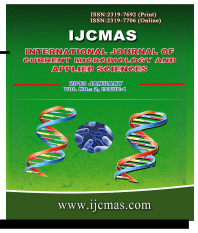
Int.J.Curr.Microbiol.App.Sci (2013) 1(1):86-93

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 1 (2013) pp. 86-93**



Original Research Article

Studies on pathogenic *Listeria monocytogenes* from marine food resources

R.Vinothkumar, K. Arunagiri* and T. Sivakumar

Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Krishnapuram, Kancheepuram - 631 551, Tamil Nadu, India.

***Corresponding author:** arunagirikumar01@gmail.com

KEYWORDS

Marine bacteria; Isolation of *Listeria monocytogenes;* Marine fishes; Antibiotic resistant pattern.

A B S T R A C T

In the present investigation encompasses the incidence of *Listeria monocytogenes* in marine food samples, a human pathogen in the outbreak of listeriosis disease worldwide. This report is concerned with the isolation and identification of *Listeria monocytogenes* from marine food resources. A total of 30 samples of dry fish, fresh fish, and crustaceans were analyzed qualitatively. These samples were tested using bacteriology method to detect the presence of *Listeria monocytogenes*. Out of 30 samples, 20 samples showed positive results for *Listeria* species. Antibiotic susceptibility was carried out for the isolated *Listeria monocytogenes* from selected food samples. This study indicated that these food samples were cross contaminated with *Listeria monocytogenes*. This study had also demonstrated that colonization of refrigerator by *Listeria monocytogenes* in a potential source of contamination of food products.

Introduction

Listeriosis is an atypical food borne illness of major public health concern because of the severity of the disease of meningitis, septicemia, and abortion, a high case fatality rate, a incubation time, and a predilection for individuals who have an underlying condition which lead to impairment of T-cell mediated immunity.

Listeria monocytogenes differ in many respects from most other food borne pathogens; it is widely distributed, resistant to reverse environmental condition, including low pH and high NaCl concentration, and psychrotrophic. The various ways the bacterium can enter into food processing plants, its ability to survive for long periods of time in the environment (soil, plants, and water), on foods, and in food processing plants, and its ability to grow at very low temperature (2ºC to 4ºC) and to survive in or on food for prolonged periods under adverse conditions (Bala swaminathan, 2001).

Listeria monocytogenes is known to be secreted in milk by both diseased and healthy animals (Wagner *et al*., 2000). In general the presence of *Listeria monocytogenes* in raw milk is more during winter season is high (Farber *et al*., 1998; Husu 1990). Contaminated of milk may also be due to environmental factor and poor hygiene practice (Sanaa *et al*., 1993).The multiplication potential for *Listeria monocytogenes* in meat and poultry products depends on the type of meat, the pH, and type and cell population of competitive flora. Poultry supports the growth of Listeria monocytogenes better than other meats. Contamination of animal muscle tissue may occur either from symptomatic or asymptomatic carriage of *Listeriamonocytogenes* by the food animal before slaughter (Farber and Peterkin, 1999).Cooked, ready-to-eat meat and poultry products have been implicated as the source of sporadic and epidemic as the source of sporadic epidemic Listeriosis on several occasions (Schwartz *et al*., 1989).Several reports indicate that fish and fishery products can be frequently contaminated with *Listeria monocytogenes*; major outbreaks associated with these products have been reported. In fish *Listeria monocytogenes* is found on such external surface as skin, gills, heads and slime and the contaminated of fish most likely depends on the presence of the bacteria in the surrounding water (Eklend *et al*., 1995).

Listeria monocytogenes is pathogenic for animals and human being the clinical manifestation for listeriosis is sepsis, meningitis and miscarriage in susceptible host (Schlech *et al*., 1996). Many animals' species infected with *Listeria monocytogenes* (Slutsluer *et al.,* 1999). In ruminants the clinical manifestation present as meningoencephalitis, septicemia, and abortions, mastitis in cattle and sheep. Both disease and healthy cattle shed *Listeria monocytogenes* in milk (Schoder *et al.,* 2003; Stephan *et al*., 2000). Listeriosis characteristically occurs in persons with a predisposing condition of disease, such as pregnancy, neonatality, malignancy, transplantation, alcoholism among other (Schuchat *et al.,* 1992).

In the past, those individuals who develop listeriosis have usually been treated with penicillin or ampicillin in conjunction with an amino glycoside (Charpentier and Courvalin, 1999), although tetracycline, erythromycin or chloramphenical alone or incombination, have also been used (Hof, 1991). Current therapy of all forms of listeriosis is a combination of ampicillin and gentamycin (Lorber, 1997). *Listeria monocytogenes* have been reported as susceptible to antibiotics active against of resistant in Listeria monocytogenes have published such increase in antibiotic resistance among *Listeria monocytogenes* are in line with a general worldwide pattern of an increasing prevalence of antibiotic resistance, including multiple antibiotic resistance among many groups of bacteria (Franko Abulin *et al.,* 1994). Keeping in this view, the present study was carried out to isolation the *Listeria monocytogenes* from marine food resources and to evaluate the antibiotic susceptibility pattern.

Materials and Methods

Collection of seafood samples

Marine food samples such as fresh fishes, crustaceans (shrimp and crab) and dry fishes were collected from different sites in Chennai and Kanchipuram fish market. All marine food samples were transported in individually labeled and sealed new

plastics bags to avoid contamination. The samples were placed in sealed contains with dry ice and transported to the laboratory for bacterial analysis.

Sample processing for bacteriological examination of marine foods

Finfish and crustaceans were washed thoroughly with sterile distilled water prior to bacteriological examination. The heads and tails of the fishes were cut into small pieces using sterile scissors and the guts were removed. The crustaceans and finfish samples were then homogenized in blenders, and 25g of each homogenate was placed in 225ml of *Listeria* enrichment broth, and incubated at 37˚C for 24 hours.

Isolation and identification of *Listeria monocytogenes*

A loopfull of culture broth from different sample was taken from Listeria enrichment broth and inoculated into agar medium (Palcam agar and Nutrient agar). The plates were incubated at 37˚C for 24 hours. The isolated bacterial species of listeria were identified by gram staining, motility performed to determine the nature of the bacterial species. Biochemical test such as Indole, methyl red, vogus prosker, citrate test, catalase, oxidase, TSI, nitrate reduction, carbohydrate fermentation test and bile esculin test were performed using the isolated species of bacteria.

Antibiotic susceptibility test

The Kirby- Bauer technique was used to determine susceptibility to antibiotics. The isolated colonies were transferred into 10ml of brain heart infusion broth, incubated at 37˚C for 24 hours. The culture inoculated plates were held at room temperature for 10 minutes to allow evaporation of free surface liquid as adopted by Anon (1997).Antibiotic disc containing 10µg ampicillin (A10), 30µg chloramphenicol (C30), methicillin, 10 units pencillin G (P10), 10µg streptomycin (S10), 30µg tetracycline (TE30) and vancomycin were placed on the surface of each inoculated plate using a sterile forceps. After incubation for 24 hours at 37˚C, the diameter of the zone around each disc was measured, and interpreted in accordance with the National Committee for Clinical Laboratory Standards (NCCLS).

Haemolytic activity

Listeria monocytogens strain to produce haemolysin was tested on blood agar supplemented with 5% sheep blood with anticoagulant. All the selected cultures were seeded in blood agar plates, and the plates were incubated at 37˚C for 24 hours. Hemolytic activity it's were determined (as described by Kishishita *et al.* (1998).

Results

Preliminary attempts were made to isolate *Listeria monocytogenes* and other *Listeria* species in marine food samples collected from shops in around Chennai and Kanchipuram.

Isolation of *Listeria monocytogenes*

The positive and negative samples obtained from different food samples were tabulated in Table.1; Figure.1.

Cultural characterization of *Listeria monocytogenes*

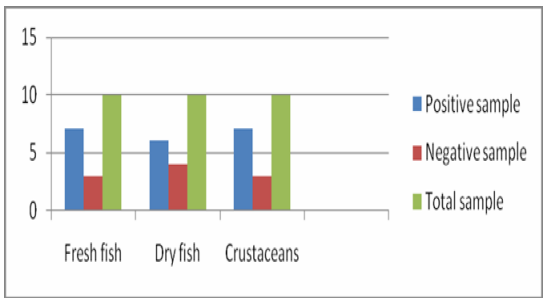
The *Listeria monocytogenes* isolated from different food samples were characterized based on colony morphology staining. Biochemical reaction and carbohydrate

test. The colony morphology of *Listeria monocytogenes* was showed on PALCAM agar, nutrient, blood agar plates.

Table.1 Number of positive and negative samples obtained from different food samples.

S.No	Food samples	Total number of positive samples	Total number of negative samples	Total number of samples
1.	Fresh fish	7 (70%)	3	10
2.	Dry fish	6 (60%)	4	10
3.	Crustaceans	7 (70%)	3	10

Figure. 1 Number of positive and negative samples obtained from different food samples



Gray green colonies with black centered, surrounded by black halo was noticed on PALCAM agar plate. Translucent, dew drop like opalescent colonies was no noticed on nutrient agar plate. Alpha haemolysis colonies were noticed on sheep blood agar. *Listeria monocytogenes* hydrolyse esculin to esculetin and glucose, these esculetin reacts with ferric ions and turns the medium to black colour.

Identification of *Listeria monocytogenes*

The biochemical characteristics of *Listeria monocytogenes* results were tabulated in table 2. Sugar fermentation results were tabulated in table 3. Based on the above results of microscopic examination and biochemical characterization of the isolates was confirmed as *L. monocytogenes*. The identification of Bacteria was based on Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Antibiotic susceptibility test

The pattern of resistant to various antimicrobial substances in *Listeria monocytogenes* isolated from various food samples in given in table 4. Among the strains tested against 7 antibiotics almost all strains were resistant to methicillin, streptomycin, and ampicillin, and penicillin G. The intermediate resistant was higher against chloramphenicol and tetracycline. Sensitive pattern was observed in vancomycin.

Table. 2 Biochemical test for identification of *Listeria monocytogenes.*

S.No	Name of the test	Reaction of *Listeria monocytogenes*
1.	Gram staining	Gram positive rods
2.	Motility	Motile
3.	Catalase	+
4.	Oxidase	-
5.	Indole	-
6.	MR	+
7.	VP	+
8.	Citrate	-
9.	TSI	A\A
10.	Nitrate	-

Table. 4 Antibiotic susceptibility test (Hirby Bauer method)

S.No	Sample	Name of the bacteria	A (10)	C (30)	P (10)	M (10)	S (10)	T (30)	V (30)
1.	Ff1	Ls	-	+	±	-	-	-	+
2.	Ff3	Lm	-	±	-	-	-	±	+
3.	Ff4	Ls	-	-	+	-	-	+	+
4.	Ff5	Lm	-	±	±	-	-	-	-
5.	Ff7	Ls	-	+	±	-	-	-	+
6.	Ff8	Lm	-	±	-	-	-	±	±
7.	Ff10	Lm	-	+	-	-	-	±	+
8.	Df1	Lm	-	±	-	-	-	-	-
9.	Df2	Lm	±	±	-	-	-	-	+
10.	Df5	Lm	±	+	-	-	-	±	-
11.	Df7	Ls	-	±	±	-	-	+	+
12.	Df8	Lm	±	+	-	-	-	±	+
13.	Df9	Ls	-	±	±	-	-	+	+
14.	Cu2	Lm	±	+	-	±	-	±	+
15.	Cu3	Ls	-	+	±	-	-	+	+
16.	Cu5	Lm	-	±	-	-	-	±	+
17.	Cu6	Ls	-	±	±	-	-	-	-
18.	Cu7	Lm	-	+	-	±	-	±	+
19.	Cu9	Lm	-	+	-	±	-	±	+
20.	Cu10	Lm	-	+	-	-	-	±	±

A=Ampicillin, C=chloramphenicol, P=Penicillin, M=Methicillin, S=Streptomycin, T=Tetracycline, V=Vancomycin. Lm= *Listeria monocytogenes*, Ls=other species of *Listeria.* +=Sensitive, - = Resistant, ± =intermediate resistant, Ff=Fresh fish, Df=Dry fish, Cu=Crustancea

Table. 3 Carbohydrate fermentation test

S.No	Types of sugars	Reactions
1.	Glucose	+
2.	Sucrose	+
3.	Fructose	+
4.	Manitol	+
5.	Maltose	+
6.	Galactose	+
7.	Xylose	-
8.	Mellibiose	-
9.	Dextrose	+
10.	Lactose	+

(+) indicates positive,
(-) indicates negative.

Discussion

Most of the bacteria food poisoning caused by *Listeria monocytogenes* and is contracted from fresh seafood samples and other foods. As this pathogen get transmitted through consumption of contaminated marine food resources. In the current study, *Listeria monocytogenes* and other *Listeria* species were isolated from fresh fish, dry fish and crustaceans. Out of 30 dry fish, fresh fish and crustaceans samples. 6 dry fish, 7 fresh fish samples and 7 crustaceans was positive for Listeria species. In this out of 13 isoates 4 from dry fish, 4 from fresh fish and 5 from crustaceans were hemolytic on blood agar and identifies as *Listeria monocytogenes*. The remaining 7 isolates were other species of *Listeria*. Jeyasekaran *et al.*, (1996) reported higher occurrence of *Listeria monocytogenes*, 12% in fresh fishes and 17.2% in fresh finfish from tropical seafoods from India.

Dhanashree *et al*. (2003) isolates *Listeria innocua* in 30.8% and *Listeria monocytogenes* in 1.3% of fresh raw fish samples from Mangalore, India. Less than 70% of the *Listeria monocytogenes* strains showed resistance to one or more antibiotics. The prevalence of antibiotic resistant microorganisms is ecologically very important and this character is plasmid borne. R Plasmids are extra chromosomal genetic elements determining resistance to antimicrobial drugs.

Numerous general of bacteria, including *Pseudomonas,Aeromonas*, *Listeria*, *Vibrio*, *Streptococcus* and *Enterobacteriaceae* have been found to carry transferable drug resistance the data suggest that antibiotic resistant *Listeria monocytogenes* may survive better than sensitive organism in marine foods. In general, resistant of bacteria to antibiotics may be due to enzymatic destruction of the antibiotic, impermeability of the cell wall to the antibiotic, addition of chemical group to an antibiotic.

The isolated *Llisteria monocytogenes* were tested against 7 antibiotics almost the isolated were resistant to streptomycin, ampillin, tetracycline, penicillin, is confirmed by standard chart (NCCLS). The intermediate results were higher against choloramphenicol. Sensitive pattern was observed only in vancomycin. The overall incidence of antibiotic resistant in *Listeria* species is still relatively low. i.e., less than 7% in the case of tetracycline, the most frequently observed resistance. However, the study does confirm that since the first report of antibiotic resistant strains of *Listeria monocytogenes* (Poyart slmeron, 1990). There has been a continuing pattern of the emerged of strains of *Listeria* species, Isolated from food, the environment or from clinical cases of Listeriosis, which are resistant to one or more antibiotics

(Aprin *et al.,* 1992; Charpentier *et al*., 1994).

The high incidence of this pathogen in commercially important food samples is to be noted with concern from public health point of view, since most sample of listeriosis and other diarrhoreal disease are attributable to consumption of contaminated foods. It seems virtually impossible to protect food samples from such defilement, but with proper case in handling and processing the "infection disease" problem can be practically controlled to a very large extent. The most important means of controlling human infection lies in simple hygienic measure aimed at preventing multiplication of the *Listeria* species in food samples. The information available to date provided fascination insight in the ecology, systematic, epidemiology and pathogenicity of *Listeria*.

From the available reports it is concluded, that low temperature may provide suitable environment for *Listeria* and secondary contamination are also reflected the frequency of isolation. It is clear that unless the ecology of the *Listeria* is fully, understood, complete control of disease caused by *Listera* particularly *Listeria monocytogens* will not be possible.

References

Anon.S., 1997. Performance standards for antimicrobial disk susceptibility Test 6th ed. *Approved Standard document.* Wayne, PA: NCCLS.

Arpin, C., C. Carlier, P.Courvalin and Quentin, C. 1992. Analysis of antibiotic resistant plasmid from a clinical isolate of *Listeria monocytogenes*. Abstract 26/ C3 In:12 Reunion Inter disc Cimiother Anti Infect, Paris, France.

Bala, S., 2001. Food Microbiology and Frontier, 2nd Edn. pp.383-409.

Charpentier, E., and Courvalin, P. 1999. Antibiotic resistance in *Listeria* species. Antimicro Chemo. 43: 2103-2108.

Charpentier, E., G. Gerbaud,J. Rocourt and Courvalin, P. 1994. Incidence Antibiotic resistance in *Listeria* species. J .Inf . Dis. 172**:** 277-281.

Dhanashree, B., S.K. Otta, I. Karunasagar and Goebel. W. 2003. Incidence of *Listeria* species in clinical and food samples in Mangalore. India. Food Microbiol. 20: 447 – 453.

Eklend, M.W., F.T. Poysky, R.N. Paranjpye, L.C. Lashbrook, M.E. Peterson and Pelroy, G.A.1995. Incidence and sources of *Listeria monocytogenes* in cold smoked Fishey products and processing plants. J.Food. Prot**.** 58: 502 -508.

Farber, J.M., 2000. Present situation in Canada regarding *Listeria monocytogenes* and Ready to eat seafood products. Int. J. Food Microbial. 62: 247 – 251.

Farber, J.M., and Peterkin, P.I. 1999. Incidenec and behavior of *Listeria monocytogenes* in meat products, in E.T.Ryser and E.H.Marh *Listeria*, listeriosis and food safety. Marcel Dekker, Inc., Newyork. p. 505 – 504.

Franco Abuin, C.M., E.J. Quinto Fernandez, C. Fente Sampayo, J.L. Rodriguez Otero, L. Dominguez Rodriguez and Cepeda Saez, A. 1994. Susceptibilities of *Listeria* species isolated from food to nine antimicrobial agents. Antimicro chemo. 38: 1655 – 1657.

Hof, H., 1991. Therapeutic activities of antibiotics in listeriosis. *Infections.* 19: 229-233.

Husu, J., 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the faces of dairy cattle. J. Vet. Med. 4: 276-282.

Jeyasekaran, G., and Karunasagar, I. 1996. Incidence of *Listeria* species in tropical fish. Int. J. Food Microbiol. 31: 333-340.

Lorber, B., 1997. Listeriosis. Clinical Inf. dis. 24**:** 1-9.

Poyert-Salmeron, C., C. Carlier, A. Trieu-Cout, L. Courtien and Courvalin, P. 1990. Transferable plasmid mediated antibiotic resistance in *Listeria monocytogenes*. Lancet. 335: 1422-1426.

Sanna, M., B. Poutrel. J.L. Menard and Sereys, F. 1993. Risk Factors Associated with Contamination of raw milk by *Listeria monocytogenes* in dairy farms. J. Dairy Sci, 76: 2891-2898.

Schlech, W. I., P.M. Lavigne, R.A. Bprtolussi, A.C.Allen, E.V. Haldand, A.J. Won, A.W. Hightower, S.E.Johnson, S.H. King, E.S. Nicholls and Broome, C.S. 1996. Epidemic listeriosis evidene for transmission by food. N. Engl. J.Med. 308: 203-206.

Schlech. W.H., 2000. Epidemiology and clinical Manifestations of *Listeria monocytogenes* infections. In: Gram positive pathogen. American society For microbiology press, Washington DC. USA.

Schoder. D., P. Winter, A. Kareem, W. Baumgarther and Wagner. M. 2003. A case of Sporadic bovine mastitis caused by *Listeria monocytogenes* and its effect on Contamination of raw milk and raw milk cheeses produced in the on farm. Dairy. J. dairy Res. 70: 395-401.

Schuchat. A., K.A. Deaver, J. D. Wenger, B.D. Plekaytis, L. Mascola, R.W. Pinner, A.L. Reingold and Broome, C.V. 1992. Role of foods in sporadic listeriosis. Case, control study of dietary risk factors. J.Appl.Med. *267*: 2041-2045.

Schwartz, B., D. Hexter, A. Hightower, R.Hischorn, J. Porter, P. Hayes, W. Bibb, B. Losher and Fairs,D. 1989. Investigation of an outbreak of listeriosis; New hypotheses for the etiology of epidemic *Listeria monocytogenes* infection. J. Infect. Dis. 159: 680-685.

Stephan R., D. Senczen, C.H. Muller and Feusi, D. 2000. Isolierung von Listeria species and *Aspergillus, fumigates.* Zwei Fallberichte aus der Mastitisdignastic. Schweizer Arch. Tierhilkd. 142: 387-90.

Wagner. M., L. Podstatzky-Lichtenstein, A. Lethner, H. Asperger, W. Baumgarther and Brand, E. 2000. Prolonged excretion of *Listeria Monocytogenes* in a subclinical Case of mastitis. Milchwissenschaft. 55: 3-6.