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Isolation and Identification of Listeria Monocytogenes in Bangalore City from Various Food Samples

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The current research emphasis on the isolation and differentiation of Listeria monocytogenes from different food samples most frequently infected with Listeriosis outbreaks. Crude chicken meat, raw milk, pasteurized cheese, ice cream and raw fish are samples from the city of Bangalore. The selective medium mainly used for the isolation of Listeria is oxford agar. Using isolated L. monocytogenes from food samples, morphologic and biochemical identification was carried out. 2 samples (fresh milk and Ice cream) were positive out of 5 samples; 3 samples (raw chicken meat, raw fish, and pasteurized cheese) were negative. The results conferred during this study indicate the contamination of Ice- cream and Raw Milk samples with L. monocytogenes.

Keywords: Contamination; Food samples; Food borne pathogen; Listeria Monocytogenes; Listeriosis.

Listeriosis is one among the least food borne disease compared to all other food borne illness. Listeriosis caused by the bacterium *Listeria monocytogenes*. It is one among the food borne pathogen and Listeriosis is one among the least food borne disease compared to all other food borne illness. Listeriosis caused by the bacteria, *L. monocytogenes.* It is one among the food borne pathogen and ubiquitous in nature. *L. monocytogenes* is Gram positive, rod shaped, Catalase positive, non-spore forming, facultative anaerobic, Oxidase negative, motile bacterium. It is widely distributed in water, soil and also present in various animal foods and food products[1]. *L. monocytogenes* withstand at various temperatures for survival and forms flagella at above 27 °C. It can grow in a pH range from 4.6 to 9.5 and grow in low water activity environments[2]. Foods include unpasteurized dairy products , ready to eat foods , sea foods. It is an opportunistic pathogen for human beings and animals and although mortality rate that reaches 20-40% and incidence of critical sickness is very high[3]. Food is an important vehicle of transmission in 99% of Listeriosis cases. The most affected peoples are immune compromised persons, pregnant women, neonates and children. *Listeria* is an emerging pathogen during the early 1980s, it's difficult to detect, number of efforts to develop both cultural and rapid methods for its detection. The best choice for Listeriosis detection is Antibiotic therapy. Some of the antibiotics to treat Listeriosis disease are Ampicillin, Gentamicin etc[4]. As a food borne pathogen, is a serious threat for human health and food safety. The consumption of raw food and

food products that carry Listeria monocytogenes is a cause of Listeriosis. To control the Listeriosis consumer should follow WHO (World Health organization) rules and regulation for the food safety and healthy life. Consumption of proper cooked food (meat and meat products), proper handling of food, adequate heating and cooling of the food is very important aspects of controlling the food borne pathogen[5]. The aim of this study is to isolate and identifies the *L. monocytogenes* from food samples & biochemical characterization for further identification.

MATERIALS AND METHODS

Sample collection

In the present study, samples were collected from Dairy shop, Fish and Chicken markets from different areas in Bangalore city. The samples including the Milk products (Pasteurized Amul cheese, Ice-cream, Raw milk), poultry product (Raw Chicken), sea food (Raw Fish). The samples were suspected to be contaminated with Listeria. The samples were collected in sterile container (Box) under aseptic precautions and also the samples collection box at 4°C and were transported to the laboratory directly[6].

Processing of sample to Enrichment media

25 g of samples were taken aseptically and homogenized using sterile mortar and pestle later the crushed sample was inoculated on to 225 ml of enrichment media Fraser Broth (Table 1), Whereas solid sample was inoculated as into Frazer broth and incubated at 37°C for 24 hours[6].

Isolation of the Listeria spp

The selective media used for isolation of *L. monocytogenes* is Oxford agar. The media were ready as per the composition (Table 2). The culture (1ml) is taken from enrichment medium and streaked on the Oxford agar plates and incubated at 37°C for 48 hours. Colonies formed after incubation, were likely to be *L. monocytogenes* and that them were sub cultured on to nutrient agar for more identification[6].

Identification methods

In addition, on the basis of their morphological observation, staining and biochemical characterization, cultures of isolated bacteria (*L. monocytogenes*) have been classified[7].

RESULTS

The isolation of *L. monocytogenes* is carried out by using Fraser Broth enrichment as selective enrichment media followed by Oxford agar. Totally few different samples were collected from different places such as super market, vegetable market and food shops from different areas in Bangalore and sample details were represented in Table 3. Oxford agar is a solid medium, mainly used for the selective medium for isolation and identification of *L. monocytogenes* in food samples. *L. monocytogenes* hydrolyze the esculin to esculetin. Esculetin reacts with ferric ammonium citrate resulting in a black precipitate and a positive reaction.

Out of five different samples were processed for isolation of sample and only Ice-cream and Milk samples showed positive results for *L. monocytogenes*. Several authors observed

Table 1. Fraser broth composition (mg/l)

S No	Name of the Chemicals	Concentration (g/l)
1	Meet peptone	5.0 g
2	Sodium chloride	20.0 g
3	Krypton	5.0g
4	Yeast extract	5.0 g
5	disodium hydrogen phosphate	12.0g
6	Potassium dihydrogen phosphate	1.35g
7	Aesculin	0.5g
8	Distilled water	1000ml
9	pH	± 7.2

Table 2. Oxford agar composition (mg/l)

S No	Name of the Chemicals	Concentration (g/l)
1	Aesculin	0.5 g
2	Sodium chloride	5.0 g
3	Peptone	28.0g
4	Starch	1.0 g
5	Ferric Ammonium citrate	0.5g
6	Lithium chloride	15.0g
7	Agar	15.0g
8	Distilled water	1000ml
9	pH	7.2

that Fraser broth is the effective enrichment broth for *L. monocytogenes*. The isolated colonies on Oxford agar plates were used for morphological, identification and staining, motility and biochemical characterization of typical *L. monocytogenes* colonies. The staining shows Gram positive rods and motile nature (Table 4).

In Biochemical confirmation miscellaneous test (Indole, Methyl red and Voges-Proskauer), Fermentation test (Sugar –Glucose and lactose), Substrate utilization test (Citrate Utilization and H_2S), and Enzymes tests (Urease, Catalase and Oxidase).

For isolation of *L. monocytogenes* from different food samples from Bangalore city, using conventional methods: as per the growth on Oxford agar medium, Gram staining and Catalase test; totally 2 samples (40%) suspected *L. monocytogenes* were isolated from 5 samples.

Five samples were analyzed for the detection of a pathogenic micro-organism. *L. monocytogenes* isolated from 2 samples Milk and Ice-cream. The 2 isolates recovered from the samples were known supported the morphological, cultural and biochemical characteristics (table 4). It has been found that about 30 percent of milk products are infected with Listeria. According to our report, ice-cream (15%) samples tainted with *L. monocytogenes* are raw milk (26%).

DISCUSSION

Listeriosis is one of the zoonotic diseases and is contracted principally from the consumption

Table 3. Collected Sample details and occurrence of *L. monocytogenes*

S. No	Name of the Food sample	Total Number of the sample	Total Number of positive samples	Total Number of Negative sample
1	Chicken	01	-	01
2	Fish	01	-	01
3	Milk	01	01	-
4	Ice –Cream	01	01	-
5	Cheese	01	-	01

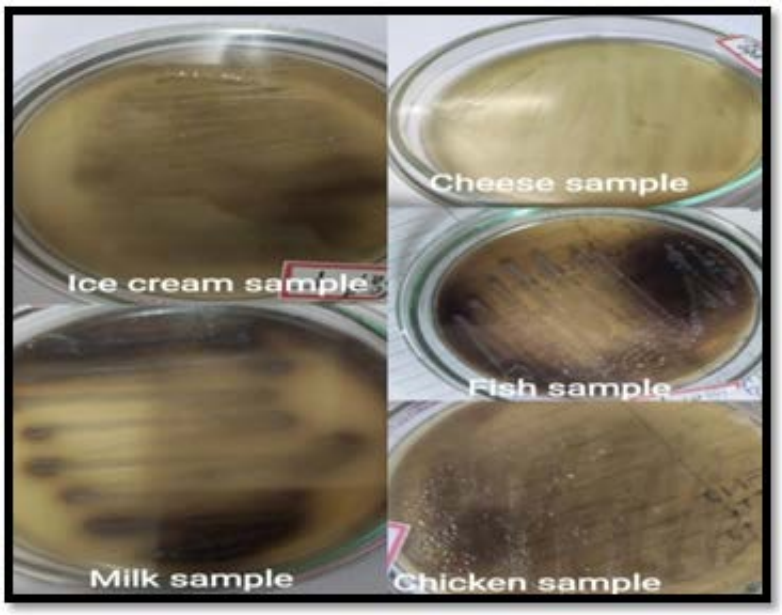

Fig. 1. Result plates of L. monocytogenes growth on Oxford agar plates

of contaminated food products[8]. The existence of *L. monocytogenes* is the atmosphere is omnipresent. Increasing evidence suggests that food borne transmission of Listeria monocytogenes is attributable to a sustainable part of the human Listeriosis cause[9]. There are several sporadic and outbreak breaks globally involving foods contaminated with Listeria that are reportable. The current study analyzed total 5 different food samples raw fish; raw milk, raw chicken meat, pasteurized cheese, and Ice cream were analyzed. Out of 5 samples only 2 samples (Raw milk, Ice cream) showed positive result. 3 samples (Raw chicken meat, raw fish, and pasteurized cheese) showed negative result. Two samples are raw milk (26%), Ice cream (15%) were positive for *L. monocytogenes*. In the present study *L. monocytogenes* were isolated from the different food samples by using selective Oxford agar media. Typical eubacteria colonies on the selective agar plates were sub cultured on the Nutrient agar plates. And additional identification had done by performing series of tests to substantiate Morphological, biochemical characteristics. *L. monocytogenes* is a gram positive, motile, encapsulated, glucose fermenting, Facultative anaerobic, Urease positive, Oxidase negative, Catalase positive organism belonging to the Eubacteria family. The isolated *L. monocytogenes* colony was identified by Gram's staining, colony characteristics and biochemical tests such as Catalase test Urease test, Indole test, H_2S test, Motility, Citrate test, Oxidase disc test, Aesculin hydrolysis test, VP test, MR test. In the present work, Raw milk and Ice –cream samples showed positive results for all biochemical tests, raw milk was found to be the main source of *L. monocytogenes*. About 25% raw milk was contaminated with Listeria. This is due to the lack of hygiene, environmental contamination and milk handlers. And other sample Ice creams (15%) were contaminated with Listeria. Ice cream is produced from Milk, Fruits, eggs these components may

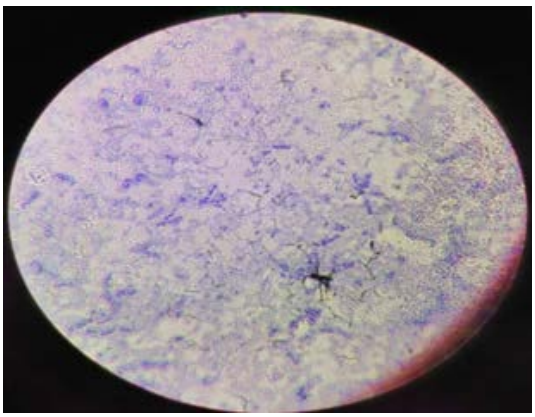

Fig. 2. Gram staining of isolated bacterial culture was carried out to detect the morphology and to examine whether the bacteria are gram negative or gram positive under microscope of 45X

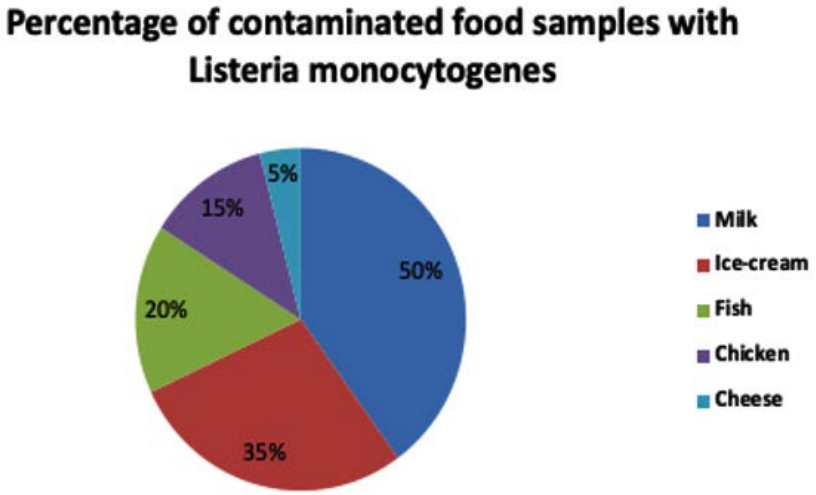

Fig. 3. The isolation percentage of different food samples contaminated with L. monocytogenes from different shops at Bangalore city

Table 4. Showing Biochemical characterization of *L. monocytogenes*

Biochemical Tests	Samples				
	Chicken	Fish	Milk	Ice	Cheese
Indole test	-	-	-	+	-
VP test	-	-	+	-	+
Catalase test	+	+	+	+	-
H_2S test	-	-	-	-	-
Urease test	-	-	-	-	-
Methylred test	-	+	+	+	-
Vogesproskauer test	-	-	+	+	-
Citrate test	-	-	-	-	-
Oxidase test	-	+	-	+	-
Flagella	Flagellated	Flagellated	Flagellated	Flagellated	Flagellated
Nitrate Reduction test	-	-	-	-	-
Motility test	Motile	Motile	Motile	Motile	Motile

provide adequate nutritional support for Listeria growth and multiplication[10].

Some of important work carried out by Schlech, III W F. *et al.,* 2000, studied that Food includes ready to eat foods, raw vegetables, unpasteurized dairy products, sea foods, meat products and 40% of traditional foods contaminated with *Listeria monocytogenes* about 40%[11]. Gunasena *et al*., 1995, were studied the presence of *L. monocytogenes* in market samples different food items indicated that 38% of the samples contained *L. monocytogenes* of them 34% of chickens and 26% of dairy products were contaminated with *L. monocytogenes*[12]. Rahimi 2012 *et al*., were studied that highest prevalence of Listeria was found in traditional Ice-cream (16.7%), followed by cheese (15.0%) samples[13]. Enurah *et al.,* 2013 were studied the prevalence of *L. monocytogenes* in fresh raw milk (15%)[14]. Molla *et al.,* was they reported a prevalence of 32.6% *Listeria* species in all food samples[15]. These findings were comparable to the results of surveys undertaken in other countries[16-20]. This suggests that the presence of a significant public health joined to the consumption of foods contaminated with *L. monocytogenes*. The overall findings were almost comparable with Gunasena *et al.,* 1995. This study has incontestable the presence of *Listeria* in several types of raw and prepared to eat food products in Bangalore city. Also suggests the requirement for improved food safety is important aspect of controlling food borne pathogen.

Summary

Food from different sources in Bangalore city was collected to analyze for detection of *Listeria monocytogenes*. Samples were collected with adequate measures and taken to the laboratory for the isolation and identification, biochemical characterization. Totally 5 samples were collected for the identification of *Listeria monocytogenes*, samples are (raw chicken meat, raw milk, raw cheese, raw fish and Ice- cream samples). Among the 5 samples two samples Ice-cream (15%) and raw milk (25%) was showed positive results for the all biochemical tests. According to researches *Listeria monocytogenes* were highly contaminated with milk and milk products.

CONCLUSION

Introducing hygiene measures from production to consumption at the lowest level, the current study jointly sets out the criteria for increasing food safety. Listeria monocytogenes can survive extreme environmental conditions and pose a high risk to human health. A large proportion of the food samples were silently infected with L. *monocytogenes* recommend measures to improve the hygiene, proper handling and cooling storage conditions of the area unit when treating the infection.

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Author contribution

Vedavati and Nagalambika Prasad conceptualized the study. Vedavati and Nagalambika Prasad Collection and assembly. Vedavati and Nagalambika Prasad drafted the Manuscript. Nagalambika Prasad and B.M. Kanthesh helped Data analysis and interpretation with the Manuscript and Discussion.

Conflicts of interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. Barbuddhe S. B, Malik S.V.S, Kumar J.A, Kalorey D.R and Chakraborty T. Epidemiology and risk management of Listeriosis in India. *International Journal of Food Microbiology*. **154**:113-118 (2012).
2. Buchanan R, Lindquist R, Ross T, Smith M, Todd and Whiting R. Risk assessment of *Listeria monocytogenes* in ready to eat foods. 2004. Microbiological Risk Assessment Series,4. Food Agriculture Organization of the United Nations, Rome (Italy).
3. Conner D. E, Scott V. N, and Bernard D. T. Growth inhibition and survival of *Listeria monocytogenes* as affected by acidic conditions. *Journal of Food protection.:* **53**:652-655 (1990).
4. EFSA (European Food safety Authority). Trends and sources of Zoonses, Zoonotic agents and antimicrobial resistance in the European Union in 2010. *EFSA Journal.* 2597 (2012)
5. Ray B., *et al*. Fundamentals Food Microbiology (3rd ed). 2004. Boca Raton, FL: CRC Press.
6. Alsheikh A. D. I, Mohammed G.E and Abdalla M.A. Isolation and identification of *Listeria monocytogenes* from Retail Broiler Chicken Ready to Eat Meat Products in Sudan. *International Journal of Animal and Veterinary Advances*.; **5**(1): 9-14, 2013 (2012).
7. Aneja, K. R. Experiments in Microbiology and plant pathology and Biotechnology. 2009. New Age International private limited Publishers.
8. Farber J.M. *et al*. Present situation in Canada regarding *Listeria monocytogenes* and ready to eat sea food products. *International Journal of Food Microbiology.;* **62**:247-251 (2000).
9. Kozacinski L, Hadiosmanovic M, Meiotic B, *et al.* The meaning of Listeria monocytogenes in Veterinary sanitary inspection, *Veterinary Achievement Journal.:* 70-71 (2000).
10. Doyle, M.P. Effect of Environmental and Processing conditions of Listeria monocytogenes. *Food technology.;* **42**: 169-171 (1988).
11. Schlech III W F. Food borne Listeriosis. *Clinical Infectious Diseases.;* **31**:770-775 (2000).
12. Gunasena Deepthi K, Chandra P. K, Kumudu G and Widanapathirana S. Occurrence of *Listeria Monocytogenes* in Food Sri Lanka. *Journal of National Science Foundation of Sri Lanka*; **23**(3):107-114 (1995).
13. Rahimi E, Momtaz H, Sharifzadech A, Behzadnia A, Ashtari M. S, Zandi Esfahani S, Riahi M, Momeni M. Prevalence and antimicrobial resistance of *Listeria* species isolated from traditional dairy products in Charar Mahal and Bakhtiary, Iran. *Bulgarian Journal of Veterinary Medicine*; **15**(2):115-122 (2012).
14. Enurah L. U, Aboaba O. O, Nwachukwu S. C. U, Nwosuh C. I. Antibiotic resistant profiles of food (fresh raw milk) and environmental (abattoir effluents) isolates of *Listeria monocytogenes* from the six zones of Nigeria. *African Journal of Microbiology Research.;* **7**(34):4373-4378 (2013).
15. Molla B, Yilma R, Alemayehu D. *Listeria monocytogenes* and other *Listeria* species in retail meat and milk products in Addis Ababa, Ethiopia. *Ethiopia Journal Health Dev.;* **18**: 131-212 (2004).
16. Uyttendaele M, Troy P. D and Debevere J. Incidence of *Listeria monocytogenes* in poultry and poultry products obtained from Belgian and Freanch abattoris. *Food Microbiology;* **14**:339-345 (1997).
17. Cordano A.M and Rocourt J. Occurrence of *Listeria monocytogenes* in food in Chile. *International Journal of Food Microbiology.* **70**: 175-178 (2001).
18. Soultos N, Koidis P and Madden R.H. Presence of *Listeria* and *Salmonella* spp. in retail chicken in Northern Ireland. *Letters in applied Microbiology.* **37**:421-423 (2003).
19. Bhilegonkar K. N, Killshrestha S. B, Kapoor K. N, Ashok Kumar, Agarwal R.K, Singh B.R and Kumar A. Isolation of *Listeria monocytogenes* from milk. *International Journal food Science and technology Mysore.*; **34**: 248-250 (1997).
20. Jalali M and Abedi D. Prevalence of *Listeria* species in food products in Isfahan, Iran. *International Journal of Food Microbiology.*; **122**: 336-340 (2008).