PREVALENCE AND ANTIBIOTIC RESISTANCE OF PATHOGENIC *VIBRIOS* IN FISH AND SHELLFISH FROM COCHIN MARKET

Project work by

AJITHA V A

AB21ZOO030

Under the guidance of

Parvathy K R

Department Of Zoology, St Teresa's College (Autonomous) Ernakulam
Kochi – 682011

Submitted to St. Teresa's College (Autonomous),

Affiliated to Mahatma Gandhi University,

Kottayam in partial fulfilment of the requirement

for the Degree of Bachelor of Science in Zoology

2023-24

CERTIFICATE

This is to certify that the project report entitled "**PREVALENCE AND ANTIBIOTIC RESISTANCE OF PATHOGENIC *VIBRIOS* IN FISH AND SHELLFISH FROM COCHIN MARKET**" submitted by Ms. AJITHA V A, Reg No: - **AB21ZOO030** in partial fulfilment of the requirement of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this is her original effort.

Dr. Soja Louis

Head of the Department of Zoology

St.Teresa's College (Autonomous)
Ernakulam

Ms.Parvathy.K.R

Assistant Professor

St.Teresa's College (Autonomous)
Ernakulam

EXAMINERS

1)

2)

DECLARATION

I, hereby declare that this project work entitled "**PREVALENCE AND ANTIBIOTIC RESISTANCE OF PATHOGENIC *VIBRIOS* IN FISH AND SHELLFISH FROM COCHIN MARKET"** is submitted to St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirements of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

NAME: AJITHA V A

REGISTRATION NUMBER: AB21ZOO030

SIGNATURE

ACKNOWLEDGEMENT

The success and final outcome of this project required a lot of guidance and assistance from many people and I am extremely privileged to have got this all along the completion of my project. All that I have done is only due to such supervision and assistance and I would not forget to thank them.

I thank God Almighty for showering his blessings upon me abundantly and for giving me the strength, ability, and opportunity to understand and complete this project work.

I am grateful to Dr. Soja Louis, the Head of the Department of Zoology, for her encouragement and support throughout this project. I owe my deep gratitude to my project guide, MS. PARVATHY K R having taken a keen interest in our research and guiding us along to build a good project work, by providing all the necessary information, the best available resources and moreover for having supported us throughout the study.

I thank all the teaching staff of the Department of Zoology, St Teresa's College, Ernakulam who were always willing to encourage and help us in all our efforts.

My project work would not have been a success without the constant encouragement from my parents and friends.

ABSTRACT

The genus *Vibrio* consists of gram-negative bacteria with curved- rod shape. They are commonly found in marine environments. Some *Vibrio* species are harmless, but some causes diseases like Cholera, Gastroenteritis, Wound Infections etc. The most common pathogenic species are *Vibrio cholerae* which causes a severe diarrheal infection called Cholera. Other species include *V. parahaemolyticus* and *Vibrio vulnificus* that causes Wound Infections and Gastroenteritis. They are mainly transmitted to humans through the intake of contaminated food, water, especially through raw or uncooked fishes and shellfishes that have nourished in contaminated water. The study "Prevalence and antibiotic resistance of pathogenic *Vibrios* in fish and shellfish from Cochin markets" accounts to detect the presence of different species of *Vibrio* and to understand their antibiotic resistance. A total of 14 samples were taken for the study. Two sampling were done from two different markets in Cochin. The first set of samples were enriched in Alkaline Peptone Water (APW) and streaked in Thiosulphate Citrate Bile salt Sucrose (TCBS) agar for the isolation of Vibrio. The second set of samples were enriched in Zobell Marine Agar and TCBS is used for the isolation. Five species of *Vibrios* such as *Vibrio cholerae*, *Vibrio alginolyticus*, *Vibrio fluvialis*, *Vibrio vulnificus* and *Vibrio parahaemolyticus* were detected. The rate of incidence of *Vibrio vulnificus* is found to more from the collected samples and *Vibrio alginolyticus* was found to have less occurrence in samples. The isolates of species were subjected to antibiotic sensitivity assays. All of them were sensitive towards antibiotics like Chloramphenicol, Erythromycin Streptomycin. Isolated species were found resistant towards Ampicillin and Penicillin. The *Vibrio cholerae*, *Vibrio fluvialis* and *Vibrio parahaemolyticus* showed complete resistance to Tetracycline. Only the species *V.vulnificus* and *V.alginolyticus* indicated sensitivity towards Tetracycline. The conclusion of the study indicated the high prevalence of pathogens of *Vibrio* in fishes and shellfishes from 2 Cochin markets and the emergence of antibiotic resistant strains.

TABLE OF CONTENTS

SI NO	TITLE	PAGE NO
1	Introduction	1
2	Review of Literature	4
3	Methodology	8
4	Observation and Result	10
5	Discussion	17
6	Conclusion	19
7	Reference	21

INTRODUCTION

Vibrio is a genus of halophilic, mesophilic, gram-negative bacteria possessing a curved rod (comma) shape and 2 chromosomes which is unusual for bacteria (1, 10). It is also ubiquitous bacteria found in wide variety of aquatic and marine habitat (3). All the members are motile and good swimmers, so that they can attach to various fishes and shell fishes (including oysters, mussels, clams) inhabiting in water (1). Several *Vibrio* species have been recognized as human enteropathogens (11). Beside their potential pathogenicity to man, they are described as important fish and shell fish pathogens. The infection caused by *Vibrio* species is called Vibriosis. When ingested, *Vibrio* bacteria can cause watery diarrhoea, often accompanied by abdominal cramping, nausea, vomiting, fever, and chills. Usually, these symptoms occur within 24 hours of ingestion and last about three days. Severe illness is rare and typically occurs in people with a weakened immune system (25). The Infection normally acquired through exposure to sea water, associated with consuming raw or undercooked contaminated seafood, also it can enter the body through a wound or break in skin of a person while handling *Vibrio* infected raw or shell fish caught from the water. The infection cases of *Vibrio* species occur during warmer months.

The human diseases caused by *Vibrio* bacteria can be Cholera and Non-Cholera infections. *V. Cholerae* is the aetiological agent of Cholera, leads to diarrhoea illness usually caused by intake of contaminated food or water, also person to person transmission (3, 19). Non-Cholera Vibriosis caused by bacteria such as *V. parahaemolyticus* and *V. vulnificus*. It will lead to mild gastroenteritis or primary septicaemia by raw or undercooked contaminated food (3). The antibiotics are extensively used to prevent the disease caused by *Vibrio* species, so there is a risk of developing antibiotic resistance in the bacteria. The resistance pose threat to public health globally. In developing countries like India, recent hospital and community-based data showed increase in burden of antibiotic resistance of bacteria(9).

A wide range of enrichment and selective media for the isolation of *Vibrio* has been developed. These media are reviewed with respect to their ability to recover and differentiate the target different *Vibrio* species. Alkaline peptone water (APW) remains the recommended enrichment medium for *Vibrio* in parallel with either Salt Polymyxin Broth (SPB) or Glucose Teepol Salt Broth (GTSB). Thiosulphate Citrate Bile salt Agar (TCBS) in parallel with Polymyxin Mannose Tellurite (PMT) or Sodium dodecyl sulphate Polymyxin Sucrose Agar (SPS) are the recommended as selective plating media(2).

Vibrio bacteria colonies that grow on the TCBS media would vary in colour, including green, yellow, transparent green, orange and bluish green. *Vibrio alginolyticus* and *Vibrio cholerae* form turbid yellow colour (19). *Vibrio parahaemolyticus* has greenish-blue colony colour, and the centre of the bacterial colony is dark colour (10). *Vibrio fluvialis* form yellow colour. *Vibrio vulnificus* appear green in colour (11). *Vibrio* species that cannot ferment sucrose are characterised by green colonies and those species which can ferment sucrose are characterised by yellow colonies (6).

Vibriosis has been a nationally notifiable disease since 2007(20). According to World Health Organization, globally the *Vibrio parahaemolyticus* and *Vibrio vulnificus* represent important human pathogens associated with consumption of seafood and Cholera remains a global threat to public health and an indicator of inequity and lack of social development. Researchers have estimated that each year there are 1.3 to 4.0 million cases of cholerae, and 21,000 to 1,43000 deaths worldwide due to cholera(22).

People who eat food like poke, sashimi, sushi that are made up of raw fish are more prone to Vibriosis. Sushi is Japanese delicacy prepared using raw fish. Recently sushi is getting much popularity in other countries also. Raw fish, shellfish and mollusc are active habitats for variety of microorganisms and parasites. Hence, it is important to detect the prevalence of the pathogens. The present study focuses on understanding the occurrence of various species of *Vibrio* bacteria that might be present in commonly consumed fish, shellfish, and mollusc.

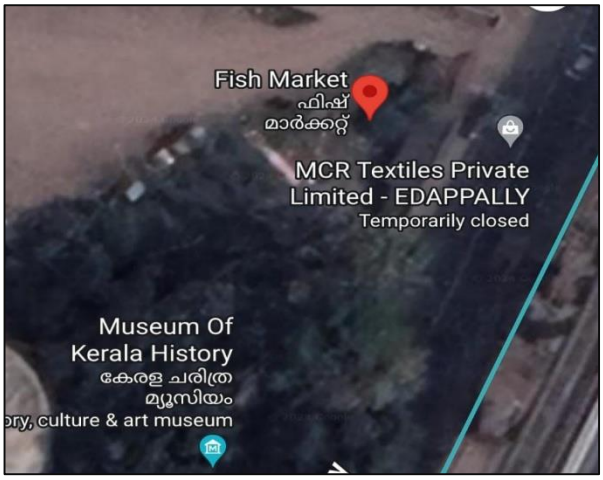
OBJECTIVE

The study aimed to determine the prevalence of different species of *Vibrio* in fish and shellfish collected from Cochin. It also examined the antibiotic sensitivity of isolated *Vibrio* species.

SAMPLING STATIONS

Kalamukku fish landing site and Pathadipalam fish market, Cochin, Kerala was selected as sampling sites for studying.

9.9848570, 76.2421778



10.038304,76.315106

REVIEW OF LITERATURE

Christian Scarano et al. in 2013 conducted a study on the topic "Antibiotic resistance of *Vibrio* species isolated from Sparus aurata reared in Italian mariculture". The study was intended to conduct multi-site survey on the antibiotic resistance of species of *Vibrio* that were isolated from skin, gills, and intestinal content of 240 Gilthead Seabream. About 150 strains were randomly selected and they were sequenced for species identification. By broth microdilution method, resistance against 15 antimicrobial agents were tested. *Vibrio harveyi* and *Vibrio alginolyticus* accounted for 36.7% and 33.3% from total isolates respectively. Multiple resistance to the tested drugs was showed in 96% of the strains. In these strains, *Vibrio aestuarianus* and *Vibrio harveyi* were found to be resistant to 9-10 antibiotics respectively. Most of them tested *Vibrio* species showed low resistance towards antibiotics like ampicillin, amoxicillin, erythromycin, and sulfadiazine.

Etinosa O. Igbinosa & Anthony I. Okoh investigated on the "*Vibrio fluvialis*: an unusual enteric pathogen of increasing public health concern" in 2010. The study concluded that the amount of wastewater effluents being discharged into watershed was increased over the period in developing countries. Among different species of *Vibrio*, *Vibrio fluvialis* is a very infectious pathogen that is a serious threat to the public health. This work mainly emphasized on the epidemiology of the pathogen, pathogenesis of its disease and its clinical expression in humans.

Anisa J. Silva & Jorge A. Benitez in 2016 studied the "*Vibrio* cholerae biofilms and cholera pathogenesis". The study reviewed the evidence for biofilm formation during infection the virulence of gene expression and the coordinate regulation of biofilm. The work discussed about a model for the role of *Vibrio* cholerae biofilms in pathogenicity.

Burak Ekrem Citil et al. in 2015 analyzed on the topic "*Vibrio* alginolyticus associated Chronic Myringitis acquired from Mediterranean waters of Turkey". The particular specie of *Vibrio* species was not found to be pathogenic to humans. But a case of Chronic Myringitis is reported to 47 years old female due to *Vibrio alginolyticus*. By automatized BD phoenix system and Kirby-Bauer disk diffusion method, the investigation and antimicrobial susceptibility were performed. This *Vibrio* specie was sensitive to all antibiotics. The clinical isolates were isolated from superficial wounds or the external ear infections.

Tarfa Abdalla. et al. in 2022 studied the prevalence of *Vibrio spp*. in fish and shell fish samples in Subtropical Arid countries(United Arab Emirates) that was 64.5 % and 92% respectively. However, *Vibrio parahaemolyticus* was detected in a mere 7.5% and 13% of the samples, respectively. *Vibrio mimicus* was detected in 1.5% and 8.5% of the samples. The 6 antibiotics except Sulfamethoxazole-Trimethoprim was not effective against fish Vibrio spp isolates. 3 antibiotics namely Penicillin, Daptomycin and Vancomycin were ineffective against the shellfish isolates.

S Sudha. et al. in 2012 studied the "Prevalence and antibiotic resistance of pathogenic *Vibrio* in shellfish included Shrimp, Clams and Crab from Cochin market". The result showed the presence of 65.5% of *Vibrio* species such as *V. parahaemolyticus* (68.1%), *V. alginolyticus* (18.1%), *V. vulnificus* (2%), *V. cholerae* (1%), *V. harveyi* (1%) and *V. mimicus* (1%). Overall percentage of incidence of *Vibrio* species was highest in clams (84.2%) followed by shrimps (63.8%) and Crabs (37.5%). When isolates were tested for their susceptibility to antibiotics, highest incidence of antibiotic resistance was evident against Ampicillin and Colistin followed by Amoxycillin, Carbenicillin, Ceftazidime and Cephalothin & lowest sensitivity was showed against Chloramphenicol, Tetracycline and Nalidixic Acid. Multiple drug resistance was prevalent among the isolates.

Mona A. El-Zamkan. et.al. in 2023 analyzed "The molecular characterization of *Vibrio* species isolated from dairy and water samples". The study aimed to detect the presence of *Vibrio cholera* and *Vibrio parahaemolyticus* in raw milk, dairy products and water samples and also examined the virulence factor, antibiotics resistance and biofilm formation. The result showed that *Vibrio cholera* and *Vibrio parahaemolyticus* was detected in 1.25% - 1.5% respectively out of the total samples. The strains of *Vibrio cholera* and *Vibrio parahaemolyticus*, showed the Penicillin resistance, with a MAR index ranging from 0.16 to 0.5. The *Vibrio cholera*e showed the virulence genes, ctxAB, hlyA, & tcpA were found in 60% to 80% isolates respectively. Although, tdh +/trh + associated virulence genes were discovered in 33.3 % of *Vibrio parahaemolyticus* isolates.

Yiqin Deng. et.al. in 2022 studied "The Prevalence, virulence genes, and antimicrobial resistance of *Vibrio* species isolated from diseased marine fish in South China". Here, 70 potential *Vibrio* pathogens belonging to 9 species were isolated, dominated by *Vibrio harveyi* Subsequently, the prevalence of 11 virulence genes and the resistance to 15 antibiotics in these strains were determined. Most strains possessed a typical virulence gene in addition to

typical virulence genes. Notably, hfk and chiA originating from *V. harveyi*, and faC associated with *V. anguillarum* were detected in more than 40% of strains. Multidrug resistance was widespread. 64.29% strains were resistant to more than 3 antibiotics, and the multi-antibiotic resistance index ranged from 0.00 to 0.60. The proportions of strains resistant to the antibiotics Vancomycin, Amoxicillin, Midecamycin, and Furazolidone all exceeded 50%.All strains were sensitive to Florfenicol, Norfoxacin, and Ciprofloxacin. both virulence genes and antibiotic resistance were more prevalent in the isolates.

Rajapandiyan. et.al. in 2009, investigated on "The prevalence and distribution of *Vibrio vulnificus* in fishes caught off Chennai, Indian Ocean. This study determined the prevalence of *Vibrio vulnificus* in fishes caught off Chennai coast of Indian Ocean. The *Vibrio* isolated in the study are, *V. mediterrenei*, *V. campelli*, *V. alginolyticus* and *V. vulnificus*. The 3 key characteristics for the identification of these species from *V. vulnificus* are the ONPG reaction, sucrose and lactose fermentation. The *Vibrio* found up to 19 - 39 percent of the total aerobic flora. The prevalence of *V. vulnificus* is about 13 percent of total *Vibrio* were isolated from the fish. Other isolates are *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. The data regarding the environmental occurrence, niches, survival, seasonality and strain diversity will be helpful for developing the different sea food strategies and that in turn helps to eradicate the probability of *V. vulnificus* in transporting sea food.

Yama Gxalo. et.al. in 2021 conducted a study on "Virulence and antibiotic resistance characteristics of *Vibrio* isolates from rustic environmental freshwater. The result showed that presence of antimicrobial resistance genes and determinants in *Vibrio* species from different freshwater sheds in rustic milieu. About a total of 118 *Vibrio* including *Vibrio fluvialis* (n=41), *Vibrio mimicus* (n=40) and *V. vulnificus* (n=37) was identified by amplification of vmh, ToxR and hsp60 genes. The study points out that the antimicrobial resistance was more for Polymyxin B (100%), Azithromycin (100%) and least in Ciprofloxacin (16.1%). Among the isolates, 5.2 % and 44.3 % of Chloramphenicol resistance genes (cmlA1 and catII) were detected. The findings in this study revealed the occurrence of antimicrobial resistance genes and virulent Vibrio species in an aquatic environment which pose a threat to human and animal life.

Claudia villicana. et.al. in 2019 studied on the occurrence and abundance of Pathogenic *Vibrio* species in Raw oysters at retail seafood markets in Northwestern Mexico. The study evaluated the multiplicity and presence of 3 species mainly *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* in 68 raw oysters that collected from retail seafood markets in Sinaloa, Mexico. The result showed that all the samples, tested contained at least 1 *Vibrio* species. *Vibrio parahaemolyticus* is more prevalent (77.9%) in the overall sample tested. It indicated the widespread presence of pathogenic *Vibrio* species in raw oysters from retail seafood markets in Mexico. The consumption of these raw oysters without proper cooking may lead to the causing serious foodborne illness caused by pathogenic *Vibrio* bacteria.

Balwinder. et.al. in 2018, conducted a study to evaluate the "Prevalence, potential pathogenicity and antimicrobial resistance of *Vibrio* isolates from 65 soil/water/fish samples collected from inland saline aquaculture areas. Depending on the sample type, presumptive *Vibrio* counts ranged from 2.50 to 6.16 log10 CFU/ml. Among the 119 confirmed *Vibrio* isolates, *Vibrio cholerae* was found to most dominant (91.6%) and it was detected in all the samples from inland saline areas. Seven other *Vibrio spp*. including *Vibrio parahaemolyticus* and *Vibrio vulnificus* were also detected. Except one O139 serotype, rest of the *V. cholerae* isolates were found belonging to non-O1/non-O139 serogroups. None of the *V. cholerae* isolate was found positive for ctx gene. Antimicrobial susceptibility testing against 7 commonly used antibiotics revealed highest resistance (50.4%) against Ampicillin. Very high intermediate resistance (87.4%) was also observed against Erythromycin. Contrary to previous studies, high susceptibility (>70%) to Chloramphenicol, Nalidixic Acid, Tetracycline and Trimethoprim was observed in *Vibrio* isolates obtained in present study. Almost 20% of *Vibrio* isolates were resistant to two or more antibiotic classes with multiple antibiotic resistance (MAR) index value of ≥0.28. Presence of *V. cholerae* isolates with very high MAR index value of 0.85 also suggested that these multidrug-resistant environment isolates could serve as reservoir of antibiotic-resistant genes in aquatic systems. The presence of multiple drug resistance Vibrio in emerging inland saline aquaculture systems emphasizes the need for their routine monitoring for developing the risk assessment and mitigation strategies.

METHODS AND METHODOLOGY

SAMPLING

Fish and shellfish samples were collected from 2 different sampling sites (Kalamukku fish landing site and Pathadipalam fish market) in Cochin located in Kerala, South India. 14 Samples of fish and shellfish were collected during the months of February and March, 2024. *Penaeus indicus, Perna viridis, Sardinella longiceps, Rastrelliger kanagurta, Selar crumenophthalmus, Portunus sanguinolentus, Nemipterus randalli, Lactarius lactarius, Carangoides malabaricus, Charybdis feriata*. The samples were collected aseptically in sterile zip-lock bags and transported to the laboratory in an icebox.

Sampling Day 1- 19.02.2024

Sampling processing and enrichment

First sampling was completed on 19.02.2024, 11:45 am from Kalamukku landing site, Cochin. The collected samples were *Penaeus indicus*, *Perna viridis*, *Sardinella longiceps*, *Rastrelliger kanagurta, Selar crumenophthalmus*, *Portunus sanguinolentus*, *Nemipterus randalli.* 1g of sample flesh was carefully taken, Homogenized and each sample was serial diluted into 2 dilutions for further enrichment. Alkaline peptone water (APW) was used as enrichment media for growth of *vibrio* species. APW was prepared by combining 1.5g of sodium chloride and 1.5g of peptone in 150 ml of distilled water. 1ml of each dilution were inoculated into two separate test tube containing APW and incubated at 37^0C for 24 hours for isolation of *vibrio* species.

Isolation and identification of *Vibrio* species

For the isolation and the identification of *Vibrio* species, Thiosulphate Citrate Bile salt Sucrose (TCBS) agar was used as selective medium. 13.45g of TCBS agar was mixed with 150ml of distilled water and poured into petri plates. A loopful of enrichment broth from each dilution were aseptically streaked onto sterile surface of TCBS agar plates and incubated at 37^0C for 24 hours.

Sampling Day 2- 14.03.2024

Sample processing and enrichment

Second sampling was conducted on 14th march, 2024 from Pathadipalam fish market, Cochin. The collected samples included *Penaeus indicus, Rastrelliger kanagurta, Selar crumenophthalmus, Nemipterus randalli, Lactarius lactarius, Carangoides malabaricus, Charybdis feriata*. 1g of sample flesh was carefully taken, Homogenized and each sample was serial diluted into 1 dilution for further enrichment. For growth Zobell Marine Agar was used. It was prepared by combining 8.3g of Zobell Marine in 150 ml water and poured into petri plates. 0.1ml from 10-1 dilution was inoculated into dried Zobell Marine Agar plates by spread plate method and incubated at 37^0C for 24 hours.

Isolation and identification of *Vibrio* species

For the isolation and the identification of *Vibrio* species Thiosulphate Citrate Bile salt Sucrose (TCBS) were used. 13.45g of TCBS agar was mixed with 150ml of distilled water and poured into petri plates. The distinct colonies that have grown on the Zobell Marine Agar plates were picked and then streaked into TCBS plates and incubated overnight at 37^0C.

Antibiotic sensitivity test of isolated of *Vibrio sp*.

Antibiotic sensitivity assays were performed using Kirby Bauer disk diffusion method. A total of 6 antibiotic disks were used to test the sensitivity of the *Vibrio* isolates such as Streptomycin, Tetracycline, Ampicillin, Chloramphenicol, Penicillin, Erythromycin. Nutrient agar was used as base media for culturing the isolates. Nutrient agar was prepared by mixing 2.8g of Nutrient Agar with 100ml of distilled water. The pure culture of *Vibrio* isolates was streaked over sterile surface of Nutrient Agar plates using a sterile cotton swab. All 6 Antibiotic disk was allocated into culture plates and incubated for 24 hours. The effect of the antibiotic disk was observed by measuring the diameter of inhibition zones after 24 hours.

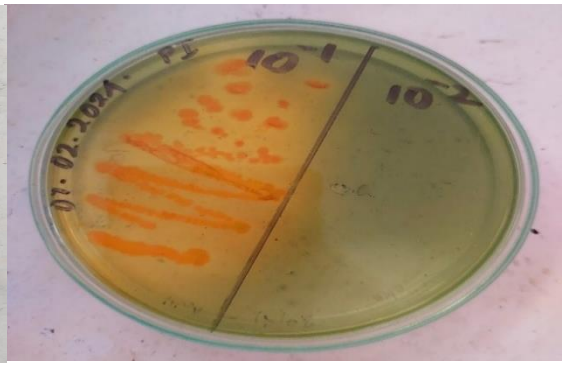
OBSERVATION AND RESULT

In fourteen samples of fish and shellfish, five species of *Vibrio* were found to exist such as *Vibrio cholerae*, *Vibrio fluvialis*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. The incidence of *Vibrio vulnificus* was higher among 14 samples and *Vibrio alginolyticus* was less prevalent. All the isolated species were subjected to antibiotic sensitivity assays by following Kirby- Bauer disk diffusion method. The different antibiotics taken are Ampicillin, Chloramphenicol, Erythromycin, Penicillin, Streptomycin and Tetracycline and the zone of inhibition was noted. All of species were sensitive towards chloramphenicol, erythromycin, streptomycin and show resistance towards penicillin and ampicillin.

SAMPLING 1

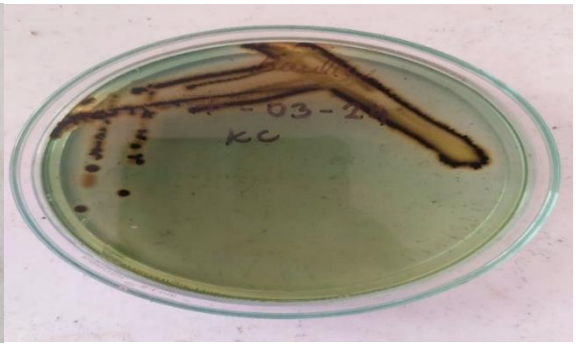
Nemipterus randalli

Portunus sanguinolentus

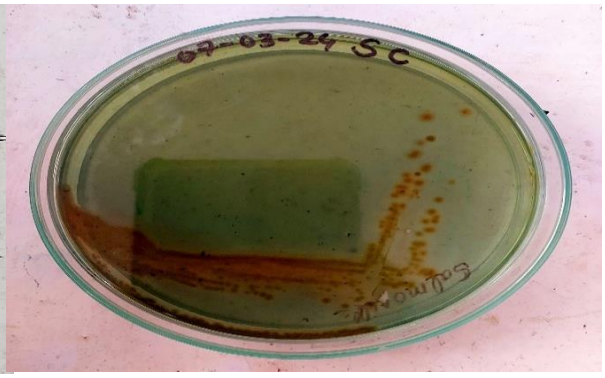



Penaeus indicus

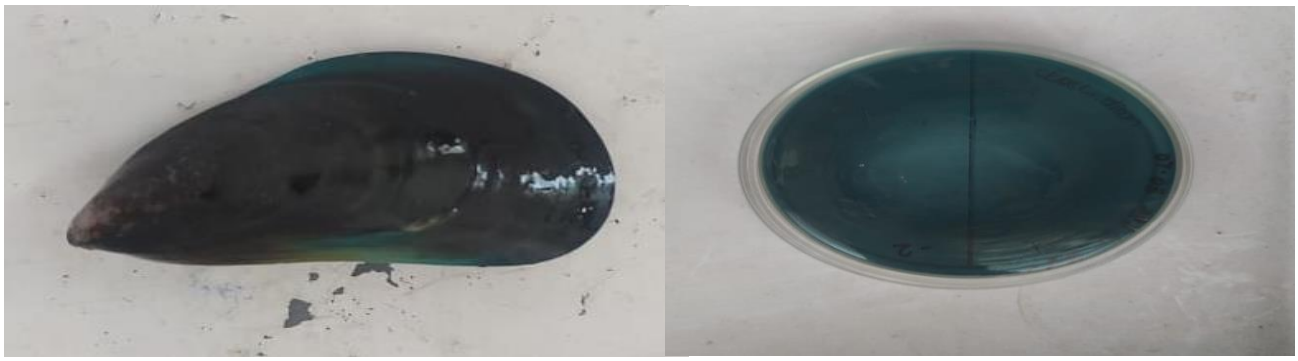

Sardinella longiceps

Johnius belangerii

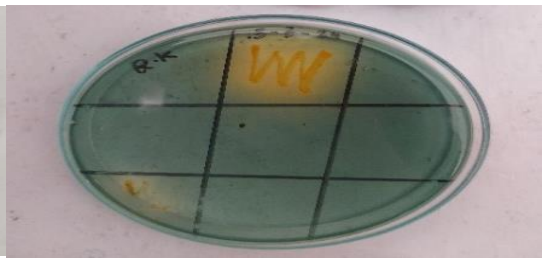
Selar crumenophthalmus



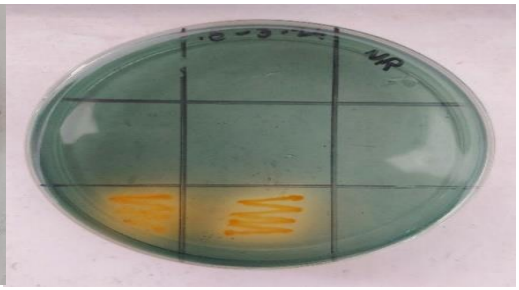
Perna viridis

FISH	*VIBRIO* SPECIES				
	Vibrio alginolyticus	***Vibrio fluvialis***	***Vibrio vulnificus***	***Vibrio cholerae***	***Vibrio parahaemolyticus***
Sardinella longiceps	Absent	Present	Absent	Absent	Absent
Selar crumenophthalmus	Present	Absent	Absent	Absent	Absent
Portunus sanguinolentus	Absent	Absent	Present	Present	Absent
Nemipterus randalli	Absent	Absent	Absent	Present	Absent
Penaeus indicus	Absent	Absent	Present	Absent	Absent
Perna viridis	Absent	Absent	Absent	Absent	Absent
Johnius belangerii	Absent	Absent	Absent	Absent	Present

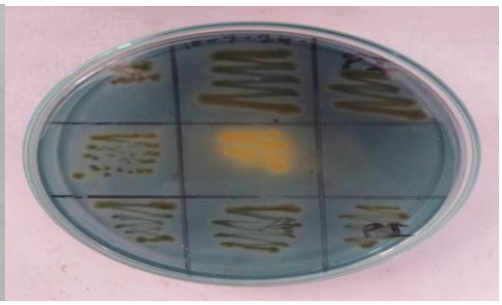
SAMPLING 2

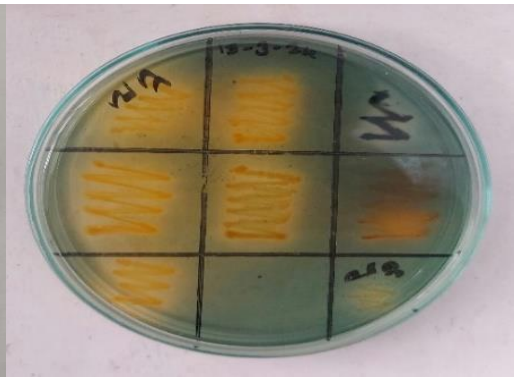
Rastrillegar kanagurta

Nemipterus randalli

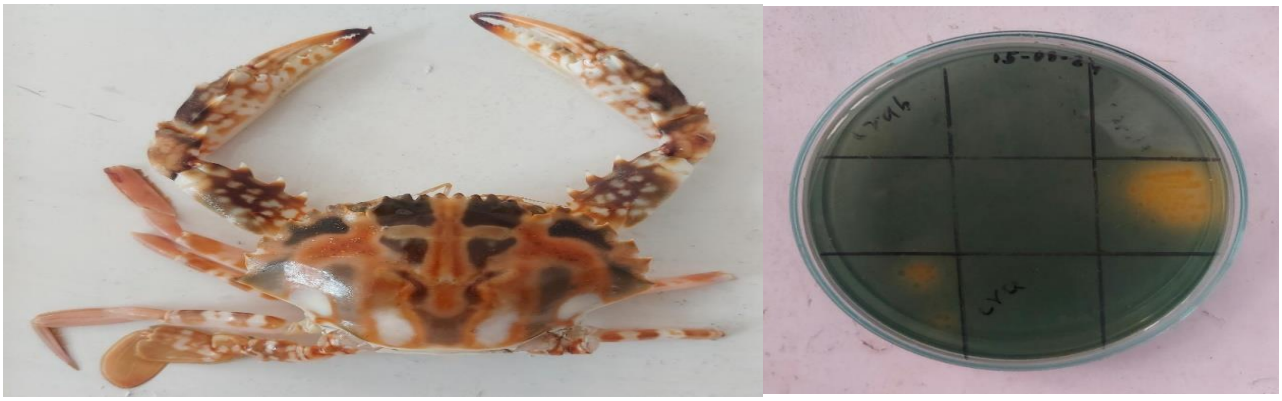



Penaeus indicus

Carangoides malabaricus

Selar crumenophthalmus



Charybdis feriatus

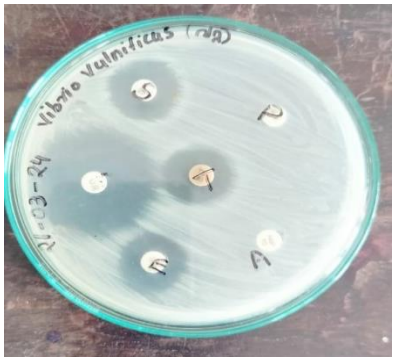
Lactarius lactarius

FISH	*VIBRIO* SPECIES				
	Vibrio alginolyticus	***Vibrio fluvialis***	***Vibrio vulnificus***	***Vibrio cholerae***	***Vibrio parahaemolyticus***
Penaeus indicus	Absent	Absent	Present	Present	Present
Nemipterus randelli	Absent	Absent	Present	Absent	Absent
Rastrilleger kanagurta	Absent	Absent	Present	Absent	Absent
Lactarius lactarius	Absent	Present	Absent	Absent	Absent
Carangoides malabaricus	Absent	Present	Present	Present	Present
Charybdis feriatus	Absent	Absent	Absent	Present	Present
Selar crumenophthalmus	Absent	Absent	Absent	Absent	Absent

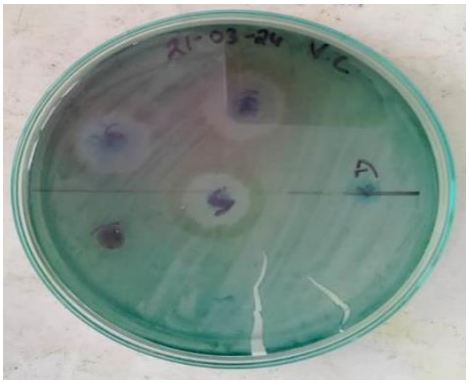
EFFECT OF ANTIBIOTICS ON *VIBRIO* SPECIES

V.fluvialis

V.parahaemolyticus



V.vulnificus

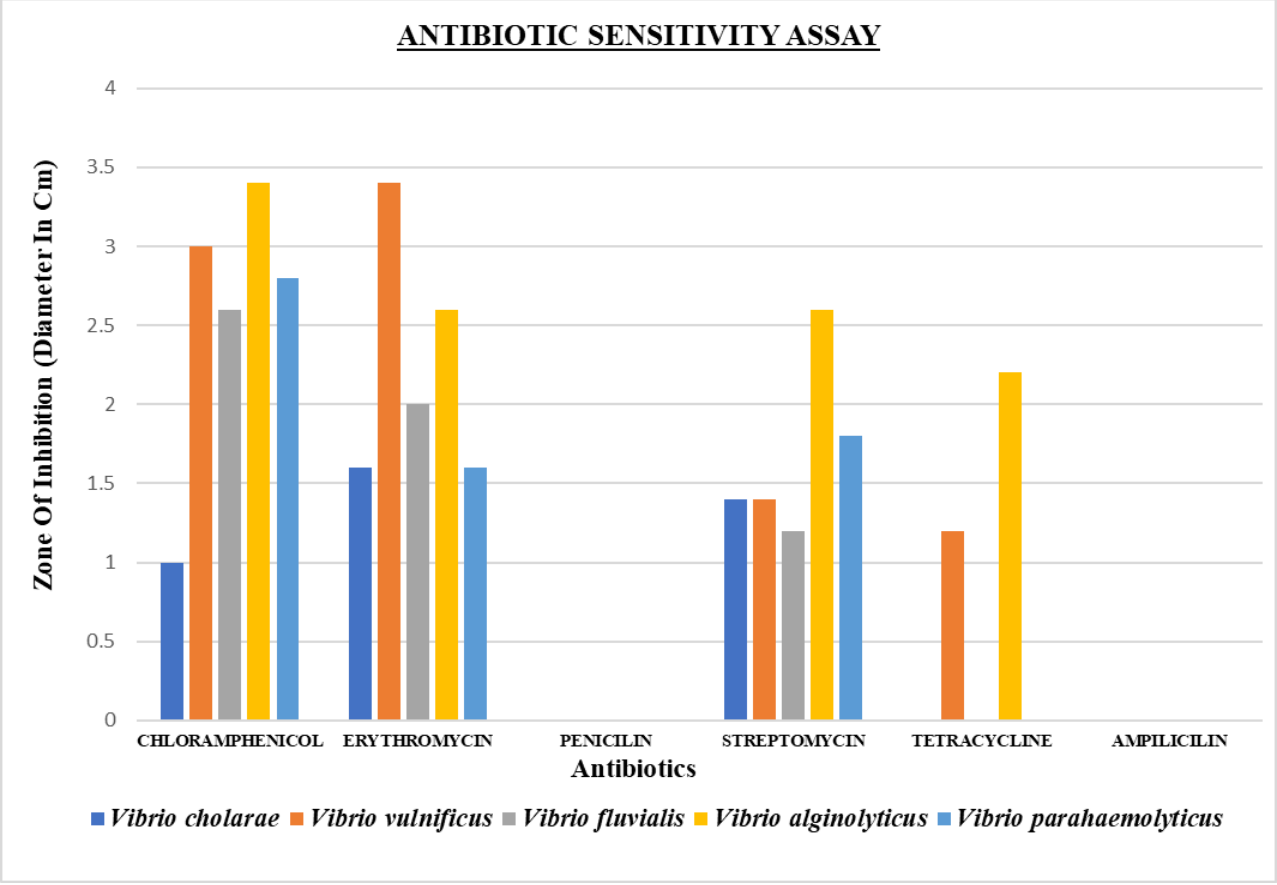


V.cholerae

V.alginolyticus

ANTIBIOTIC SENSITIVITY ASSAY (Kirby Bauer disk diffusion)

Antibiotics	Zone Of Inhibition (Diameter in cm)				
	Vibrio species				
	Vibrio cholerae	*Vibrio vulnificus*	*Vibrio fluvialis*	*Vibrio alginolyticus*	*Vibrio parahaemolyticus*
Ampicillin	0	0	0	0	0
Chloramphenicol	1	3	2.6	3.4	2.8
Erythromycin	1.6	3.4	2	2.6	1.6
Penicillin	0	0	0	0	0
Streptomycin	1.4	1.4	1.2	2.6	1.8
Tetracycline	0	1.2	0	2.2	0



DISCUSSION

Vibrio is a genus of halophilic, mesophilic, gram-negative bacteria. Several *Vibrio* species have been recognized as human enteropathogens such as, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio alginolyticus*. They mainly cause infections like Cholera, septicemia, Gastroenteritis, Ear Infections, Wound Infections, etc. Apart from this, antibiotic-resistant strains are becoming more prevalent in recent times.

The study conducted by S Sudha et.al. 2013 on the topic "Prevalence and antibiotic resistance of pathogenic *Vibrios* in shellfishes from Cochin market" revealed the prevalence of different pathogenic *Vibrios* and their antibiotic resistance. For this study 110 shellfish samples including Clams, Crab and Prawns were gathered between September 2010 and March 2011. Six potentially harmful *Vibrio* species were found in 65.5% of the samples. Clams showed the highest total proportion of *Vibrio* species (84.2%), followed by prawns (63.8%) and crabs (37.5%). Antibiotic resistance was most noticeable against Ampicillin and Colistin and least noticeable against Chloramphenicol, Tetracycline, and Nalidixic acid. Amoxycillin, Carbenicillin, Ceftazidime and Cephalothin were the next most common antibiotics to show signs of resistance. The isolates exhibited a high multiple drug resistance. The findings show that potentially dangerous *Vibrio* species are present in Cochin retail markets, which may affect the quality and safety of seafood products.

Our study also exhibits similar results like the presence of *Vibrio* species in fish and shellfishes that were collected from two markets in Cochin, one being Kalamukku fish landing site and the other being Pathadipalam fish market. Further test was conducted to understand the antibiotic resistance of isolated *Vibrio* species. For this study, 14 samples of fish were collected from the two sampling stations. After processing of samples followed by subsequent culturing of bacteria, five species of pathogenic *Vibrio* were identified namely, *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *V. Cholerae* and *V. fluvialis*. The incidence rate of species V. *vulnificus* is more and *V. alginolyticus* was found to be rare in the samples. All these isolated pathogenic *Vibrios* were subjected to antibiotic sensitivity assay using Kirby- Bauer Disk diffusion using 6 selected antibiotics. The antibiotics used are Ampicillin, Chloramphenicol, Erythromycin, Penicillin, Streptomycin and Tetracycline. All species were resistant to Ampicillin and penicillin. All the species were found sensitive towards Chloramphenicol, Erythromycin, and Streptomycin.

The species *V. cholerae*, *V. fluvialis* and *V. parahaemolyticus* were completely resistant towards the antibiotic Tetracycline. Only *V. vulnificus* and *V. alginolyticus* were sensitive towards the antibiotic Tetracycline.

This study accounts the high prevalence of *Vibrio* pathogens in fishes and shellfishes in Cochin market which causes contamination issues and the also indicates the emergence of antibiotic resistance strains.

CONCLUSION

Vibrio are a group of bacteria that are commonly found in marine habitats. The important *Vibrio* species include *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio alginolyticus* and *Vibrio fluvialis*. These pathogenic species cause various diseases in humans like Gastroenteritis, Septicemia, Wound infections, Cholera etc. The pathogens mainly enter into human body through consumption of contaminated food and water, seafood especially raw or undercooked fish, shellfish and molluscs, and through open wounds that come in contact with contaminated environment. Therefore, the prevalence of this pathogenic species of *Vibrio* is a threat for human health. Nowadays most of the species are becoming resistant towards various antibiotics. This Antibiotic resistance has emerged as a result of continued use of antibiotics.

In the present study, the samples of fishes were collected from two sites in Cochin, Kerala. In first sampling, alkaline peptone water (APW) is used for enrichment and Thiosulphate Citrate Bile salt Sucrose (TCBS) for the isolation of different species of *Vibrio*. In second sampling, Zobell Marine Agar and TCBS agar for enrichment and for isolation respectively. Antibiotic assays were performed by disk diffusion method with six antibiotics namely Streptomycin, Tetracycline, Ampicillin, Chloramphenicol, Penicillin and Erythromycin. The isolated are streaked on nutrient agar plates and the Disk impregnated with antibiotics is placed in the plates. After the incubation period, inhibition zones were measured. This study reveals the incidence of five *Vibrio* species from 14 different samples of fishes and shellfishes. *V. cholerae*, *V. Parahaemolyticus*, *V. Alginolyticus*, *V. vulnificus* and *V. fluvialis* were isolated. The species *V. vulnificus* is more prevalent among them, while *V. alginolyticus* is less prevalent. The results of the antibiotic sensitivity test indicate that five isolated *Vibrio* species was resistant to antibiotics such Ampicillin and Penicillin as well as sensitive towards Chloramphenicol, Erythromycin and Streptomycin.

The results emphasize the need for stronger regulations and better hygienic measures in the seafood business to reduce the danger of *Vibrio* contamination and also it is very important to have surveillance programs to track the antibiotic resistance of pathogenic *Vibrio* in seafood. The antibiotic sensitivity test for *Vibrio* help to determine the effective antibiotics against each specific strains. Knowing the effective antibiotics ensures proper treatment against diseases caused by them. It also enables in prescribing the most appropriate

antibiotics and it reduces the treatment failure against diseases caused by them. It also enables in prescribing the most appropriate antibiotics and it reduces the treatment failure.

REFERENCE

1. Abdalla, T., Al-Rumaithi, H., Osaili, T. M., Hasan, F., Obaid, R. S., Abushelaibi, A., & Ayyash, M. M. (2022). Prevalence, Antibiotic-Resistance, and Growth Profile of Vibrio spp. Isolated from Fish and Shellfish in Subtropical-Arid Area. Frontiers in microbiology, volume 13, 861547. https://doi.org/10.3389/fmicb.2022.861547

2. Balwinder, singh., Anuj, Tyagi., Naveen, K. Billekallu, Thammegowda., Meera, D. Ansal. (2018). Prevalence and antimicrobial resistance of vibrios of human health significance in inland saline aquaculture areas. Aquaculture research, 49(6), 2166-2174.

3. Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). Vibrio spp. infections. Nature reviews. Disease primers, 4(1), 8. https://doi.org/10.1038/s41572-018-0005-8

4. Deng, Y., Xu, L., Chen, H., Liu, S., Guo, Z., Cheng, C., Ma, H., & Feng, J. (2020). Prevalence, virulence genes, and antimicrobial resistance of Vibrio species isolated from diseased marine fish in South China. Scientific reports, 10(1), 14329. https://doi.org/10.1038/s41598-020-71288-0

5. El-Zamkan, M. A., Ahmed, A. S., Abdelhafeez, H. H., & Mohamed, H. M. A. (2023). Molecular characterization of Vibrio species isolated from dairy and water samples. Scientific reports, 13(1), 15368. https://doi.org/10.1038/s41598-023-42334-4

6. Farida, Hikmawati., Ari, Susilowati., Ratna, Setyaningsih. (2019). Colony morphology and molecular identification of Vibrio spp. on green mussels (perna viridis) in Yogyakarta, Indonesia tourism beach areas. Biodiversitas, 20(10), 2891-2899.

7. Gxalo, O., Digban, T. O., Igere, B. E., Olapade, O. A., Okoh, A. I., & Nwodo, U. U. (2021). Virulence and Antibiotic Resistance Characteristics of Vibrio Isolates From Rustic Environmental Freshwaters. Frontiers in cellular and infection microbiology, 11, 732001. https://doi.org/10.3389/fcimb.2021.732001

8. Igbinosa, E. O., & Okoh, A. I. (2010). Vibrio fluvialis: an unusual enteric pathogen of increasing public health concern. International journal of environmental research and public health, 7(10), 3628–3643. https://doi.org/10.3390/ijerph7103628

9. Kumar, S. G., Adithan, C., Harish, B. N., Sujatha, S., Roy, G., & Malini, A. (2013). Antimicrobial resistance in India: A review. Journal of natural science, biology, and medicine, 4(2), 286–291. https://doi.org/10.4103/0976-9668.116970

10. N. R. Rukmana., G. Mahasri., S. N. Hidayah., M. F. Ulkhaq., H. Kenconojati. (2019). Bacterial identification from marine ornamental fish in fish quarantine, quality control and fishery product safety class I Denpasar, Bali .The 1st International Conference on Fisheries and Marine Science

https://www.researchgate.net/publication/331223684_IOP_Conference_Series_Earth_and_Environmental_Science_Towards_sustainable_development_resource_approach_through_interaction_of_peri-urban_and_surrounding_areas_Towards_sustainable_development_resource

11.Okada, K., Roobthaisong, A., Hearn, S. M., Okada, P. A., Doung-Ngern, P., Wongboot, W., Nakkarach, A., Morita, M., Kodama, T., & Iida, T. (2023). Emergence of Vibrio parahaemolyticus serotype O10:K4 in Thailand. Microbiology and immunology, 67(4), 201–203. https://doi.org/10.1111/1348-0421.13055

12. Steven L. Percival, David W. Williams, in Microbioogy of Waterborne Diseases (Second Edition), 2014.

13. Ramamurthy, T., Mutreja, A., Weill, F. X., Das, B., Ghosh, A., & Nair, G. B. (2019). Revisiting the Global Epidemiology of Cholera in Conjuction With the Genomics of Vibrio cholerae. Frontiers in public health, 7, 203. https://doi.org/10.3389/fpubh.2019.00203

14. Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., & De Santis, E. P. (2014). Antibiotic resistance of Vibrio species isolated from Sparus aurata reared in Italian mariculture. The new microbiologica, 37(3), 329–337.

15. Silva, A. J., & Benitez, J. A. (2016). Vibrio cholerae Biofilms and Cholera Pathogenesis. PLoS neglected tropical diseases, 10(2), e0004330. https://doi.org/10.1371/journal.pntd.0004330

16. S. Rajapandiyan., K. Sudha., Kantha, D. Arunachalam., Burak, Ekrem, Citil., Serhan, Derin., Funda, Sankur., Murat, Sahan., Mahmut, Ugur, Citil. (2015). Vibrio alginolyticus Associated Chronic Myringitis Acquired in Mediterranean Waters of Turkey. African Journal of Microbiology Research, 3(10), 622-625. https://doi.org/10.1155/2015/1872122009

17. S. Sudha., C. Mridula., Reshma, Silvester & A. A. M. Hatha. (2013). Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market. Indian Journal of Geo-Marine Sciences, 43(5), 815-824.

18. Villicaña, C., Amarillas, L., Soto-Castro, L., Gómez-Gil, B., Lizárraga-Partida, M. L., & León-Félix, J. (2019). Occurrence and Abundance of Pathogenic Vibrio Species in Raw Oysters at Retail Seafood Markets in Northwestern Mexico. Journal of food protection, 82(12), 2094–2099. https://doi.org/10.4315/0362-028X.JFP-19-237

19. Vibrio infecions, https://www.vdh.virginia.gov/epidemiology/epidemiology-fact-sheets/vibriosis-non-cholera/

20. Vibrio characteristics, https://www.wikipedia.org/

21. Encyclopedia of food microbiology
https://www.sciencedirect.com/book/9780123971692/molecular-medical-microbiology

22. Vibriosis, https://www.cdc.gov/vibrio/index.html

23. Vibrio bacteria, https://en.wikipedia.org/wiki/Vibrio

24. Incidence of vibriosis, https://www.who.int/

25. vibrio infection, https://www.medicinenet.com/vibrio_infection_vibriosis/article.htm