International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Isolation and identification of pathogenic *Vibrio alginolyticus* from marine food resources

L. Banupriya and K. Arunagiri

Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Knacheepuram, Tamil Nadu, India

*Corresponding author e-mail: *akarungirikumar01@gmail.com*

Abstract

The development of antimicrobial resistant among pathogenic bacteria has emerged as a major public health concern, which has led to an intesitfication of discussion about the prudent use of antimicrobial agents especially in medicine, agriculture and veterinary. In this report encompasses the incidence of *V.alginolyticus* in sea foods as a human pathogen, in contribute to the onset of sporadic and epidemic outbreaks of diarrhoeal disease in humans. The following aspects were covered during the present investigation. Isolation of *V alginolyticus* in sea food (Finfish and Shrimps) samples collected from Kasimedu, Chennai. Antibiotic resistant pattern of isolated *V.alginolyticus*, Hemolytic activity of randomly selected in *Vibrio alginolyticus and* To surveillance of the antimicrobial resistance status of *Vibrio alginolyticus.* High lights of the pattern investigations are,*V.alginolyticus* was readily recoverable in all the 2 samples tested viz., Finfishes and crustaceans. All the biochemical test was carried out. Among 2 categories of samples, crustaceans harboured high number (80%) and finfishes (73%) of *V.alginolyticus*. Antibiotic resistant pattern was observed in Penicillin, Erythromycin, tetracycline, streptomycin, amoxyclav ampicillin, vancomyvin, and novobiocin. No resistant pattern was observed in Validixic acid, Gentamycin, Nitrofurantoin, Chloramphenical. A total of 15 strains were tested for hemolytic reaction 12 strains were positive for hemolytic production in blood agar plates and the remaining 3 strains were failed to lyses blood erythrocytes.

Keywords *V.alginolyticus*, Hemolytic activity, Antibiotic resistant pattern.

Introduction

The genus *Vibrio* includes more than 35 species, mostly in marine and natural habitats of sea water, and this species is broadly distributed throughout the world. There are halophylic marine bacteria of this genus known to cause in humans. *Vibrio parahaemolyticus* cause food borne gastroenteritis associated with seafood. Other halophylic *Vibrios,V.alginolyticus, V.fluvialis, V.metschnikovii* are also pathogenic for humans, while *V.anguillarium* is a pathogen fish and other marine animals (Selmin Ozer *et al*., 2008).

Members of the *Vibrio* genus are gram-negative, halophilic bacteria indigenous to coastal marine systems (Thompson *et al*., 2003). While these common bacteria persist as a natural component of the marine microbial flora, a small percentage of environmental isolates carry the genetic

determinants for human pathogenesis. Currently, *Vibrio* infections are the leading cause of seafood-borne bacterial gastroenteritis in the United States and together, *Vibrio cholerae* (non-O1 and non-O139), *V. parahaemolyticus* and *V. vulnificus* account for the majority of those infections (Mead *et al*., 1999). Among the 4,754 *Vibrio* infections reported to the Centers for Disease Control and Prevention (CDC) from 1997-2006, 3,544 (75%) of those infections were foodborne in origin and 1,210 (25%) of those infections were non-foodborne in origin.

V.alginolyticus has not been confirmed as a pathogen in marine fish. Experimental infections causing gilt-head sea bream have demonstrated the presence of cytotoxic and neurotoxic effects for fish and cell lines. The characterization of *V.alginolyticus* isolated from cultured fish or marine water (Zorrilla *et al*., 2000). It is a ubiquitous organism of the saprophytic micro biota. However it has also been suggested the species is a pathogen of several marine animals and humans (Carmen Balebona *et al*., 1998). *V.alginolyticus* as a pathogen of shellfish rather than fish (Colorni *et al.*, 1981) adequately the recovery of *V.alginolyticus* from diseased sea bream *(Sparus aurata)* in Isrel. The characteristics were in complete agreement with the description of *V.alginolyticus* (Shewan and veron 1974). It is usually isolated in the spring and summer from marine sources. *i.e.* it depend on water temperature greater than 10˚C. *V.alginolyticus* as well as *V.parahaemolyticus* should be considered in tissues infections exposure to ocean water (Sally JoRubin *et al.*, 1975).

Aim and objectives

Bacteria of the *Vibrio sp.* are becoming increasingly important as causative agents of human disease. Unlike most pathogenic enteric species usually associated with faecal contamination, *Vibrio sp* are common members of the coastal environments. Thus, the role of the environments, its association with seafood has become a considerable importance, since these are responsible for diarrhoeal diseases. Many studies related to this organism were centered on their occurrence and distribution in environmental samples. Also, their associations with commercially important seafoods which fetch high economical value have been well documented. Recent investigation in many countries indicates that contamination of natural environments and cooked food through raw seafood with potentially pathogenic *Vibrios* are of great public health importance. Long term survivals of *Vibrio*s in cold stored seafoods also indicate a serious public health risk. Hence the present study has therefore been undertaken in the following objectives.

To isolate the pathogenic bacteria mainly *Vibrio* sp from sea foods
To find out the hemolytic activity of randomly selected isolates.
To characterize and identify pathogenic *Vibrio alginolyticus*
To know the resistant pattern of isolated *Vibrio alginolyticus* against various antibiotics.
To study ecological aspects of *Vibrio alginolyticus*

Materials and methods

Sample collection

Fishes and crustacean (Shrimp and crab) of marine origin were randomly purchased from a number of vendors in local fish market at Kasimedu coastal area, Chennai. All the samples were transferred individually into new polythene bags and immediately transported to the laboratory in a portable ice pack.

Sample processing for bacteriological examination

Bacteriological analysis of collected sea foods were performed within 2hours of sampling. The samples such as Fish and crustaceans were washed thoroughly with sterile distilled water prior to bacteriological examination, Fish samples were cut into small pieces using sterile scissors. For shrimp, whole specimen after removing the head region was used. Approximately 25g of each sample was homogenized and mixed with 225 ml of APW and incubated for 18hours at 37^0C. At the end of

incubation period, two loops full of culture from pellicle of each flask were then streaked on to TCBS agar plate. After overnight incubation at 37^0C presumptive *Vibrio*s colonies were subcultured into nutrient agar containing 2% NaCl.

Identification of fish and crustacean

The following are the generic level identification of the seafood samples used in the present study. All fishes and crustacean were identified in Centre of Advanced study in Marine Biology, Annamalai University Parangipettai.

A. Fishes

1. *Caranx sp.*
2. *Sardinella sp.*
3. *Dussumeria sp.*
4. *Stolephorus sp.*
5. *Upneans sp.*
6. *Thryssa sp.*
7. *Ambassis sp.*
8. *Nemipterus sp.*

B. Shrimp

1. *Peneaus monodon*

Identification of *Vibrio alginolyticus*

All bacterial colonies obtained from the entire water and sea food samples were maintained on nutrient agar supplemented with 2% of NaCl. The isolated bacterial strains (mainly *Vibrio alginolyticus*) were identified by studying the morphological, cultural and biochemical characterization. Colony morphology, Gram staining, Motility test and biochemical test *viz*., catalase test, oxidase test, indole production, Methyl red test, Voges-Proscauer test, Citrate utilization, Triple sugar iron test (TSI), nitrate reduction test, Starch hydrolysis, gelatin liquefaction, carbohydrate fermentation test , amino acid decarboxylase test and halophilisms test with various concentration of NaCl. The above test results matched with standard bacteriological and laboratory manuals (Food and Drug Administration, 1992) to identify bacterial strains.

Hemolytic activity

Ability of randomly selected *Vibrio sp* (*Vibrio alginolyticus*) to produced hemolysin was tested on blood agar supplemented with 5% sheep blood. All the selected cultures were spot inoculated on blood agar plates and zone of hemolysis was read after 18-24h incubation.

Study of antibiotic resistant pattern

The entire isolated *Vibrio* sp (*Vibrio alginolyticus*) both from seawater and seafoods were grown in nutrient broth containing 2% NaCl. Muller –Hinton (MH) agar medium (Hi-Media) was used for antibiotic resistance pattern. Disc diffusion assays for antibiotic susceptibility were conducted following the method described by Anon (1970). The following antibiotic discs viz. Trimethoprim, 5; Ampicillin, 10; Penicillin, 10 units; Tetracyclin, 10; Nalidixic acid (Na 30), 30; Bacitaracin, 10 units; Gentamycin, 10; Ceftazidime, 30; contromoxazole, 25; Pefloxacin, 5; Chloramphenicol, 30; Amoxycillin, 30; Ciprofloxacin, 5; and Kanmycin, 30 (the number represent the concentration of anitibiotic in µg and purchased from Hi-Media) . The discs were dispensed using alcohol dipped and flamed forceps onto the surface of MH agar medium, previously seeded with 18-24h cultures of *Vibrio alginolyticus.* All plates were left for 20 minutes and then incubated in an inverted position at 35°C for 18-24hours. The results were recorded by measuring the inhibition zones and scored susceptibility ranges.

Results

Isolation and identification of *Vibrio alginolyticus*

The pathogenic bacterium *Vibrio alginolyticus* was isolated from seafood obtained from various places and it was identified by studied their cultural, morphological and biochemical characterization (Table 1).

Among the 15 fish samples tested, 11 samples showed positive result for *Vibrio sp*. In case of 5 Crustaceans tested, 4 shown positive for *Vibrio sp.*

The percentage for occurrence of *Vibrio sp* in fish and Crustaceans were 73% and 80% respectively (Table 2). Among the 15 fish samples tested, *Vibrio alginolyticus* was recovered from 11 samples. In case of 5 Crustaceans tested, 4 shown positive for *Vibrio alginolyticus* (Table 2, Figure 1,2).

Hemolytic activity

Among the 15 strains tested, 12 strains (80%) showed positive hemolytic activity on blood agar plates (Figure 3) and remaining 4 strains were non-haemolytic activity were observed (Table. 3)

Study of antibiotic susceptibility pattern

The antibiotic susceptibility pattern for various strains of *Vibrio alginolyticus* isolated from sea foods samples were given in (Table 4, Figure 4). Among the strains tested against 12 antibiotics resistant pattern was observed in penicillin (10 strains), Ampicillin (6 strains) Erythromycin (6 strains), Tetracycline (8 strains), Streptomycin (7 strains), Amoxyclav (6 strains), Vancomycin (9 strains), Novobiocin (6 strains). No resistant pattern was observed in Nalidixic acid, Gentamycin, Nitrofuratoin, Chloramphenicol.

Discussion

Vibrio alginolyticus, a halophilic gram- negative bacterium that inhabits marine and estuarine environments. The human diseases caused by *Vibrio alginolyticus* include gastroenteritis, soft tissues, otitis media, food intoxication and septicemia. Furthermore, it is one of the main *Vibrio* pathogens affecting marine animals, such as marine fish, shrimp and shell fish, which leads to large economic damage. Thus, it is essential to explore an effective protective pathway against by this microorganisrn) *Vibrio alginolyticus* was reported to be the most prevalent species with in *Vibrio spp* in mussels and sea water samples. According to another study, toxigenic *Vibrio alginolyticus* strains were isolated as a predominant species from Chennai coastal waters and sea food has been produced and consumed by bathing in this area. Therefore, researchers have stated that these potentially pathogenic isolates may significantly contribute to the onset of sporadic and epidemic outbreaks of diarrhoeal disease in humans. The clinical significance of these potentially pathogenic *Vibrio* strains are in association with gastroenteritis and invasive septicemia and usually spread to human via consumption of raw or under cooked shell fish, wound infections acquired by contact with fish, shell fish and sea water. In the present study, the several species of Finfishes and crustaceans were collected from the market found to harbour *Vibrio alginolyticus* and other *Vibrios.* From available reports it is well known that sea foods were found to be they carrier of *Vibrios* and the ubiquitous distribution of this pathogenic Bacteria in sea foods. Bacterial adhesion to host surface has been described as one of the initial steps in microbial pathogenesiss. It has been suggested that hydrophobicity is a determining factor in the adhesive process and in the survival of pathogen in cells.

The strains of *Vibrio alginolyticus* assayed did not agglutinate sea bream erythrovytes were agglutinated. Similarly, chen and Hanna reported that only two of the *Vibrio* species which they tested, *Vibrio alginolyticus* and *Vibrio anguillaram*, attached to cells and tissues from great tiger prawn. The results obtained in this study, the isolated gram negative halophilic bacterium *Vibrio* alginolyticus requires at least 3% Nacl and can tolerate up to 10% Nacl for growth. Sea water and sea food is an important vehicle of transmission -of this species and other *Vibrio*s and the possible spread of *Vibrio*s to marine invertebrates.

Antibiotic susceptibility of *Vibrio alginolyticus* were determined by disc diffusion on Muller Hinton agar (Merck) supplemented with 3% Nacl. The diamerer of the national committee for clinical laboratory standards. Antibiotic resistant pattern was observed in Ampicillin, Tetracycline, and Chioramphenical. The prevalence of antibiotics resistant microorganisms is ecologically very important and this character is plasmid borne. R plasmid is responsible for antibiotics resistant characters. These R plasmids have been found in *Vibrio alginolyticus* and other *Vibrios* and carry

Table 1. Cultural, morphological and biochemical characteristics of *Vibrio alginolyticus*

S. .No	Test name	Results
1.	Gram staining	Gram Negative rods
2.	Motility	Motile
3.	Indole	+Ve
4.	Methyl Red	+Ve
5.	Voges proskauer	+Ve
6.	Citrate Utilization test	+Ve
7.	Triple sugar iron agar	Acid/Acid, and H_2S
8.	Catalase	+Ve
9.	Oxidase	+Ve
10.	Nitrate reduction test	+Ve
11.	Gelatin hydrolysis test	+Ve
12.	Starch hydrolysis test	+Ve
13.	Carbohydrate Fermentation test	
	Glucose	+Ve
	Maltose	+Ve
	Mannitol	+Ve
	Sucrose	+Ve
	Lactose	-Ve
14.	Amino acid decarboxylase test	
	Arginine	-Ve
	Lysine	+Ve
	Ornithine	+Ve
	Leucine	-Ve
	Valine	-Ve
15.	Trypticase soy agar	+Ve
16.	Halophilisms test	
	0%NaCl	-Ve
	1%NaCl	+Ve
	3%NaCl	+Ve
	6%NaCl	+Ve
	8%NaCl	+Ve
	10%NaCl	+Ve

‘+’ Positive; ‘-‘Negative

Table 2. Incidence of *Vibrio alginolyticus* in different groups of finfishes and crustaceans collected from fish market.

S. No.	Samples	Total No. of Samples	Total No. of Positive for *Vibrio*s	No. of Strains Isolated for *Vibrio alginolyticus*	Total No. of Negative sample.
1	Fin fishes	15	11 (73%)	11	4
2	Crustaceans (Shrimp & Crab)	5	4 (80%)	4	1

Figure 1. Incidence of *V.alginolyticus* in different groups of fin fishes and crustaceans collected from fish market

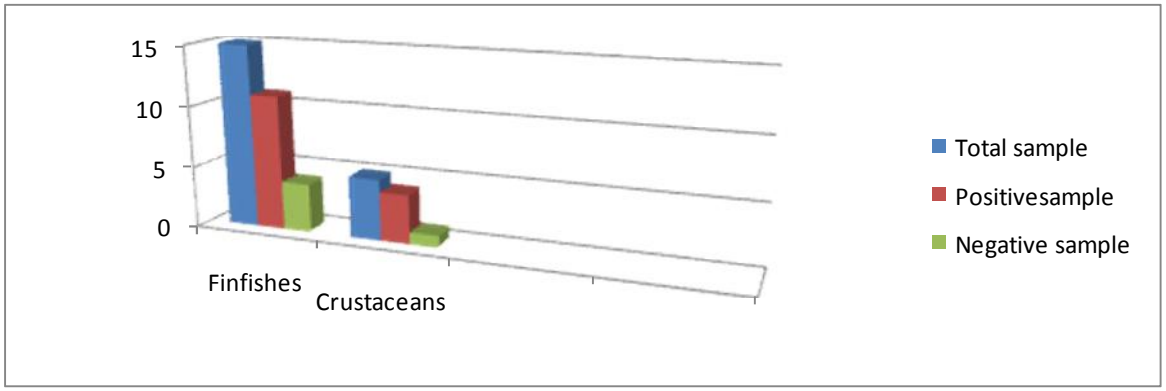

Table 3. Hemolytic activities of randomly selected Isolates of *Vibrio alginolyticus*

S. No.	Strain No.	Name of the Organisms	Source	Hemolysin activity
1	SF1	*Vibrio alginolyticus*	Fin fishes	+
2	SF2	*Vibrio alginolyticus*	Fin fishes	+
3	SF3	*Vibrio alginolyticus*	Fin fishes	+
4	SF4	*Vibrio alginolyticus*	Fin fishes	+
5	SF5	*Vibrio alginolyticus*	Crab	+
6	SF6	*Vibrio alginolyticus*	Fin fishes	-
7	SF7	*Vibrio alginolyticus*	Fin fishes	+
8	SF8	*Vibrio alginolyticus*	Shrimp	+
9	SF9	*Vibrio alginolyticus*	Crab	-
10	SF10	*Vibrio alginolyticus*	Fin fishes	-
11	SF11	*Vibrio alginolyticus*	Fin fishes	+
12	SF12	*Vibrio alginolyticus*	Fin fishes	+
13	SF13	*Vibrio alginolyticus*	Fin fishes	+
14	SF14	*Vibrio alginolyticus*	Fin fishes	+
15	SF15	*Vibrio alginolyticus*	Crab	+

'+' Positive; '-'Negative

Table 4. Antibiotic susceptibility pattern of *Vibrio alginolyuticus* isolated from sea food samples

S.No.	St.No.	P	E	Na	G	Nf	C	T	S	AM	A	V	N
1	SF1	+ (14)	+ (20)	+ (21)	+ (17)	+ (14)	+ (13)	-	+ (14)	-	+ (15)	-	+ (19)
2	SF2	-	-	+ (20)	+ (15)	+ (17)	+ (14)	-	+ (07)	-	+ (18)	-	+ (18)
3	SF3	+ (21)	+ (21)	+ (17)	+ (17)	+ (18)	+ (23)	+ (17)	-	+ (08)	-	+ (08)	-
4	SF4	-	-	+ (14)	+ (13)	+ (11)	+ (15)	+ (18)	-	+ (10)	-	+ (12)	-
5	SF5	+ (14)	+ (17)	+ (13)	+ (20)	+ (19)	+ (16)	+ (20)	+ (13)	-	-	+ (09)	+ (25)
6	SF6	-	+ (14)	+ (14)	+ (14)	+ (17)	+ (20)	+ (28)	-	+ (30)	-	-	-
7	SF7	-	+ (13)	+ (15)	+ (13)	+ (15)	+ (18)	-	+ (14)	+ (28)	+ (215)	+ (01)	-
8	SF11	-	-	+ (14)	+ (15)	+ (16)	+ (25)	-	-	+ (15)	+ (13)		+ (15)
9	SF12	-	-	+ (18)	+ (16)	+ (18)	+ (26)	-	+ (16)	-	+ (17)	-	-
10	SF14	+ (15)	-	+ (19)	+ (20)	+ (21)	+ (22)	+ (06)	+ (12)	+ (16)	+ (21)	-	-
11	SF16	-	+ (14)	+ (21)	+ (14)	+ (20)	+ (11)	-	-	+ (18)	-	-	+ (18)
12	SF17	-	-	+ (18)	+ (15)	+ (24)	+ (23)	-	-	+ (14)	+ (14)	-	+ (21)
13	SF18	+ (18)	+ (15)	+ (17)	+ (18)	+ (30)	+ (16)	-	-	+ (13)	+ (18)	+ (07)	+ (22)
14	SF19	-	+ (16)	+ (14)	+ (14)	+ (15)	+ (14)	+ (14)	+ (15)	-	-	+ (15)	+ (04)
15	SF20	-	+ (18)	+ (12)	+ (16)	+ (13)	+ (23)	+ (18)	+ (20)	-	-	-	+ (10)

Measured Zone diameter (mm) and susceptibility status of isolates, + = Sentive, - = Resistant, Antibiotics P = Penicillin, E = Erythromycin, NA = Nalidixic Acid, G = Geyamycin, C = Chloramphenical, T = Tetracyline, NF = Nitrofurantion, S = Streptpmycin, AM = Amoxyclav, A = Ampilicin, V = Vancomycin, NO = Novobiocin.

Figure 2. *Vibrio alginolyuticus* isolated from sea food samples

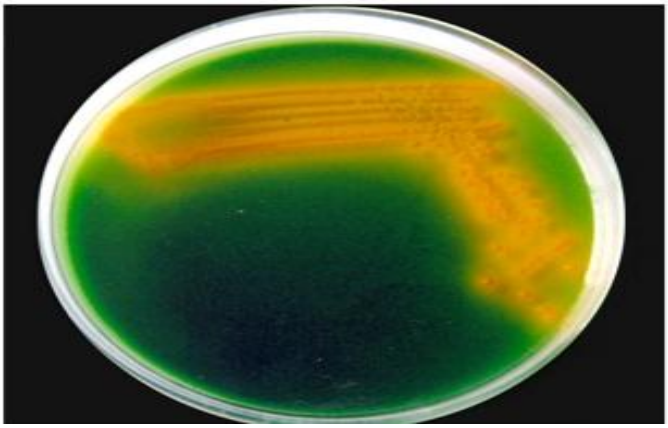

Figure 3. Haemolytic activity of *Vibrio alginolyuticus*

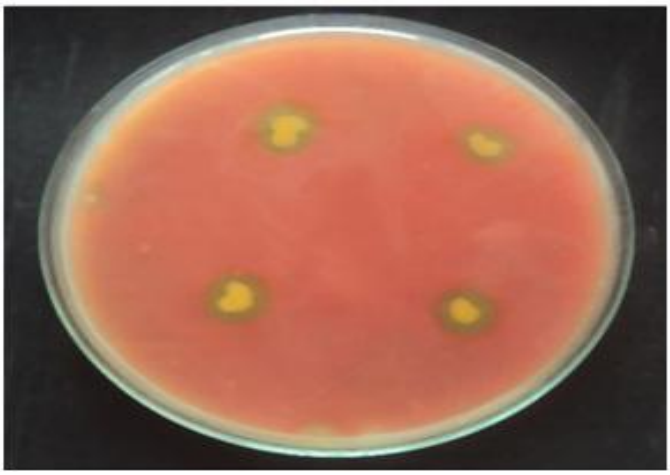

Figure 4. Antibacterial activity of *Vibrio alginolyuticus*

transferable drug resistance. The data suggest that antibiotic resistant *Vibrio alginolyticus* may survive better than sensitive organisms in surface water. In general, resistance of bacteria to antibiotics may be due to enzymatic destruction of antibiotics impermeability of the cell wall to the antibiotics destruction of antibiotics impermeability of the cell wall to the antibiotics additional of chemical group to antibiotics of the 15 strains *Vibrio* tested 12 strains 80% were positive for hemolytic activity.

Conclusion

Food security is a complex issue; where various factors pose a condition os risk to fish food safety and they range from contamination from environ ment where it is cought up to contamination by the consumer before eating. The sea foods are safe from contamination. Fish handlers and the public should be educated on the possible microbial hazards of fishes and methods for their prevention. The importance of sanitation is during handling, storage, transportation and marketing. The sea foods are thoroughly cooked. Avoid cross contamination of cooked sea food and other raw sea foods. Avoid exposure of open wound or broken skin to warm salt or brackish water.

In this present study shows and indicate shrimps could serve as delivery vehicles of antimicrobial resistance to pathogenic Vibrios from aquatic environment to human. Public health professionals at the levels of government should lead a multi disciplinary approach to prevent the recreational water illness that includes surveillance, health education, epidemiologic studies, laboratory support and environmental health research.

References

Carmen Balebona. M,Manuel.Andreu,J. Angeles Bordas, Irene Zorrila, Miguel.A.Morinigo, and Juan.J, Borrego 1998. Pathogenicity of *Vibrio alginolyticus* for cultured Gilt head sea Bream *sparus aurata L. Appl. Environ.Microbiology*, 64:4269-4275.

Colorni. A.Paperna, I.Gordin.H 1981. Bacterial infections in gilt-head seabream sparus aurata cultured at *Elat.Aquaculture*, 23:257-267.

Feingold, M.H., Kumar, M.L 2004. Otitis media associated with *V.alginolyticus* in a child with pressure equalizing tubes.pediatr.*Infect.Dis.J*, 23:475-476.

Food and drug Administration 2004. *Vibrio.Bacteriological amalytical manual of line* 8th *Ed Revision A.* 1998 online.

SallyJo Rubin, Richard C, Tilton 1975 Isolation of *V alginolyticus* from wound infections,J. clini. *Microbiol*,2:556-558.

Selmin Ozer, Gonul Aslan, seda Tezchn, pinar Sevim Bulduklu. Mehmet sami serin, Gurol Emekdas 2008.Genetic Hetero genetic and Antibiotic susceptibility of *V alginolyticus* strains isolated from Horse-Mackerel *Trachurus trachurus L* 1758,*J.Vet.Anim.Sci*, 321.

Shewan.J.M, Veron, M 1974.Genus I. *Vibrio pacini* 1854,411. In *Bergey"s Manual of determinative Bacteriology 8th edn.ed*. Buchanan, R.E.and Gibbons,N.E.,PP.340-345,Baltimore:Williams & Wilkins.

Topic Popovic,N. Benussi Skukan, A. Dzidara, P. Coz-Rakovac, R. Strunjak-Perovic, I. Kozacinski, L. Jadan, M. Brlek-Gorski, D 2010. Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic coast of Croatia , Veterinarni Medicina, 55, 5: 233–241

Zorrilla.I 2000. Characterization intraespecifica de aislados de *Vibrio alginolyticus* procedentes de peaces Marinos cultivados.Ph.D.Thesis.Malaga, Spain: *University of Malaga.*

Zorrilla.I, M.A. Morinigo,D. Castro, M.C.Balebona and J.J.Borrego., 2003.Intraspecific characterization of *V alginolyticus* isolates recovered from cultured fish in spain.*J.Appl.Microbiol*, 95:1106-116.