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A Study of Identification *Listeria monocytogenes* Bacteria Isolated from Raw Vegetables in Madhya Pradesh, India

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ABSTRACT

The modern lifestyle pronounced changed eating habits around the world, today raw vegetables are used for major cravings but the packaging and storage conditions of this output can favor the growth of bacteria *Listeria spp*. or LM which is a foodborne pathogen. that's why it is necessary to check as they are difficult to identify and differentiate solely by biochemical tests and molecular methods. The aim of the present study is to explore identification of LM in various raw vegetables from wet and hypermarkets available in selected Madhya Pradesh districts using the different Biochemical test and 16s rRNA method. A variety of available vegetables (n = 312) consumed in the minimally processed states in Madhya Pradesh have examined ten types of selected raw vegetables for the presence of LM to found information on the presence of these organisms in these raw vegetables. In the years 2019-2020, a total of 147 of the 312 different raw vegetable samples collected tested positive for *L. spp*. or *L. monocytogenes*. Our findings demonstrated that the presence of *L. spp*. in selected vegetable products was generally medium, on average 47.11% in the years studied including *Listeria monocytogenes*.

Key words*: Listeria spp., L. monocytogenes, Biochemical test, Molecular methods.*

Introduction

Listeriosis Disease is a significant bacterial infection, which is caused by LM Pathogen from which affected humans and animals, mainly from ingestion of Microbiologically contaminated vegetables pose a risk, especially the processing of raw vegetables. Known to be the human pathogenic bacterium Listeria, it can be found in fresh vegetables (Golberg *et al*., 2011). Which are contaminated if they come in contact with contaminated soil, manure, irrigation water. *Listeria monocytogenes* leads to sepsis, miscarriage, new borne baby, perinatal infections, meningitis, gastroenteritis, and meningoencephalitis, especially in immunocompromised individuals and the geriatric. The genus Listeria is a Gram-positive bacterium based on the low G + C content of the genome and currently exists recognized all 21 additional species *L. monocytogenes, L. rocourtiae, L. welshimeri, L. marthii, L. ivanovii, L.grayi, L. fleischmannii, L. seeligeri L. innocua, , L. grandensis, L. riparia L. foridensis, L. cornellensis, L. riparia, L. aquatica and L. booriae, L. thailandensis, L. weihentephanensis L. newyorkensis, L. goaensis*. *L. newyorkensis*. But only 2 *spp*. The genus *Listeria monocytogenes* in humans and *L. ivanovii* in animals are generally considered pathogens (Quereda *et al*., 2020). Based on previous data on the ecology and physiology of *L. monocytogenes* and its manifestations such as listeriosis, the number has increased over the past two decades. Applying this new information in food production and processing may reduce the prevalence

of listeria infection in industrialized countries around the world. (Schlech *et al*., 1983; Swaminathan *et al*., 2007). There is evidence for cases of Listeriosis (Gaul *et al*., 2013) caused by the consumption of contaminated vegetables and fruits. on the list of *Listeria monocytogenes* or Listeria spp. of food samples and this explains why it remained unnoticed as a major food pathogen until recently. this study was to raw vegetables investigate the presence of *L. monocytogenes*. was taken from different types of vegetables are in two types (wet and Hyper) market at different locations in the selected District of Madhya Pradesh, India.

Materials and Methods

Sample collection

Raw vegetables samples were collected in and around Madhya Pradesh State and checked for the presence of *Listeria species*. Samples (n=312), which was divided into 10 varieties, Cabbage (38), Tomato (36), Potato (34), Spinach (32), Yardlong been (24), Carrot (30), Green peas (36), Eggplant (24), Okra (28), Sweet potato (30).

Bacteria isolation

The microbiological analyses for presence of *Listeria monocytogenes* in fresh vegetable samples were done by standard method EN ISO 11290- 2. (Becker *et al*., 2006; Midelet-Bourdin *et al*., 2007). To find out *Listeria monocytogenes* 25 g of raw vegetable sample was homogenized in 225 g of Pre- Enrichment After transferring 0.1 ml of the initial stock solution to 10 ml of Brain Heart Infusion (secondary selective agar) at 30 °C for 24 ± 2 h and incubated in the incubator for 48 hours at 37 °C. Both primary and 2nd selective agar suspensions were inoculated into PALCAM agar (HiMedia) and incubated in the incubator at 37 °C for 24-48 hours. To confirm the presence of *Listeria.* specific colonies were selected and inoculated into PALCAM Agar tryptone medium (Merck, Germany) and incubated at 37 ° C for 18-24 hours.

Biochemical characterization

Oxidase test

The investigated bacterial colony was applied to filter paper pre-saturated with a freshly prepared oxidase reagent. A positive oxidase test result was recorded as the appearance of a blue-purple color within 10 seconds (Cheesbrough, 1991).

Catalase test

Gas bubbles are detected within 10 seconds after adding the purified bacterial culture to 5 ml of hydrogen peroxide solution, which is considered a positive test for catalase (Cheesbrough, 1991).

Urease test

Tilted two milliliters of urea medium, which are placed in McCarthy jewelry bottles, are used to incubate bacterial colonies at room temperature. The red-pink color of the medium was considered a positive test for urease induction (Cheesbrough, 1991).

Indole test

The appearance of a bright red and yellow color resulting from the addition of 0.5 ml of Kovac reagent to the incubated bacterial culture at 35 °C for 24 h on BHI medium indicates positive and negative results, respectively (Cheesbrough, 1991).

Methyl red (MR) test

After adding the methyl red indicator solution (BHI, Himedia) to culturing and incubation media at 35 °C for up to 4 days, a change in color to red indicates that MR has good appearance of tested bacteria.

Gelatin hydrolysis

A gelatin stab method was applied according to (Edison *et al*., 2012). Heavy inoculums of test bacterium are absorbed into tubes containing nutrient gelatin, liquefaction gelatin is a positive-results for bacterial gelatin hydrolysis.

PCR amplification of the 16S rRNA

Samples for Listeria monocytogenes were used using 16S rRNA primary pairs: 5 'CCT AAG ACG CCA ATC GAA 3' and 5 'AAG CGC TTG CAA CTG CTC 3'. For this purpose, DNA extracted from a previously preserved *L. monocytogenes* culture was used as a control regulator in each PCR test. All heat transfer operations were performed with the Bio-Era thermal cyclizer. To analyze the PCR product, 10 µl of PCR product was applied at 100 V for 28 min in 1.0% agarose gel. The gel was then stained with bromide ethinide and observed under ultraviolet light. DNA molecular ladder (100 bp ladder).

Statistical analysis

Past-4 software (version 16.0) was used to analyze the data and determine whether there was a significant *L. monocytogenes* between sampling sites and vegetable types and level of consequences was determined at P.

Results and Discussion

A total of 312 raw vegetables were analyzed. Based on biochemical tests (as shown in Table 1), an isolated examination, the targeted LM reflected different biochemical characteristics. Positive for oxidase test and negative for catalase, indole, MR, gelatin hydrolysis and urease test identified the data and determine whether there was a significant *L. monocytogenes* (Fig. 1) between sampling sites and vegetable types and the level of significance determined in P <0.05 *L. monocytogenes* is widespread in the environment (Schlech *et al.*, 1983). Analyzed for the prevalence and microbial load of *L. monocytogenes* using MPN – PCR where PCR targets 16S rRNA for *Listeria monocytogenes*. Fig. 2 shows a representative image of gel electrophoresis of PCR amplification of the 16S rRNA gene. total detected 59.67% in most of the wet market vegetable samples (111 samples were positive out of 186 totally Different raw vegetable samples). While in Hypermarket *Listeria* Was detected 28.57% (36 samples were positive out of 126 Different vegetable samples) In general, observing all locations, 47.11% vegetable samples were positive of *Listeria*. However, there were no significant differences between the sampling locations when statistically analyzed. Of the 10 types of vegetables, *Listeria spp*. Was more prevalent, while *L. monocytogenes* was more prevalent in yardlong beans at 31.3%, cabbage at 27.2% and potato at 25% (Graph 1). The microbial load for *L. monocytogenes* was listed using MPN-PCR and was found to vary from. *L. monocytogenes* was calculated by MPN-PCR and was found to range from <3 to 1100 MPN / g and most samples (96.4%) contained *Listeria*. There was a microbial load of <100 MPN/g for *L. monocytogenes*. It was also found that 2.9% of

Table 1. Isolates *L. monocytogenes* biochemical tests results.

Test	Results
Oxidase test	+
Catalase test	-
Urease test	-
Indole test	-
Methyl Red (MR) test	-
Gelatin Hydrolysis	-

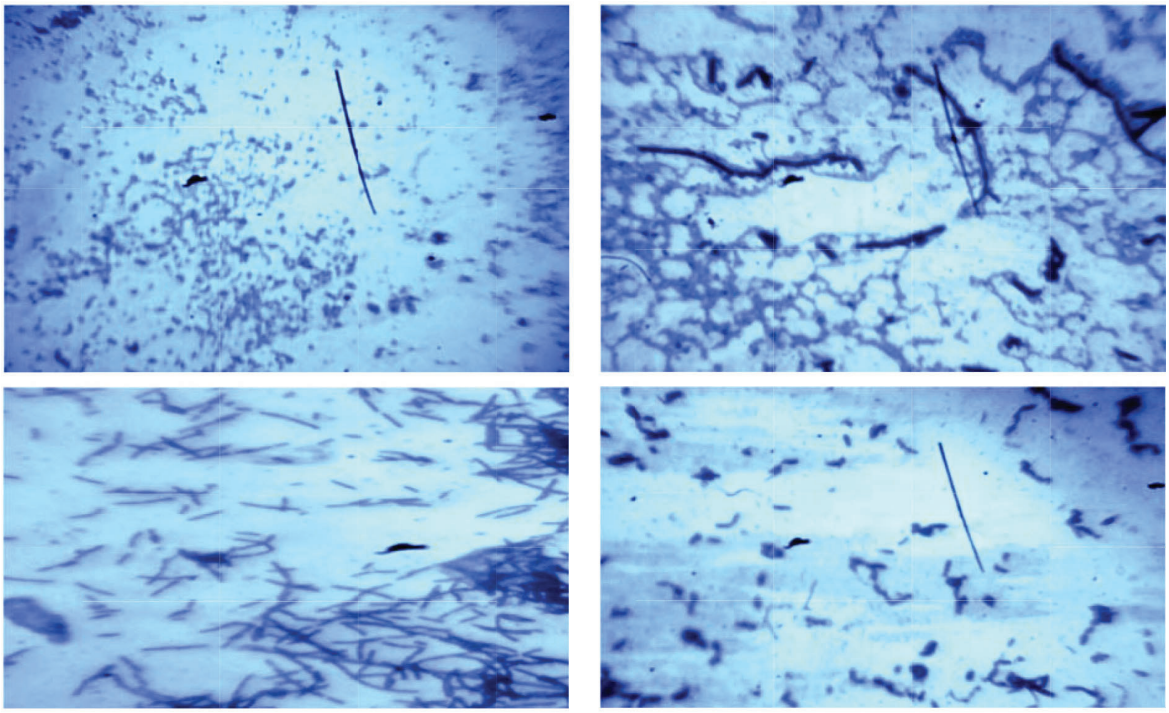
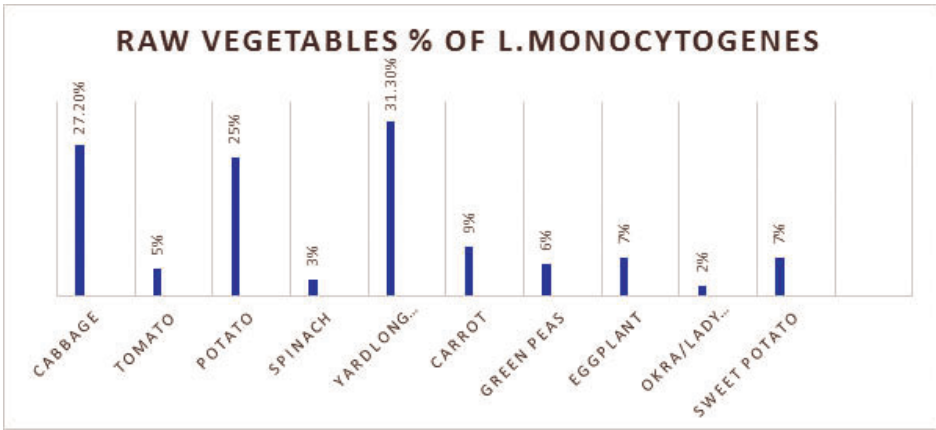


Fig. 1. Observed Microscopic Picture of *Listeria monocytogenes* by Oil immersion lens 100x.



Graph 1. *L. monocytogenes* percentage of used raw vegetables sample

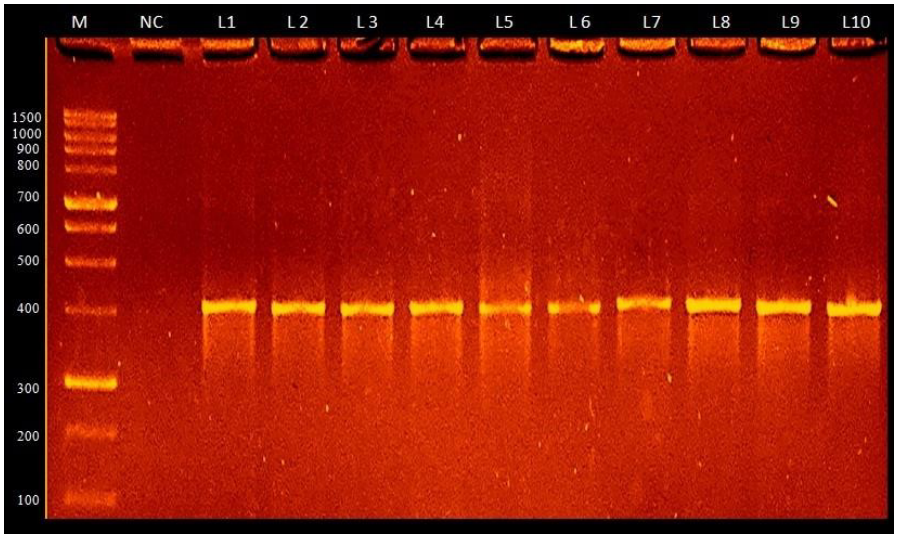


Fig. 2. Representative 16S rRNA amplification for the identification of Listeria monocytogenes. where M shows the medium range DNA ladder. NC shows negative control and L1 to L10 shows MPN broth DNA.

the samples had a microbial load of 100 to 1000 MPN / g, while 0.7% had a microbial load of over 1000 MPN / g. *L. monocytogenes* was estimated to have lower MPN / G in the Hypermarket.

Conclusion

The samples of raw vegetables analyzed from retail fresh and hypermarket originating from Madhya Pradesh were found to be contaminated LM is spacious distributed in the environment (Farber and Peterkin, 1991) and this can lead to contamination of vegetables during cultivation, harvesting, post-harvesting, processing or distribution. Fresh vegetables can pose a significant risk because they are eaten raw. Raw vegetables on the wet market are likely to harbor bacteria, as the main habitat of *L. monocytogenes* appears to be soil and vegetation, where bacteria lead to saprophytic existence, and the soil acts as a reservoir for infections transmitted to animals and humans. (Fenlon, 1986). The results of the reported study provided scientific evidence for the prevalence of LM in raw vegetables, which is of great importance for the prevention of the incidence of listeriosis.

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Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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