iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** VII **Month of publication:** July 2019

DOI: http://doi.org/10.22214/ijraset.2019.7215

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue VII, July 2019- Available at www.ijraset.com

Detection of Virulent Efficiency of L. Monocytogenes Species Present in Retail Meats in North Eastern Region of India

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Abstract: ***A total 0f 1005 meat samples were collected from the different street markets of North East states of India and screened for L. monocytogenes. The isolates were characterized by PCR for virulent gene hlyA and iap. Out of all samples 46 (4.6%) were found to be positive for L. monocytogenes and belonged to virulent species. This study investigated the presence of potentially human pathogenic strains of Listeria spp. in the meats commercialized in street markets of North East states of India that may represent a damning health risk to the consumers.***
Keywords: Listeria monocytogenes; meat; virulence factors; PCR.

I. INTRODUCTION

Listeria monocytogenes is an emergent food-born pathogen responsible for major outbreaks and sporadic food-related cases of listeriosis world wide [1]. Listeriosis is usually sporadic, but large outbreaks related to consumption of contaminated foods have been reported [2]. The natural habitats of the bacterium L. monocytogenes are soil, water and plant material, particularly plant material undergoing decay [3]. L. monocytogenes has been recognized as an animal pathogen for over 70 years [4], but only for 20 years as a food-borne human pathogen [5]. In the 1990s, it was frequently isolated from all the main food sectors, such as unfermented dairy products [6], [7], cheese and other dairy products [8], meat products [9], poultry and egg products [10], products of plant origin [11] and fish and seafood products [12]. The available current literature shows that L. monocytogenes has been reported from a wide variety of contaminated food types and clinical samples in various country of the world [13], [14]. As a widely distributed environmental contaminant, L. monocytogenes may contaminate food at any point in the food chain.

Reports indicate that listeriosis has emerged as an important disease in developed countries but it is reported less frequently in developing countries [15]. But information on the occurrence and distribution of L. monocytogenes is very limited both in veterinary and public health in North Eastern hilly region, one developing state of India. Moreover most of the people living in the difficult terrains in the highland are tribal, having no idea about the hygienic food processing system. The contaminated food may play a major bed impact in health of the population. The aim of our present study was to identify the occurrence of virulent L. monocytogenes species that is present in the raw meat samples in the different state of North East India using the multiplex-PCR method.

II. MATERIALS AND METHODS

A. Collection of Meat Samples

A total 0f 1005 meat samples viz beef, chicken, mutton and pork were collected from different commercial shop located in Assam, Tripura, Meghalaya, Manipur, Nagaland, Mizoram and Arunachal Pradesh in the year 2009. All samples were collected aseptically and maintained 4°C temperature until microbiological analysis.

B. Isolation of Strain

One gm. of each sample was aseptically taken and was homogenized in 10ml of UVM (University of Vermont) (Oxoid, Basingstoke, Hampshire, UK). Samples were cultured with Listeria selective enrichment supplement, UVMI, (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 h. One loop of enriched culture was streaked on to listeria selective agar base (Oxoid, Basingstoke, Hampshire, UK), supplemented with Listeria selective supplement (Oxoid, Basingstoke, Hampshire, UK). Plates were incubated at 37°C for 48 h followed by incubation at 4°C for 12 to 24 h. After incubation only the suspected colonies of round button shaped with blackish-grayish colored were allowed for pure culture on nutrient agar and incubated at 37°C. The single colonies obtained were confirmed as *L. monocytogenes* by biochemical tests and characterized at its molecular level by PCR analysis for *hly*A and *iap* genes.

C. PCR Analysis

1) *DNA Isolation:* One colony from the culture was inoculated in to 10 ml of Brain heart infusion broth (Himedia, Mumbai, India) and incubated overnight at 37°C. 1ml of broth culture was transferred in eppendorf tube and centrifuged at 12000 rpm for 5 minutes, and the pellet was washed with of distilled water and resuspended in another appendrof tube in 1 ml of distilled water followed by heating on water bath at 100°C for 15 minutes. After heating, the suspension was again centrifuged at 15000 rpm for 10 min, and the supernatant was used as the PCR template DNA.
2) *PCR Conditions or Virulent Genes:* The PCR primers directed towards the *hlyA* gene of 234bp and *iap* gene of 131bp were forward, 5´-ACAAGCACCTGTTGCAG-3´; reverse, 5´-TGACAGCGTGTGTAGTAGCA-3´ [16] and forward, 5´-CGGAGGTTCCGCAAAAGATG-3´; reverse 5´-CCTCCAGAGTGATCGATG-3´ [17] respectively. The reaction mixture was prepared in a total volume of 25µl consisting of mastermix (2.5X) 10 µl (Fermentas), 0.125 µl of each primer and 2.5 µl of boiled samples was used as DNA template. PCR amplification was carried out in the thermal cycler (Biorad). The PCR cycling parameters were as follows: Initial denaturation at 94°C for 4minutes (1 cycle), followed by 30 cycles of denaturation at 94°C for 1 minutes, annealing at 56°C for 1minutes, and extension at 72°C for 1minutes. Final extension at 72°C for 10 minutes; and finally the products were kept at 4°C until collection. Then the PCR products were separated by electrophoresis in 1.2% agarose gel and visualized by ethidium bromide staining (Fig 1).

III. RESULTS

Out of 1005 different meat samples analyzed, 46 (4.57%) were found to be positive for *L. monocytogenes* and were also amplified by *hlyA* and *iap* virulent genes in PCR analysis (Fig 1). The occurrence of *L. monocytogenes* in raw meat samples varied and was high in beef (9%), followed by pork (4.8%), chicken (3.1%) and mutton (2.3%) (Table 2). The occurrence of *L. monocytogenes* isolated from different state also varied and was high in Meghalaya (6.5%), followed by Nagaland (5.3%), Mizoram (5.1%), Arunachal Pradesh (4.1%), Assam (4%), Tripura (3.8%) and Manipur (2.6%) (Table 2).

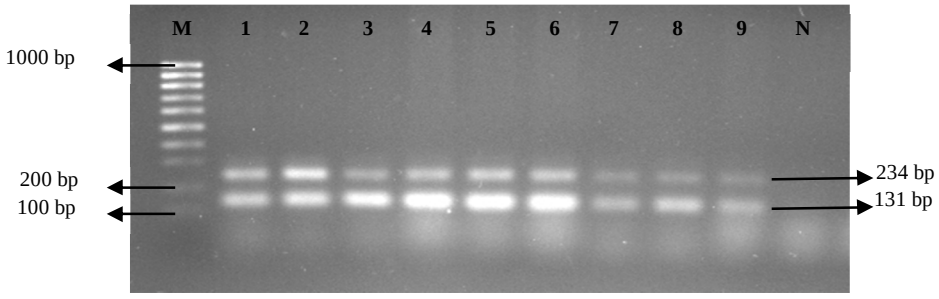

Fig 1**:** PCR detection of hlyA (234bp) and iap (131 bp) gene of L. monocytogenes. M: marker (1000 bp), lane 1-9: isolates, N: negative control.

States	Type of sample											
	Beef			Pork			Mutton			Chicken		
	Examined	+ve	%	Examined	+ve	%	Examined	+ve	%	Examined	+ve	%
Tripura	-	-	-	37	2	5.4	45	1	2.2	50	2	4
Assam	18	2	11.1	10	1	10	47	-	-	50	2	4
Meghalaya	55	6	10.9	60	3	5	15	1	6.6	55	2	3.6
Manipur	23	1	4.3	37	1	2.8	33	1	3	60	1	1.6
Mizoram	36	3	8.3	42	3	7.1	31	1	3.2	45	1	2.2
Nagaland	45	4	8.9	32	1	3.1	12	-	-	23	1	4.3
A. Pradesh	23	2	8.7	51	2	3.9	35	1	2.8	35	1	2.8
Total	200	18	9	269	13	4.8	218	5	2.3	318	10	3.1

Table 1: Number of samples collected from different states and the incidence of L. monocytogenes in different sources.

Place of collection	Number of sample examined	Number of isolate positive	(%)
Tripura	132	5	3.8%
Assam	125	5	4%
Meghalaya	185	12	6.5%
Manipur	153	4	2.6%
Mizoram	154	8	5.1%
Nagaland	112	6	5.3%
Arunachal Pradesh	144	6	4.1%
Total	1005	46	4.6

Table 2: Incidence of L. monocytogenes in different states

IV. CONCLUSIONS

A total of 1005 samples were collected from diverse commercial shops located in 7 states of NEH region of India in the year 2009. The experimental results show that the incidence of L. monocytogenes is varied in state to state and in animal to animal. The major contaminated source of L. monocytogenes in all states is beef (Assam 11.1%, Meghalaya 10.9%, Nagaland 8.9%, Arunachal Pradesh 8.7%, Mizoram 8.3%, Manipur 4.3%, and Tripura 0%). The present study indicates that the listerial contamination highly prevails in the retail meat shops as screening of 1005 samples resulted into 46 (4.57%) isolates of L. monocytogenes. From the above a clear picture about the occurrence of L.monocytogenes in the NEH region is found. The occurrence of virulence genes in most of the isolates revealed that the L. monocytogenes were equally virulent and pathogenic as found all over the world.

Findings on 4.57% isolation rate of L. monocytogenes with high pathogenic potential in the samples from different sources during the present investigation indicates the importnace of this important pathogen in the region, which is a matter of great concern from public health point of view and the occurrence of this pathogen suggests that the meat commercialized in markets may represent a health risk to the consumers.

V. ACKNOWLEDGMENTS

I express my gratitude to Division of Animal Health, ICAR, Barapani, Meghalaya, India and Assam University, Silchar, India for providing research facility to carry out the study.

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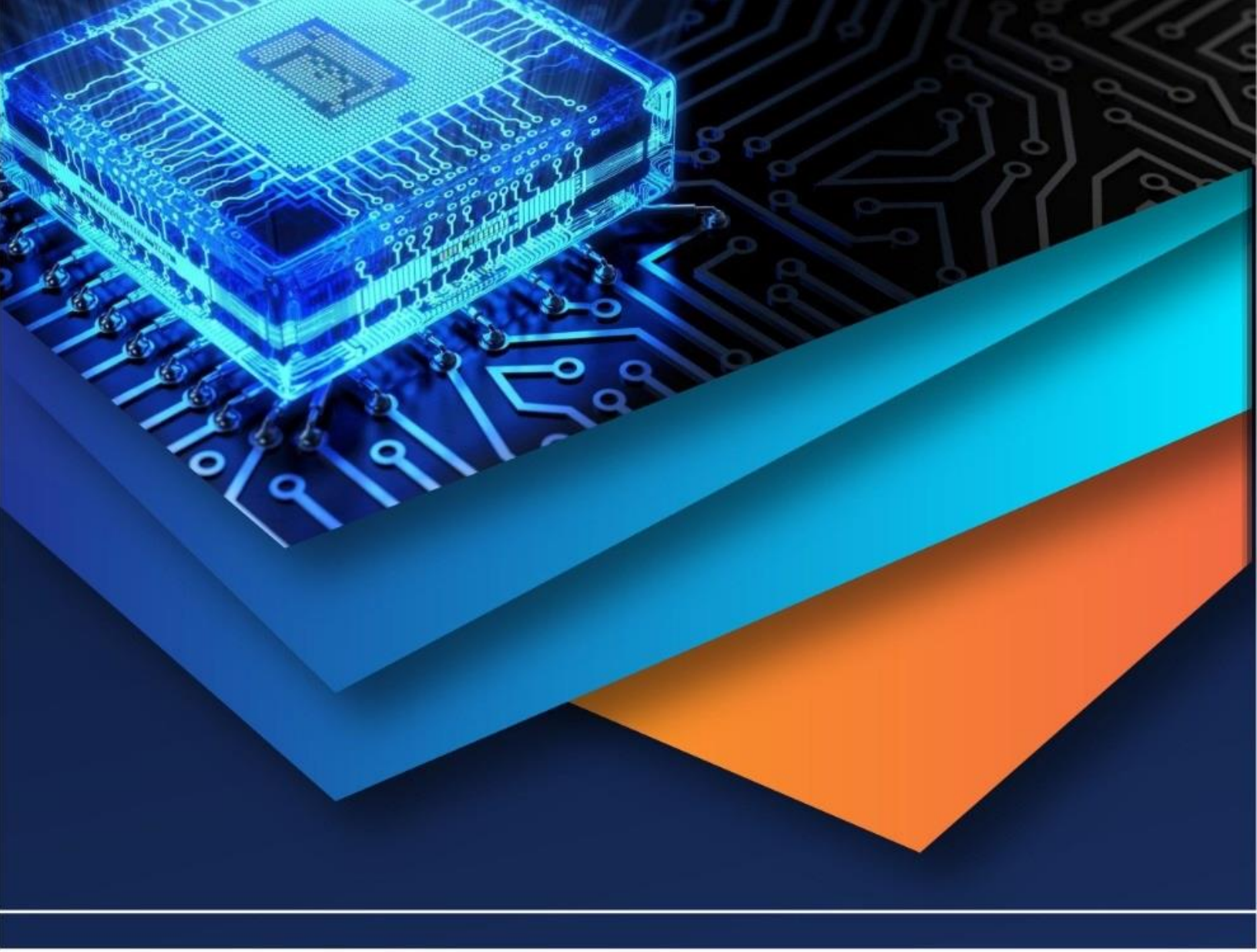

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