 • Hospitalizations: 9 • Deaths: 0 • Recall: No
3	2013	*Vibrio parahaemolyticus* illnesses associated with consumption of shellfish, United States,	• Case Count: 104 • States: 13 • Deaths: 0 • Hospitalizations: 6 • Recall: Yes
4	2012	Pandemic *Vibrio parahaemolyticus,* Maryland	• Case Count: 2 • States: 1 • Hospitalizations: 2
5	2006	*Vibrio parahaemolyticus* Infections Associated with Consumption of Raw Shellfish --- Three States, 2006	• Case Count: 177 • States: 3

1.3 Pathogenicity of particular strains of *V. parahaemolyticus*

- *V. parahaemolyticus* strains possess various virulence factors like adhesins, *tdh* and *trh* and type III secretion systems, namely T3SS1 and T3SS2. some bacterial strains gain T3SS2, *tdh* and *trh* genes paving the way for a number of strains with varying pathogenicity.
- T3SS are bacterial machinery that are needle-like, used to inject bacterial proteins directly into the membrane and cytoplasm of eukaryotic cells. The common targets of T3SS effectors include the actin cytoskeleton, innate immune signalling, and

autophagy. Depending on the pathogen's needs, the systems can be either upregulated or downregulated.

- The T3SS1 encoded in this bacterial strain helps in ensuring its survival in the environment. It has a number of virulence factors that cause lysis of the infected host cell and allows the release of important nutrients. The T3SS1 is present in all environmental and clinical *V. parahaemolyticus* strains. During tissue cell infection, T3SS1 initiates a series of events that involves autophagy, membrane blebbing, cell rounding, and lastly cell lysis.
- The T3SS2 encoded on a pathogenicity island on chromosome 2 is found in clinical isolates and is associated with pandemic *V. parahaemolyticus* strains. T3SS2 is closely associated with pathogenicity island (VPAI-7) and flanked by two *tdh* genes.
- T6SS1 and T6SS2 are located on chromosomes 1 and 2 respectively on *V. parahaemolyticus*. plays a role in the delivery of effector proteins into target cells, often during interactions between bacteria or between bacteria and host cells. It is reported that the T6SS gene was used as a virulence marker to differentiate pandemic and non-pandemic strains isolated in Japan. The T6SS gene was present in all pandemic strains, whereas the majority of the non-pandemic strains had a partial set of T6SS genes.
- In the case of *V parahaemolyticus*, certain serotypes, including O3:K6, have been associated with outbreaks of gastroenteritis.
- Based in dose response curve and the construction it has been assumed that *V. parahaemolyticus* positive for *tdh, trh* and negative for *tdh, trh* both behave in a similar way. However unpublished data on growth and survival between *tdh* positive and *tdh* negative *V. parahaemolyticus* from oysters showed that growth rate of *tdh* negative *V. parahaemolyticus* is higher than *tdh* negative ones. Hence, from risk assessment point of view growth rate is not a significant factor to differentiate *V. parahaemolyticus* based on the presence or absence of *tdh*; neither does it have any ecological significance for growth and survival as a very low proportion of *tdh* is present.
- The clinical strains of this bacterium isolated from humans with gastroenteritis are different from the environmental strains based on the ability of the strain to produce TDH which can lyse red blood cells on Wagatsuma blood agar knowing Kanagawa phenomenon.

2. Growth and survivability of foodborne AMR microorganism(s) in the food commodity production to consumption Continuum.

- Factors that influence the growth and survivability of foodborne AMR *V. parahaemolyticus* in the food commodity production-to-consumption continuum include aquaculture practices, sea surface temperatures, salinity conditions, and the ability of the strains to adapt to cold temperatures. Environmental parameters such as water temperature, salinity, total chlorophyll, dissolved oxygen, and pH are associated with an increased abundance of *Vibrio parahaemolyticus*.
- Additionally, genetic markers for *V. parahaemolyticus* have been enumerated to determine their occurrence and seasonality in water, oysters, and sediment samples. The growth and survival of *V. parahaemolyticus* strains at refrigeration temperatures vary, with some strains able to survive but not grow, and others able to grow at certain temperatures.
- *V. parahaemolyticus* infection are increasing in different parts of the world and climate changes could be one of the reasons. The *V. parahaemolyticus* pathogenic strains could be spreading because of climate changes and oceanic currents; which could also lead to new strains coming to new areas to establish and cause infection

Table 2: Physiological conditions favouring survival of *Vibrio parahaemolyticus*

Physiologic Characteristics	*V. parahaemolyticus*
Temperature range (°C)	5-43
Optimum temperature (°C)	37
Inactivation temperature (°C)	>63
pH range	5-11
Optimum pH	7.96 ± 0.1
Minimum water activity (aw)	0.94
NaCl (%)	0.5-8.0
Salinity (PSU- practical salinity units)	5-30

2.1 Virulence and linkages to resistance

- Multiple studies have reported the presence of virulence genes, such as *tlh* and *toxR*, in *V. parahaemolyticus* isolates. However, the specific virulence genes *tdh* and *trh*, which are associated with enterotoxicity, were not detected in all isolates. Virulence genes does not seem to have any great difference between the ones that possessed *tdh, trh* and the ones that did not. In growth experiments their ability to produce hemolysins, growth in sea water, fresh water, brackish water remained unaffected whether isolate from muscles or clamps.
- In a study conducted in Maryland, 2-10% of environmental *V. parahaemolyticus* samples collected from along the marine and coastal base from water sediments, oyster blue crab showed positive for *tdh* and *trh* genes. On performing MIC and whole genome analysis it was observed that 10-15% of isolates were resistant to ampicillin and few isolates were resistant to tetracycline. It was inferred that the presence of the *tdh* & *trh* genes need not always confer resistance to particular antibiotics. however, there is a correlation between antibiotic resistance and T3SS
- Few strains of *V. parahaemolyticus* showed resistance to ampicillin, tetracycline and this resistance was found to be associated to type T3SS, not *tdh* and *trh*. *V. parahaemolyticus* isolated from fin fish showed more resistance to antibiotics than the ones isolated from shell fishes. Isolates obtained from shrimp samples were more resistant to antimicrobials than isolates recovered from crab or oyster. Also, isolates from crab were more resistant to antimicrobials compared to oyster isolates.
- Genotypic examination of the bacterial would show the presence of resistance gene, but expression of these genes in the presence of antibiotics is not well studied. Further, when multiple antibiotics are used, they would probably get selected if they carry the corresponding gene, even if was resistant at the point of time when tested phenotypically, and thus the silent resistant genes should not mislead thinking that the isolate is resistant to the antibiotic. This makes phenotypic tests much more reliable than genotypic tests. Also, several routine studies on resistance genes reveal discord between genotypic and phenotypic results. Hence, the expression of gene is more important than mere presence of the gene itself, and the circumstance under which the gene starts expressing is important.

- The presence of multidrug-resistant strains and the high prevalence of resistance to certain antibiotics highlight the potential risk to public health. It is important to note that the incidence of pathogenic and multidrug-resistant strains is relatively low. These findings emphasize the need for continuous monitoring of *V. parahaemolyticus* contamination in aquatic products and the development of guidelines for antibiotic use to ensure food safety.
- Resistance genes that were conventionally known to confer resistance to the bacteria could also be involved in physiological function in a bacterial cell. For instance, ampC, an ampicillin resistance gene is known to be involved in cell wall synthesis in gram-negative bacteria; this is true even for *V. parahaemolyticus*.

2.2 Inactivation of *V. parahaemolyticus* in food

1. Thorough cooking is one of the most effective methods to inactivate *V. parahaemolyticus*. Cooking temperatures that exceed 145°F (63°C) are recommended. Combination of steam-venting packaging and microwave cooking to inactivate *V. parahaemolyticus* in oyster meat have been used.
2. Incorporating acidic ingredients such as vinegar, citrus juices, or acidic marinades & brining can create an environment that is less favourable.
3. Ionizing radiation can be used to irradiate food products, effectively reducing or eliminating pathogens, including *V. parahaemolyticus*. This method is regulated and approved for certain food products in various countries. The use of blue light-emitting diode (LED) photodynamic inactivation (PDI) with the photosensitizer curcumin to eradicate *V. parahaemolyticus* and its biofilm have been employed.
4. Implementing HACCP principles and following good manufacturing practices are crucial in preventing contamination during food processing, handling, and distribution.
5. Lowering the temperature, especially rapid cooling can slow down or halt the growth of *V. parahaemolyticus* and reduces the risk of contamination. It is also noticed that if *tdh* positive *V. parahaemolyticus* is frozen for a longer time, the *tdh* positive ability of *V. parahaemolyticus* is lost.

6. Several other techniques like use of Residual ozone concentration (ROC), epigallocatechin gallate (EGCG), aqueous ozone & use of probiotics for controlling *V. parahaemolyticus* has been studied.

2.3 Distribution, frequency and concentrations of the AMR hazard(s) in the food chain

- *V. parahaemolyticus* is a naturally occurring bacterium in marine and estuarine environments. The presence of antimicrobial resistance in these environments can be influenced by factors such as pollution, aquaculture practices, and the use of antimicrobials in agriculture. The prevalence of AMR in *V. parahaemolyticus* within seafood can be influenced by contamination in the environment, post-harvest handling practices, and the use of antibiotics in aquaculture.
- *V. parahaemolyticus* shows high resistance to cefazolin and ampicillin, with resistance rates of 94.4% and 37.0% respectively. However, resistance to other antimicrobials, including clinically relevant agents and even imipenem, is below 3%. The overall levels of antimicrobial resistance and multidrug resistance are 95.1% and 3.3% respectively.
- The distribution of antimicrobial resistance and multidrug resistance depends on region, isolation source, human activity, such as shipping, aquatic product trade. Increased human migration between continents, has led to changes in the distribution pattern of *V. parahaemolyticus*.
- The concentration of antimicrobial-resistant strains in shellfish may be influenced by the levels of AMR in the environment. The use of antimicrobials in aquaculture for disease prevention or growth promotion can contribute to the development of antimicrobial resistance in *V. parahaemolyticus* strains associated with fish and shellfish farming.
- It is the dynamic nature of the environment and the interrelationships with the phytoplankton, the diatoms and all that playing a role in terribly reducing the number of *Vibrios* because of the presence of diatom blooms that produce antimicrobial substances.
- New clonal types of *V. parahaemolyticus* are emerging each year, among which many remain as endosymbionts with some of the algae which makes it convenient to *V. parahaemolyticus* to address to weather and climate change making its number increase to large numbers.

- In Singapore, haemolytic AMR *V. parahaemolyticus* was detected in farmed green mussels and retail shellfish, with average probabilities of illness estimated at 5.7×10^{-3} and 1.2×10^{-2} per serving for the full and partial chains, respectively.
- In South Korea, *V. parahaemolyticus* was found in seafood distribution channels, and 16.8% of the samples were positive for the pathogenic strains.
- In China, *V. parahaemolyticus* was prevalent in fish and shrimp, with 73.27% of the isolates being multidrug-resistant.
- In cases pertaining to coastal regions of India like West Bengal, pathogenic and pandemic *V. parahaemolyticus* were isolated from oysters, with 5.5% of the oyster population carrying pandemic strains.
- In Cochin, Kerala, potentially pathogenic *V. parahaemolyticus* carrying the tdh gene was isolated from seafood in retail markets. These studies highlight the presence of AMR *V. parahaemolyticus* in the food chain and the need for food safety measures to control this pathogen.
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2.4 Characteristics of the resistance expressed by the AMR microorganism(s) and/or determinant(s):

2.4.1 Resistance mechanisms and location of AMR determinants.

- Ninety-five percent of *V. parahaemolyticus* isolates are resistant to amikacin as an antibiotic. Growing in biofilms, *V. parahaemolyticus* cells are more resistant to environmental changes and have the ability to take up resistance plasmids from other species or undergo mutations that increase their resistance to antibiotics.
- The consumption of quinolone-resistant *V. parahaemolyticus* in shrimp may lessen the effectiveness of quinolone medications in the clinical treatment of this infection since quinolones are the preferred medications for the treatment of gastroenteritis brought on by this pathogen. Resistance to quinolones is mainly due to chromosomal mutations in the quinolone resistance determining region (QRDR) of genes encoding the drug target enzymes (DNA gyrase and topoisomerase IV), efflux pumps that involves the

extrusion of toxic substrates from within cells into external environment and plasmid-mediated resistance. point mutations in *gyrA, gyrB and parC* has been reported in several isolates of *V. parahaemolyticus.*

- Resistance to aminoglycoside is usually brought by mutation or by accepting resistance plasmids from other species. Resistance related gene such as *blaTEM, sul2, strA strB CARB, tetA, tetB, tetH* are commonly being reported in this organism. Resistance could be acquired by different methods and this would probably affect the relative fitness. The metabolic cost may be high if the bacteria pick a large plasmid and the metabolic cost might be less if it is just a point mutation.

2.4.2 Cross-resistance and/or co-resistance to other antimicrobial agents.

- resistance to one antimicrobial agent resulting in confers resistance to another antimicrobial agent even if the bacteria have not been exposed to the subsequent agent is referred as cross-resistance; while Co-resistance is when a bacterial strain develops concurrent resistance to many antimicrobial treatments.
- *V. parahaemolyticus* prevalent in aquatic environment has become more resistant as a result of long-term, improper usage of antibiotics. It is believed that heavy metal, fungicides and antiparasitic polluted water habitats promote co-resistance and cross-resistance. Few investigations have reported the presence of cross- and/or co-resistance in *V. parahaemolyticus* to different antimicrobial drugs.
- Ting Wang et al. identified antimicrobial resistance genes on both the chromosome and plasmid of *V. parahaemolyticus* strains from seafood, indicating the presence of cross-resistance.
- Jiaqi Jin et al. found that multidrug-resistant *V. parahaemolyticus* strains displayed resistance to multiple antimicrobials and possessed specific mutations in antimicrobial resistance genes. These findings suggest that *V. parahaemolyticus* strains can exhibit cross-resistance and/or co-resistance to multiple antimicrobial agents, highlighting the importance of monitoring and preventing the spread of antimicrobial resistance in this pathogen.

2.4.3 Transferability of resistance determinants between microorganisms.

- Antimicrobial resistance gene on both the chromosome and plasmid of *V. parahaemolyticus*, with the plasmid carrying multiple resistance genes and exhibiting high transfer frequency were reported. Plasmid-mediated resistance gene transfer are often carried on mobile genetic elements.

- Various mobile genetic elements in the *V. parahaemolyticus* genomes, including genome islands, prophage, gene clusters, integrons, and insertion sequences, indicates possible horizontal gene transfer and considerable genome variation.
- Transfer of quinolone resistance genes between *V. parahaemolyticus* & *Escherichia coli*, suggesting a recent flow of these genes between bacteria from different environments. Foodborne Vibrio spp. carrying the qnrS gene, a key plasmid-mediated quinolone resistance gene, found a diverse range of resistance elements that these isolates harboured.
- Positive correlation between multidrug resistance and multi-heavy metal resistance in *V. parahaemolyticus* isolates, suggests the potential dissemination of resistance determinants via mobile genetic elements

3. Information on the antimicrobial agent(s) to which resistance is expressed

3.1: Class of antibiotics used to treat *V. parahaemolyticus*

- The majority of *V. parahaemolyticus* infections manifest as self-limiting enteritis, but in certain severe and persistent cases—particularly in patients with compromised immunity—antibiotic therapy may be necessary. The recommended treatment for severe Vibrio infections is tetracycline, with other options being combinations of doxycycline and expanded-spectrum cephalosporins (such ceftazidime) or a fluoroquinolone. According few studies doxycycline was the most effective antibiotic against *V. parahaemolyticus* isolates as it showed maximum sensitivity.

3.2 Non-human uses of the antimicrobial agent to treat *V. parahaemolyticus*

- *V. parahaemolyticus* causes disease in aquaculture, and use of antimicrobials are restricted in aquaculture and not recommended for good management practices. Few permitted antimicrobials could only be used at the early stage of disease in aquaculture. *V. parahaemolyticus* and its pathogenicity to humans and shrimp for linkage resistance has a lot of data gap and hence has not been well studied yet. Based on the existing information about *V. parahaemolyticus* in growth, ability distribution, and concentration in the food chain it can be presumed that both resistant and susceptible *V. parahaemolyticus* have the same way in the food chain

- Although there are restrictions on use of antimicrobials in aquaculture in many countries, it is not truly followed. Therefore, much of the use of antibiotics in aquaculture sector is derived from human and veterinary information many times without any rational. There are limits stipulated for use of antimicrobials in aquaculture. The fear of being tested for some of the major antibiotics has stopped its use in aquaculture, but his does not assure that some others are not used.
- Over the years regulations on use of antimicrobials is changing rapidly and is improving in many countries that are implementing aquaculture because of the demand in the export market and requirements of the importing country.
- It would be interesting to look at the resistance pattern of *V. parahaemolyticus* in countries from Europe and the US, where there is good regulation and no use of antimicrobials in aquaculture. But, since this is essentially an organism that flourishes in tropical waters, the number of organisms and types that are encountered in Europe and the US may be limited in comparison to what is found in the tropical belt.
- Although *V. parahaemolyticus* is an important pathogen in Europe and the US, the infection is mainly human infection rather than in aquaculture. Table 3 shows the list of antibiotics resistant to *V. parahaemolyticus*.

Table 3: List of antibiotics resistant to *V. parahaemolyticus* (2022)

Class of Antibiotics	Antibiotics that show resistance to *V. parahaemolyticus*
1. β Lactams	
•Cephalosporins	•1st Generation: Cephalothin, Cefazolin •2nd Generation: Cefoxitin, Cefuroxime •3rd Generation: Cefotaxime, Ceftizoxime, Ceftriaxone, Cefpodoxime, Ceftazidime •4th Generation: Cefepime
•Monobactams	Aztreonam
•Carbapenems	Imipenem, Meropenem

•Penicillin	Ampicillin, Ampicillin-sulbactam (SAM), Amoxycillin/Clavulanic acid, Carbenicillin, Methicillin Amoxycillin, Piperacillin
2. Tetracyclines	Oxytetracycline, doxycycline, chlortetracycline
3. Quinolones	Ciprofloxacin, Nalidixic acid, enrofloxacin, ofloxacin, levofloxacin
4. Aminoglycosides	Amikacin, Streptomycin, Kanamycin, Gentamicin, Spectinomycin, Tobramycin, Netilmicin, Apramycin, Neomycin
5. Sulphonamides	Sulphamethoxazole, co-trimoxazole
6. Macrolides	Medemycin, Clindamycin, Azithromycin, erythromycin.
7. Glycopeptides	Vancomycin, Teicoplanin
8. Lipopeptides	Daptomycin
9. Ansamycins	Rifampicin
10. Nitrofurans	Nitrofurantoin, Furazolidone
11. Other Antibiotics: Colistin (Polymyxin), Chloramphenicol, Novobiocin, Trimethoprim	

3.3 Formulation of the antimicrobial agent(s).

- Developing compounds that block the mechanism of antibiotic resistance, generating new antibacterial agents with unique mechanisms of action, structurally altering well-known antibiotics to get around the bacterial-resistance mechanism, and designing new compounds that, when combined with (ineffective) antibacterial drugs, restore the action of these drugs (e.g., efflux pump inhibitors) are just a few of the different strategies that can be used for developing new antibacterial agents.
- Creating a customized and receptive drug delivery system could potentially address these issues by increasing the amount of medication that reaches the infection site. Drug targeting enables conjugates to attach to the pathogen with specificity and produce a high concentration of antibiotics locally, which kills the target. In theory, this tactic shields non-targeted bacteria (and host cells in the case of general toxins) from harmful payload concentrations. As a result, there is a decreased chance of side effects

and less accumulation of drug components in healthy tissue. Another well-established approach to combat infections is the use of antibodies or bacteriophages.

3.4 Purpose and use of antimicrobial agent(s) in feed, food, animals, and/or during food processing.

- Antimicrobial compounds serve a variety of functions in the food production process, including in feed, food animals, crop cultivation, and food processing. Antimicrobial compounds are added to animal feed for a number of purposes, such as enhancing feed efficiency and encouraging animal growth, as well as preventing and managing infections in animals raised under stressful or crowded settings.
- Antimicrobial drugs find application in livestock and poultry production as feed additives. To accelerate growth rates and minimise the risk of infection, they can be administered to animals through their water or diets, referred as "subtherapeutic use." In the production of livestock or aquaculture antimicrobial drugs are used to prevent and control illnesses, which can be prevalent in intensive farming methods. Animals are given antimicrobial medicines in isolation or in batches to stop the spread of infection and enhance overall animal health and this practise is known as “prophylactic use."
- In case of antibiotic resistance studies, it would be necessary to perform experiments both in aquatic lifeforms and water in which they are found, and check how they fare in growth and survival.
- Antimicrobial compounds are used in food processing for a variety of purposes, such as improving food safety by lowering the possibility of contamination by harmful bacteria and safeguarding food products by preventing microbial growth and spoiling. To maintain the safety and quality of food, antimicrobial agents are either added to food products or used in food processing facilities. Examples are the sanitization of food processing equipment and the use of preservatives in packaged goods.
- Management strategies includes good management practices where water quality, sediment quality feed is taken care, reducing fish feed wastage and other aspects related to aquaculture pond management.

3.5 Methods, routes of administration of the antimicrobial agent(s) (individual/mass medication, local/systemic application) and frequency.

- The 2 most common routes of administration of antimicrobial agents in aquaculture are use of medicated feed and adding antimicrobial agents directly to the water (immersion therapy), and both of these methods imply flock treatment of the animals. These practices may result in heavy use of antimicrobial agents and convey a strong selective pressure not only in the animals, but also in the exposed environments
- Few other methods are:
  - ✓ Gavage is a form of oral administration used extensively in experimental work because the precise dose is known.
  - ✓ The usual routes of injection for fish are intramuscular, intraperitoneal, and dorso-median sinus injections. Venepuncture and cardiac puncture are also possible but are not recommended for routine injections since they are always stressful to the fish.
  - ✓ Baths and dips are not as effective as some of the other treatment methods, particularly for systemic infections, because of generally poor internal absorption of the antibiotic being used. The method of application can therefore be used for surface infections such as fin rot, bacterial gill disease, superficial fungal infections and ectoparasitic infestations.

3.6 Potential extra-label/off-label, use of approved antimicrobial agent(s) and use of non-approved antimicrobial agent(s) to treat *V. parahaemolyticus*

- The majority of *V. parahaemolyticus* infection cases resolve on their own, but severe or protracted infectious disease necessitates antibiotic therapy. The recommended six antibiotic agents by the CDC for the for the clinical treatment of *Vibrio* species infections are as follows cephalosporins (ceftazidime and ceftiofur), tetracyclines (tetracycline), quinolones (ciprofloxacin), folate pathway inhibitors (trimethoprim /sulfamethoxazole), and phenicol’s (chloramphenicol). However, the pathogens' susceptibilities to the antimicrobial agent must be taken into consideration while selecting antibiotics.
- The two polymyxins that are most often utilized in clinical treatments and agricultural productivity are polymyxin E (colistin) and polymyxin B. In particular, colistin is used as a last resort to treat serious infections brought on by organisms that are resistant to drugs. When treating children for whom doxycycline and fluoroquinolones are

contraindicated, trimethoprim-sulfamethoxazole combined with an aminoglycoside are utilized.

- Although there are very limited range of antimicrobials permitted for use in aquaculture, *V. parahaemolyticus* has shown resistance to almost all anti-microbials like ampicillin, sublactams, amoxicillin, carbenicillin and also various cephalosporins first generation, second generation and even fourth generation cephalosporins, monobactams and also carbapenems, tetracycline quinolones, different type of quinolones, aminoglycosides, sulfonamides and macrolides.

3.7 Trends in the use of the antimicrobial agent(s) in the agricultural and aquaculture sectors and information on emerging resistance in the food supply.

- Antibiotic availability has become a critical factor in the management of *V. parahaemolyticus*-related diseases during the past few decades. Over the years, ciprofloxacin or tetracycline have been suggested as common treatments for severe or protracted *V. parahaemolyticus* infections.
- One of the antibiotic classes most commonly employed to combat microorganisms in aquaculture is the tetracycline family; specifically, tetracycline can be utilized to treat severe or protracted human disease caused by *V. parahaemolyticus* as well as heavy *Vibrio* infection in fisheries. Because of their excellent antibacterial activity, broad antibacterial spectrum, and lack of cross-resistance with other antibiotics, members of the third generation of quinolones, such as ofloxacin, ciprofloxacin, and norfloxacin, are widely used in clinic settings.
- Ciprofloxacin, erythromycin, and rifampicin are commonly used medications to treat vibriosis in shrimp hatcheries and/or larval production in countries in some the Asian countries. The ciprofloxacin use is now limited in aquaculture as many countries including China, Thailand & India have reported resistance and the use of ciprofloxacin in aquaculture has been banned in China. Furthermore, in some regions of Asia, quinolones are used in aquaculture. According few studies doxycycline (94.12% isolates sensitive) was the most effective antibiotic against *V. parahaemolyticus* isolates.
- Currently, antibiotic resistance has not been flagged as an issue in human cases of *V. parahaemolyticus*. Even in aquaculture, where shrimp pathogens have been reported, the AHPND causing ones because there is limited information now. There is no clear trend to see whether the AHPND strains causing Shrimp infections.

3.8 Information on the relationship between the use of the antimicrobial agent(s) and the occurrence of AMR

- *V. parahaemolyticus* is naturally occurring in aquatic environments. Pollution and discharge from industrial and agricultural processes can expose it to different antimicrobial agents, including antibiotics and other antimicrobial chemicals. The development of resistance could be enhanced by this environmental exposure.
- Human infections with *V. parahaemolyticus* usually cure naturally and do not require to be treated with antibiotics. Antibiotics, however, could be used in severe cases or if the infection extends beyond the gastrointestinal system. Antibiotics are an option for resistant strains in particular circumstances.
- In aquaculture, including seafood farming, antibiotics are occasionally used to treat or prevent bacterial infections in fish and shellfish. Antibiotic usage in aquaculture may promote the development of antimicrobial resistance (AMR) in *V. parahaemolyticus* and other bacteria that inhabit similar conditions.
- *V. parahaemolyticus* can transfer genes horizontally to develop antibiotic resistance. Treatment of severe infections might be challenging due to the resistance observed in certain strains of *V. parahaemolyticus* to particular antibiotic classes.
- Surveillance and monitoring programs are essential to track the prevalence of AMR in *V. parahaemolyticus* and other pathogens. This helps public health agencies identify emerging resistance patterns and make informed decisions about the use of antibiotics in clinical and agricultural settings.
- Monitoring the incidence of antimicrobial resistance (AMR) in *V. parahaemolyticus* and other diseases requires monitoring and surveillance programs. Sensible decisions on the use of antibiotics in clinical and agricultural settings could be adopted by public health authorities with the support of this in identifying increasing resistance trends.

4. Human uses of the antimicrobial agent(s) to treat *V. parahaemolyticus*

4.1 Spectrum of activity and indications for treatment.

- The treatment of *V. parahaemolyticus* infections depends on the severity of symptoms and the patient's overall health. Treatment with particular antibiotics may not be required in mild cases of *V. parahaemolyticus* infection. Anti-diarrheal medications are typically not advised as they can make the infection persist for longer.

- Generally, antibiotic therapy is reserved for moderate to severe *V. parahaemolyticus* infections/conditions like
  - ✓ severe high fever, blood loss, or heavy dehydration, Infections in
  - ✓ immunocompromised individuals, such as the elderly or those with underlying medical conditions.
  - ✓ infection beyond the gastrointestinal tract.
- Selected antibiotics may include fluoroquinolones (e.g., ciprofloxacin) or tetracyclines (e.g., doxycycline). However, antimicrobial susceptibility studies should be performed to determine the most appropriate antimicrobial selection.
- It is critical for people experiencing signs and symptoms of *V. parahaemolyticus* contamination to seek medical attention, specifically in the event that they have excessive signs or if they fall into excessive-threat. A healthcare company could make a right analysis and decide the most appropriate remedy plan.

4.2 Importance of the antimicrobial agent(s) including consideration of critically important antimicrobial lists.

- While most cases of V. parahaemolyticus infection heal on their own, antibiotic therapy is required for severe or long-lasting infections. The following six antibiotics are recommended by the CDC for the clinical treatment of Vibrio species infections: tetracyclines (tetracycline), quinolones (ciprofloxacin), folate pathway inhibitors (trimethoprim /sulfamethoxazole), phenicols (chloramphenicol), and cephalosporins (ceftazidime and ceftiofur). When choosing antibiotics, one must evaluate the infections' susceptibilities to the antimicrobial agent.
- Ciprofloxacin and doxycycline are regularly effective in treating infections resulting from *V. parahaemolyticus*. Colistin is used as a last option to treat severe infections caused by drug-resistant pathogens. It's additionally well worth noting that antibiotic susceptibility trying out should be achieved to guide the selection of the most suitable antibiotic based at the unique stress of *V. parahaemolyticus* inflicting the infection.

4.3 Availability of alternative antimicrobial agent(s) against *V. parahaemolyticus*

- Alternative antimicrobial agents against *V. parahaemolyticus* include Actinomycin D [, procyanidin, stevioside, troxerutin, and rutin, quercetin, and halogenated indoles such as 4-chloroindole and 7-chloroindole. Actinomycin D showed strong anti-*V. parahaemolyticus* activity and inhibited biofilm formation and motility.

- Procyanidin, stevioside, troxerutin, and rutin exhibited both high binding affinity with LolB, a protein responsible for lipoprotein transport in V. parahaemolyticus, and antibacterial activity. Quercetin demonstrated inhibitory effects on biofilm formation and motility, as well as reduced expression of genes related to flagella motility, biofilm formation, and quorum-sensing. Halogenated indoles, particularly 4-chloroindole, showed antimicrobial and antivirulence properties, inhibiting biofilm formation and causing damage to the cell membrane.
- Other alternative antimicrobial agents for pathogenic *V. parahaemolyticus* include chitooligosaccharide (COS)-tea polyphenol conjugates, essential oils from plants such as *Artemisia absinthium*, Zataria multiflora Boiss, *Pulicaria gnaphalodes*, *Trachyspermum ammi, and Cuminum cyminum*, and probiotic strains such as *Bacillus licheniformis* and *Pseudomonas aeruginosa*. Chitooligosaccharide (COS)-catechin conjugate (COS-CAT) showed high bactericidal activity against both pathogenic and non-pathogenic strains of *V. parahaemolyticus*. Several bacteriophages against *V. parahaemolyticus* have been found to be effective against this organism.
- These alternative antimicrobial agents have the potential to control pathogenic *V. parahaemolyticus* and can be considered for use in aquaculture and seafood safety practices.

4.5 Trends in the use of the antimicrobial agent(s) in humans and information on emerging diseases due to microorganism(s) resistant to the antimicrobial agent(s) or classes.

- The prevalence of antimicrobial resistance (AMR) in *V. parahaemolyticus* strains is a growing concern for public health. Studies have shown that *Vibrio* strains exhibit resistance to multiple antimicrobial classes, with some strains showing resistance to up to seven antimicrobials.
- The detection of resistance genes from 11 antimicrobial classes in Latin American *Vibrio* strains highlights the need for more efficient control measures and rational use of antimicrobials in human therapy and aquaculture. Additionally, the study of *V. parahaemolyticus* strains isolated from shrimp farming revealed high resistance to antibiotics commonly used for the prevention and treatment of Acute Hepatopancreatic Necrosis Disease (AHPND). The emergence of antibiotic-resistant

strains of *V. parahaemolyticus* poses a threat to public health, as this bacterium is a major cause of human gastroenteritis and is linked to foodborne outbreaks.

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