Biological Forum – An International Journal **15(6): 599-602(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Isolation and Biochemical Characterisation of Food Born Pathogens Listeria Monocytogenes from Beverages

Keshamma E.*
Associate Professor, Department of Biochemistry,
Maharani Cluster University, Palace Road, Bengaluru (Karnataka), India.

(Corresponding author: Keshamma E.)*
(Received: 23 March 2023; Revised: 04 May 2023; Accepted: 20 May 2023; Published: 20 June 2023)
(Published by Research Trend)

ABSTRACT: Listeriosis in human is very often associated with very high mortality, and clinical cases of listeriosis in humans caused by *Listeria monocytogenes*. Hence, current study was undertaken with the prime objective of isolation and characterization of food borne pathogens *L. monocytogenes* from different soft drinks sold at local market outlets in the city of Chikkaballapura, Karnataka. Samples comprising of different types of locally soft drinks were collected aseptically and subjected to serial dilution and inoculated to nutrient agar medium, Palcam selective agar base medium and Tryptic soy broth. The growth with the typical colony characteristics were further identified up to species level based on their morphological and biochemical characteristics. The bacteria isolated from different soft beverages was found to be gram positive bacilli, non-motile, showed positive for catalase, methyl red, and Voges Proskauer, CAMP tests. Whereas, isolated strains were negative for oxidase, indole, and citrate utilization tests. Based on the morphological and biochemical characterization isolated strain was identified as *L. monocytogenes*. In conclusion, results of the presented study suggested that *L. monocytogenes* is prevalent in beverages samples and sheds new light on the growth of food pathogens and spoilage microbes in a variety of beverages that are increased in popularity worldwide. These findings could be considered in estimating the risk associated with the production and consumption of beverages.

Keywords: Beverages, *L. monocytogenes*, Morphology, Biochemical tests, Softdrinks

INTRODUCTION

Listeria monocytogenes is a gram positive, opportunistic, intracellular food borne pathogenic bacterium that can infect humans and other mammals severely and fatally (Farber and Peterkin, 1991; Kang *et al.,* 2013). According to studies by Thomas *et al.* (2015); Jamali *et al.* (2013), pregnant women, newborns, and unborn children are the main targets of the bacterium that causes the disease listeriosis, which has a fatality rate of 20–30%.

One of the possible sources of contamination of *L. monocytogenes* in food products and food industry is due to the cross contamination. *L. monocytogenes* is one of very few pathogenic organisms which can grow at refrigerated temperatures. The bacterium is widespread in nature and can survive and grow under low temperatures and pH, high concentrations of salt or bile, oxidative stress, carbon starvation, and other adverse conditions making it a potential hazard in foods (Razavilar and Genigeorgis 1998).

Listeriosis in human is very often associated with very high mortality which may be as high as 30 % (Griffiths, 1989). Listeriosis is infectious disease of humans and animals, caused in 99% of cases by consumption of food contaminated by *L. monocytogenes*, and rarely from the environment. *L. monocytogenes* is widely spread in nature, easily enters the food and as such can lead to contamination of the food. The bacteria were isolated in the soil; vegetation; water (sweet, salty and sewerage); raw and processed food (milk and dairy products) (McLauchlin *et al.,* 2004; Todd and Notrmansb 2010). Both the outbreaks and sporadic human listeriosis is caused by transmission of this pathogen through contaminated food and the involvement of a wide variety of foods has been reported throughout the world (Linnan *et al.,* 1988; Ericsson *et al.,* 1997). Additionally, since *L. monocytogenes* has the ability to multiply and grow at low and even freezing temperatures, foods kept in a refrigerator for a long time are the factors for the presence of the pathogen (Kasalica *et al.* 2011).

Antibiotic resistance is the ability of microorganism to combat the action of one or more antibiotics that are used in clinical practice where the organism changes its response to the antibiotics as reviewed by Olaimat *et al.* (2018). In recent years, the food industries have faced a challenge with an emergence of antibiotic-resistant bacterial strains, including pathogens of public health importance such as *Salmonella, Staphylococcus aureus, Escherichia coli,* and *L. monocytogenes* as they

potentially cause deterioration of foods. American food supply is among the safest in the world, but people can still getsick from food-borne infections due to antibiotic-resistant bacteria (CDC, NCEZID, 2018).

Various regulatory bodies, including the USFDA, have imposed zero tolerance for this pathogen in ready-to-eat food products due to its enormous pathogenic potential and high mortality rate (Hitchins, 1998). Listeriosis in countries of South East Asia are scarce, either because of failure to detect, failure to report, or low incidence rate or failure to consider listeriosis for differential diagnosis by clinicians. The disease remains largely undiagnosed and underreported. However, *L. monocytogenes* has been found to be one of the etiological factors in causing spontaneous abortions and premature births in India.

The most frequent methods used to identify *L. monocytogenes* are conventional methods, which rely on the use of microbiological media to selectively cultivate and count this pathogen. These techniques are sensitive, affordable, and they produce both qualitative and quantitative results. In India's Delhi and Karnataka states, clinical cases of listeriosis in humans caused by *L. monocytogenes* have also been documented (Bhujwala *et al.*, 1973; Dhanashree *et al.*, 2003). Therefore, it is necessary to regularly check for and constantly monitor for the presence of this *L. monocytogenes* in food products, including beverages.

With this scenario, the present study has been undertaken to assess the occurrence of *L. monocytogenes* in beverages marketed in Chikkaballapura, Karnataka, and to improve the basic knowledge of the incidence and characteristics of *L. monocytogenes*.

MATERIAL AND METHODS

Sample Collection. Different types of locally sold soft drinks were collected from different places of Chikkaballapura in a sterile polythene bag under aseptic condition. Originally samples were kept under refrigerated conditions and immediately samples brought to the laboratory and processed further within 3-4 hours of collection.

Isolation and Identification. Serial dilution involves the process of taking one mL of sample and diluting it through a series of standard volumes of sterile diluent, which can either be 9 mL of distilled water or 0.9% saline. Depending on the estimated concentration of cells / organisms in a sample, the extent of dilution is determined. 0.1mL of sample was taken from the dilution like 10^4 to 10^6 inoculated on to the respective plates by spread and streak plate method. Based on their different media Nutrient agar media, Selective Palcam agar media, oxford and tryptic soy broth was used to isolate the bacteria. After incubation on respective dilution different bacterial colonies were observed.

Morphological Characterization

Macroscopic and Microscopic. Macroscopic analysis involved the observation of physical appearance of the incubated colonies viz. size, shape, color and texture of microorganisms by naked eyes. Microscopic observation involves the observation of shape (Bacilli, Cocci), size, color, and texture of bacteria fluorescent microscope after staining

Gram's staining reactivity. Gram's staining was used to characterize the potential isolates' morphology. The size, shape, arrangement, and Gram's staining reactivity of the cells were examined.

Motility test. The hanging Drop method, which involves collecting live microorganisms and removing them from a liquid medium, has been the most widely used method for studying cell movement and morphology. Using a ring of adhesive tape, circular concavities was made in a glass slide. vaseline was applied with a toothpick to the corners of the coverslip after placing a clean coverslip on its edges. In the middle of the coverslip, a loop of freshly made broth to test was transferred making sure to use a thin inoculum. To ensure that the vaseline is sealed within the concavity, the prepared glass slide or concavity slide upside-down (concavity downwards) was placed over the drops on the coverslip. The slide was flipped so that it is on top. The organism was allowed for 1 minute to settle. The droplet was seen suspended across the concavity.

Biochemical Tests. The biochemical characterization of isolated strains was carried out by conducting biochemical tests like Catalase test, Oxidase test, Indole test, Methyl red test, Voges Proskuer test, Citrate utilization test, Nitrate utilization test, Haemolysis test, Christie–Atkins–Munch-Peterson (CAMP) test, and Urease test using standardized methods

RESULTS AND DISCUSSION

Morphological Characterization. The shape of the bacteria (Bacilli, cocci), size, color, and texture of bacteria through fluorescent microscope after staining was observed. The pure colonies were observed under microscope by Gram staining technique (Fig. 1 and Table 1).

Fig. 1. Showing colonies on nutrient agar plate.

Table 1: Morphological characteristics.

Bacterial Isolates	Colony Shape	Colony Colour	Margin	Elevation	Gram Staining	Motility test
1	Bacilli	Pale yellow	Regular	Raised	Positive	Non motile
2	Bacilli	Whitish	Entire	Convex	Positive	Non motile

Biochemical Characterization. The results of biochemical characterization of isolated strains were represented in Table 2. Results depicted that isolated strains were positive for catalase, methyl red, and Voges Proskauer, CAMP tests. Whereas, isolated strains were negative for oxidase, indole, and citrate utilization tests. Identification of *L. monocytogenes* through biochemical test was a useful technique to analyze the samples in less than a week.

Table 2: Biochemical characteristics.

Sr. No.	Biochemical Tests	Results	
		Positive	Negative
1.	Catalase test	√	-
2.	Oxidase test	-	√
3.	Indole test	-	√
4.	Methyl red test	√	-
5.	Voges Proskauer test	√	-
6.	Citrate utilization test	-	√
7.	CAMP test	√	-

L. monocytogenes is a potential public health hazard in the food industry due to its capacity to grow in various food products at low temperatures and cause various types of human illnesses. According to Mereghetti *et al.* (2000), this pathogen is widespread in nature and is more resistant to various sanitizing agents, including quaternary ammonium compounds. Raw materials and food processing environments, which include various food contact surfaces where this pathogen may be able to form a biofilm, are the sources of *L. monocytogenes'* presence in food. As a significant and newly discovered food-borne pathogen, it necessitates special attention, especially in regards to the quick detection and control of its growth in foods and beverages.

In this study, it has been observed that *L. monocytogenes* is common contaminants of a beverages sold and marketed in Chikkaballapura. The findings suggests that the public health qualities of the product is doubtful. The production is quite unwholesome for human consumption. This work is similar to that of Bille (1999); Chukwu *et al.* (2006) who independently reported the isolation of *L. monocytogenes* in ready-to-eat dairy products. To the best of our literature knowledge this is preliminary report of *L. monocytogenes* contamination in a locally sold beverages. The findings in this study are similar to the previous cases reported in processed meats and ready-to-eat dairy products like cooked salami, meat loaf, suya, cheese (gouda), unpasteurized milk sold as fura-de-nunu, ice cream which are contaminated with *L. monocytogenes*. The results presented in study of Owusu-Kwarteng *et al.* (2018) demonstrates *L. monocytogenes* is prevalent in raw milk and nunu, a spontaneously fermented yoghurt-like milk beverage, in Ghana (Owusu-Kwarteng *et al.*, 2018). Acharya and Nummer (2022) reported survival of *L. monocytogenes* in commercially available refrigerated cold-brewed coffee (Acharya and Nummer 2022). Furthermore, Bartula *et al.* (2023) also found *Listeria* in a selection (coconut, almond and cashew) of plant-based milk beverages as dairy milk despite the significant differences in beverage composition (Bartula *et al.*, 2023).

The microbial load in the current study appeared to be high, which may be explained by the possibility that the raw materials, including the water used in the production process, were seriously contaminated. It's important to remember that beverages lack quality control procedures and critical control points, which makes it reasonable for highly pathogenic *L. monocytogenes* to contaminate them. According to these findings, international organizations should focus their funding on research into listeriosis transmission through food products in developing nations. The production of healthy beverages in developed and developing nations should also be a focus of the global effort to ensure the security of food products.

CONCLUSIONS

The findings of the study lead to new light on the growth of food pathogens and spoilage microbes in a variety of beverages that are growing in popularity across the globe and suggested that *L. monocytogenes* is common in beverage samples. These results might be taken into account when calculating the risk connected to beverage production and consumption.

FUTURE SCORE

This study would be of immense important in minimizing and safeguarding the life of people who consume beverages products.

Acknowledgements. Author their owes gratitude to family and friends for their support and constant encouragement rendered for excelling professional career.
Conflict of Interests. None.

REFERENCES

Acharya, S. and Nummer, B. A. (2022). Survival of *Listeria monocytogenes* in commercially available refrigerated cold-brewed coffee. *Journal of Environmental Health*, *85*(2).

Bartula, K., Begley, M., Latour, N. and Callanan, M. (2023). Growth of food-borne pathogens *Listeria* and *Salmonella* and spore-forming *Paenibacillus* and

Bacillus in commercial plant-based milk alternatives. *Food Microbiology*, *109*, 104143.

Bhujwala, R. A., Hingorani, V. and Chandra, R. K. (1973). Genital listeriosis in Delhi (India): A pilot study. *Indian Journal of Medical Research*, *61*(9), 1284–1288.

Bille, J. S. (1999). Epidemiology of listeriosis in Europe, with special reference to Swiss outbreak. In *Industrial microbiology, food borne listeriosis* (miller AJ, smith JL and Somkuli F – editors) (pp. 25–29). Elsevier Science Publications.

Chukwu, O. O. C., Ogbonna, C. I. C., Olabode, A. O., Chukwu, D. I., Onwuliri, F. C.and Nwankiti, O. O. (2006). *Listeria monocytogenes* in Nigeria Proceed Meats and ready-to-eat dairy products. *Niger. J Microbiol*., *20*(2), 900–904.

Dhanashree, B., Otta, S. K., Karunasagar, I., Goebel, W. and Karunasagar, I. (2003). Incidence of *Listeria spp.* in clinical and food samples in Mangalore, India. *Food Microbiology*, *20*(4), 447–453.

Ericsson, H., Eklöw, A., Danielsson-Tham, M. L., Loncarevic, S., Mentzing, L. O., Persson, I., Unnerstad, H. and Tham, W. (1997). An outbreak of listeriosis suspected to have been caused by rainbow trout. *Journal of Clinical Microbiology*, *35*(11), 2904–2907.

Farber, J. M. and Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews*, *55*(3), 476–511.

Griffiths, M. W. (1989). *Listeria monocytogenes*: Its importance in the dairy industry. *Journal of the Science of Food and Agriculture*, *47*(2), 133–158.

Hitchins, A. D. (1998), Chap. 10. Center for Food Safety and Applied Nutrition. Detection and enumeration of *Listeria monocytogenes* in foods. In *Bacteriological analytical manual*. United States Food and Drug Administration.

Jamali, H., Chai, L. C. and Thong, K. L. (2013). Detection and isolation of *Listeriaspp.* and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. *Food Control*, *32*(1), 19–24.

Kang, Q. H., Guo, Y., Hong, S. Q. and Jiang, L. (2013). *Listeria monocytogenes* meningitis in infants: Report of two cases. *Zhong Guo Dang Dai Er Ke Za Zhi*, *15*(12), 1150–1152.

Kasalica, A., Vukovic, V., Vranjes, A. and Memisi, N. (2011). *Listeria monocytogenes* in milk and dairy products. *Biotechnology in Animal Husbandry*, *27*(3), 1067–1082.

Linnan, M. J., Mascola, L., Lou, X. D., Goulet, V., May, S., Salminen, C., Hird, D. W., Yonekura, M. L., Hayes, P. and Weaver, R. (1988). Epidemic listeriosis associated with Mexican style cheese. *New England Journal of Medicine*, *319*(13), 823–828.

McLauchlin, J., Mitchell, R. T., Smerdon, W. J. and Jewell, K. (2004). *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiologyical risk assessment of foods. *International Journal of Food Microbiology*, *92*(1), 15–33.

Mereghetti, L., Quentin, R., Marquet-Van Der Mee, N. and Audurier, A. (2000). Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. *Applied and Environmental Microbiology*, *66*(11), 5083–5086.

Owusu-Kwarteng, J., Wuni, A., Akabanda, F. and Jespersen, L. (2018). Prevalence and characteristics of *Listeria monocytogenes* isolates in raw milk, heated milk and nunu, a spontaneously fermented milk beverage, in Ghana. *Beverages*, *4*(2), 40.

Razavilar, V. and Genigeorgis, C. (1998). Prediction of *Listeriaspp.* growth as affected by various levels of chemicals, pH, temperature and storage time in a model broth. *International Journal of Food Microbiology*, *40*(3), 149–157.

Thomas, M. K., Vriezen, R., Farber, J. M., Currie, A., Schlech, W. and Fazil, A. (2015). Economic cost of a *Listeria monocytogenes* outbreak in Canada, 2008. *Foodborne Pathogens and Disease*, *12*(12), 966–971.

Todd, E. C. D. and Notermans, S. (2011). Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. *Food Control*, *22*(9), 1484–1490.

How to cite this article: Keshamma E. (2023). Isolation and Biochemical Characterisation of Food Born Pathogens Listeria Monocytogenes from Beverages. *Biological Forum – An International Journal*, *15*(6): 599-602.