

## Occurrence of *Listeria* species isolated from raw vegetable samples and its distribution

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### ABSTRACT

The investigation focuses on *Listeria* species and *Listeria monocytogenes* from vegetable samples that are usually associated with outbreaks of food-borne disease *i.e.*, listeriosis. Different raw vegetables were collected *viz.*, *e.g.*, spinach, carrot, cabbage, cauliflower, and coriander leaves. The ISO-11290(1) method was used for the isolation and identification of *Listeria* species through morphological and biochemical analysis of isolates. The overall prevalence of total *Listeria* species and *Listeria monocytogenes* were 8.5% (34/400) and 4.8% (19/400) respectively. The frequency percentage of *Listeria* species *viz.*, Spinach, 13.8%; carrot and cabbage, 8.8%; cauliflower, 6.3% and 5%, coriander leaves. *L. monocytogenes* frequency percentage was 8.75%, spinach followed by carrot and coriander leaves, 5%; 3.75%, cabbage; and the least in cauliflower, 1.25%. The majority of *Listeria* species were isolated throughout the spring and winter seasons in terms of seasonal variations, carrots, and cabbage were the next most common foods to contain *Listeria monocytogenes* during the monsoon season after spinach and coriander leaves. From the above findings, it is concluded that spinach was found to be more prevalent for *L. monocytogenes* along with other positive samples which increases risks associated with the consumption of raw vegetables.

Figures : 04

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KEY WORDS : Food-borne disease, *Listeria monocytogenes*, Raw vegetables.

### Introduction

Listeriosis is a rare and fatal food borne illness, that can be life-threatening to both animals as well as humans and arises mainly from the ingestion of contaminated food. Listeriosis leads to infections with a high mortality rate in highly vulnerable groups including pregnant women, immunocompromised and elderly people. It also leads to septicemia, abortion, stillbirth, perinatal infections, meningitis, gastroenteritis and meningoencephalitis<sup>9,15,16,23</sup>. Immuno-suppressed patients such as organ transplant recipients, AIDS, and acute leukemia are predisposed to higher rates of infection<sup>12</sup> or Listeriosis causes serious outbreaks every year, and the cases reported in a significant fatality rate of about 20 to 40%, the rate of listeriosis in healthy about 0.7 cases per 100,000 persons<sup>3,15,16,18</sup>. The genus *Listeria* contains 10 species of which only two species *L. monocytogenes* and *L. ivanovii* are pathogenic, and

the rest are non-pathogenic, *L. monocytogenes* is a food borne pathogen that causes listeriosis which is an intracellular, Gram-positive, rod-shaped, non-spore-forming, motile, facultative anaerobic that grows in frozen vegetables<sup>8</sup> at temperature range 0.4 to 50°C, high salt concentrations while, genus *Listeria* can tolerate the food processes, as cooking and pasteurizing conditions, *Listeria* spp. is ubiquitous in the environment and isolated from different sources, water, soil, livestock and different vegetables that were irrigated with contaminated water and it enters the food chain<sup>6,15,16,23</sup>. The Regulation from the U.S. Food and Drug Administration (FDA) has zero tolerance for *L. monocytogenes* in food articles and a high mortality rate due to listeriosis, *L. monocytogenes* can penetrate the blood-brain and placental barriers, increasing the severity of the disease, compared to other food borne pathogens that predominantly infect the gastrointestinal tract<sup>9,19</sup>. Several investigators reported

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**TABLE-1 : Biochemical characterization of *Listeria* isolates**

Biochemical Tests	<i>Listeria</i> Sp.	<i>L. monocytogenes</i>
Indole test		
MR	+	+
VP	+	+
Catalase test	+	+
Urease test	-	-
Motility test	+	+
Nitrate reduction	-	-
Oxidase test	-	-
H <sub>2</sub> S test		
Sugar fermentation tests		
Rhmnose	LI +ve, LW +ve Others -ve	+
Glucose	+	+
Lactose	LI -ve, LW - NR, LIN +ve	V
Mannitol	LG +ve, (others -ve)	-
Xylose	+ (other sp.)	-
L. ivanovii- LI, L. welshimeri		

that 99% of human *Listeria* infections are of food-borne origin. *L. monocytogenes* is found to be associated with ready to eat food items or those were consumed raw as per the health professionals<sup>13,23</sup>. Usually, vegetables or food entities were consumed as raw in homes, restaurants or kept at refrigeration and handily washed that increases the pathogens survival rate. *L. monocytogenes* food borne disease reported annually in the United States, with a case-mortality rate of approximately 20%<sup>4,7,20</sup>. Alleviation of *L. monocytogenes* in fresh produce while growing in fields, orchards, or greenhouses were challenging as it can survive or

sustained natural soil, adhere to surfaces as biofilm that are resistant to desiccation, acid, heat or disinfectants *i.e.*, became a vehicle of transmission of pathogens by improper harvesting procedures and other sources of contamination from rivers, canal waters, and effluents from sewage treatments<sup>2,5</sup>.

### Materials and Methods

The study was conducted in the Department of Industrial Microbiology for investigation of *Listeria* species from different vegetable samples. The total of 400 samples of raw vegetables and salads were screened for *Listeria* species Out of eighty (80) samples each of spinach, cauliflower, cabbage, coriander leaves, and carrots were collected from vendors and vegetable shops of Prayagraj. Isolation and identification of pathogenic *Listeria* species from raw vegetable samples investigated as per the recommendation by ISO 11290-1, 1996<sup>14</sup>. Primarily samples were enriched with 225 ml of broth (Hi-Media) incubated at 30±1 °C for 24 h and secondary enrichment, 0.1 ml of broth was transferred to 10 ml of Fraser broth and was incubated at 37°C for 48 h. A loopful culture was streaked onto selective medium *i.e.*, PALCAM agar (Oxoid, Basingstoke, UK), incubated for 24-48 h at 37 °C<sup>13,14</sup>. Plates were observed for typical *Listeria* colonies *i.e.* Grey green colonies with black sunken centers. Morphological characteristics observed after Gram's staining, colonies were picked up and cultured on tryptic soy agar with 0.6% yeast extract. All isolates were subjected to standard biochemical tests that showed mannitol and xylose, fermented and rhamnose positive were considered *L. monocytogenes*<sup>8,12,15,29</sup>.

### Results and Discussion

Most people in India consume fresh and raw vegetables as salads, as a part of their diet. In the present investigation on the incidence of *Listeria* spp. and *Listeria monocytogenes* from different raw vegetable samples, a total of 400 raw vegetable samples were screened, of which 34 samples were found to be distinctive *Listeria* spp., these isolates were identified based on cultural, morphological, and biochemical characteristics and an overall prevalence of *Listeria* spp. reported in the samples was 8.5% (34/400) and prevalence of *Listeria monocytogenes* was 4.8% (19/400) (Table-1). It was reported based on its peculiar colony characters on PALCAM agar. Further in Table 2, the percentage incidence of *Listeria* spp. and *Listeria monocytogenes* has been shown in different vegetable samples. Figure 2 depicts the percentage of presumptive *Listeria* spp. (8.5%) and *Listeria monocytogenes* (4.8%) in vegetable samples. A total of 80 samples from each category, *i.e.*, Spinach, cauliflower, carrot, coriander leaves, and

TABLE- 2 Incidence of *Listeria* spp. and LM in different vegetable samples

Vegetable samples	Total Number of Samples	Positive <i>Listeria</i> spp.	Positive LM	Incidence (%)	
				<i>Listeria</i> sp. (%)	LM (%)
Spinach	80	11	7	13.8 %	8.75 %
Carrot	80	7	4	8.8 %	5 %
Cauliflower	80	5	1	6.3 %	1.25 %
Cabbage	80	7	3	8.8 %	3.75 %
Coriander leaves	80	4	4	5 %	5 %

LM= *Listeria monocytogenes*

cabbage, were screened for the incidence of *Listeria* spp. and *Listeria monocytogenes* in different vegetable samples. The highest percentage of *Listeria* contamination was reported in the Spinach, and less work has been done in Asia, mostly in India, as reported<sup>5,26</sup>. The bacterial load of *Listeria monocytogenes* in different vegetable samples was screened and found to be highest among the spinach samples followed by carrot and coriander leaves, cabbage, and the least in cauliflower samples. The frequency percentage of *Listeria* spp. was 13.8% (11) in Spinach; 8.8% (7) in both carrot and cabbage; 6.3% (5) in cauliflower and 5% (4) out of 80 samples of each category analyzed) in coriander leaves. Further, the distribution percentage of *L. monocytogenes* was 8.75% (7) in spinach, 5% (4) in carrot and coriander leaves each, 3.75% (3) in cabbage, and 1.25% (1 out of 80 samples of each category) in cauliflower (Table 3, Figure 4). (Figure 2 and Figure 3). These results follow the previous results in which the prevalence of *Listeria* spp. in different vegetable samples was reported as

8.7%<sup>13</sup>. The results are also per previous studies<sup>1</sup>, reporting 9.70% of total *Listeria* spp. and 8.5% of samples were positive for *Listeria innocua*. Similar results were reported<sup>30</sup> showing an overall prevalence of *Listeria* spp. (14%) and *L. monocytogenes* (9%)(Figure 2). A higher incidence of *Listeria* spp. 26.6% in vegetables and 7.0% of vegetable samples contaminated with *Listeria monocytogenes* were reported<sup>28</sup> earlier which is in contradiction to the present findings. Similar results were recorded as 3.03% contamination of the vegetable samples by *L. monocytogenes*<sup>8,16</sup>. As compared to the findings of the present investigation, a prevalence of *Listeria* spp. and *Listeria monocytogenes* in vegetable samples were observed ranging from 1.2%-59% respectively<sup>15,18,23,29</sup>. Variable incidence of *Listeria* sp. from as low as 0.62% in North China, 3.1% in Brazil, 12.5% in India, 14.03% in Indonesia, 25.6% in central Serbia; 33.3% in Malaysia; 37.8% in Malaysia; 42.9% in South Africa; 44.3% in Brazil; to the highest 69.2% in Nigeria has been reported<sup>14,25</sup>. A similar incidence of *L. monocytogenes*(4.7%) in vegetables and 3.8% of

TABLE-3 : Incidence of *Listeria* spp. and *Listeria monocytogenes* in different vegetable samples

Total No. of samples examined	Total number of presumptive <i>Listeria</i> spp.	<i>Listeria</i> spp. (%)	<i>Listeria monocytogenes</i>
400	34	8.5	4.8

LM= *Listeria monocytogenes*

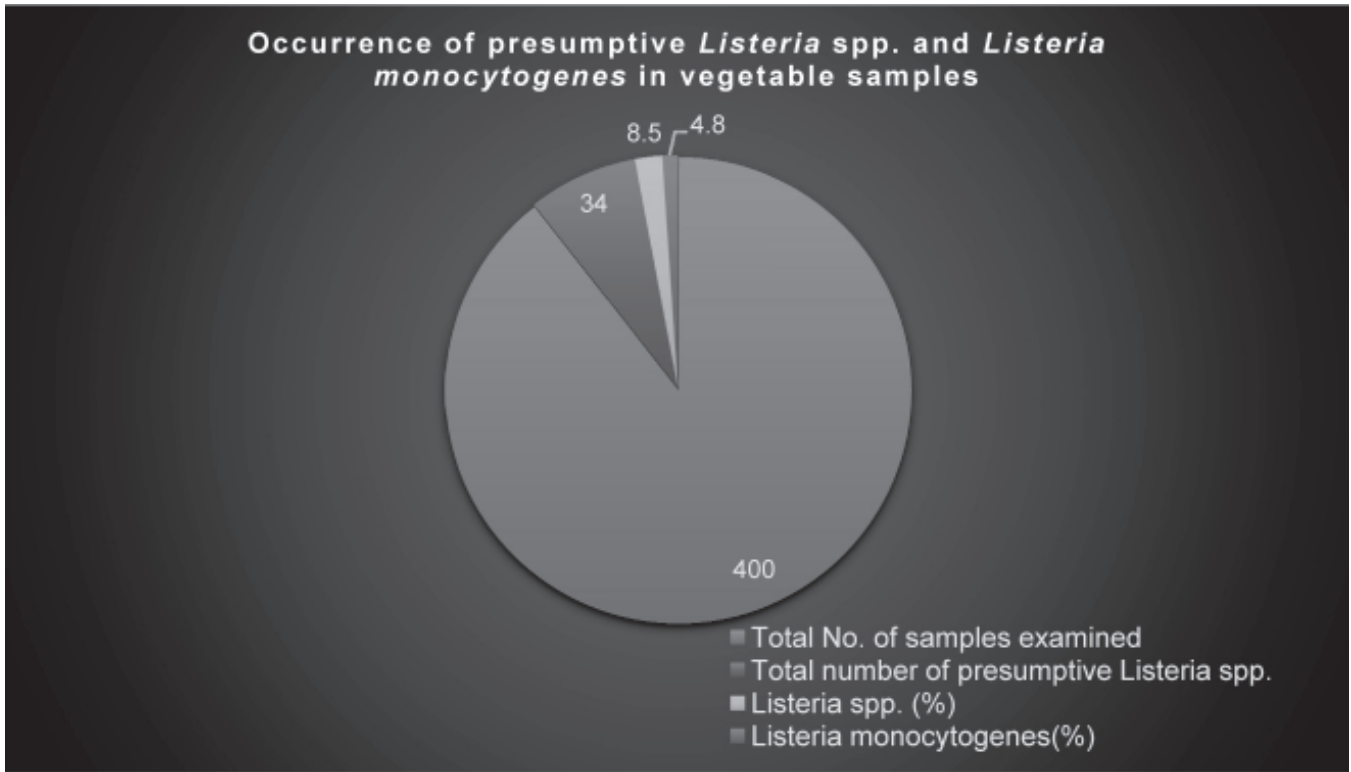


Fig. 1 : Occurrence of presumptive *Listeria* spp. and *Listeria monocytogenes* in vegetable samples.

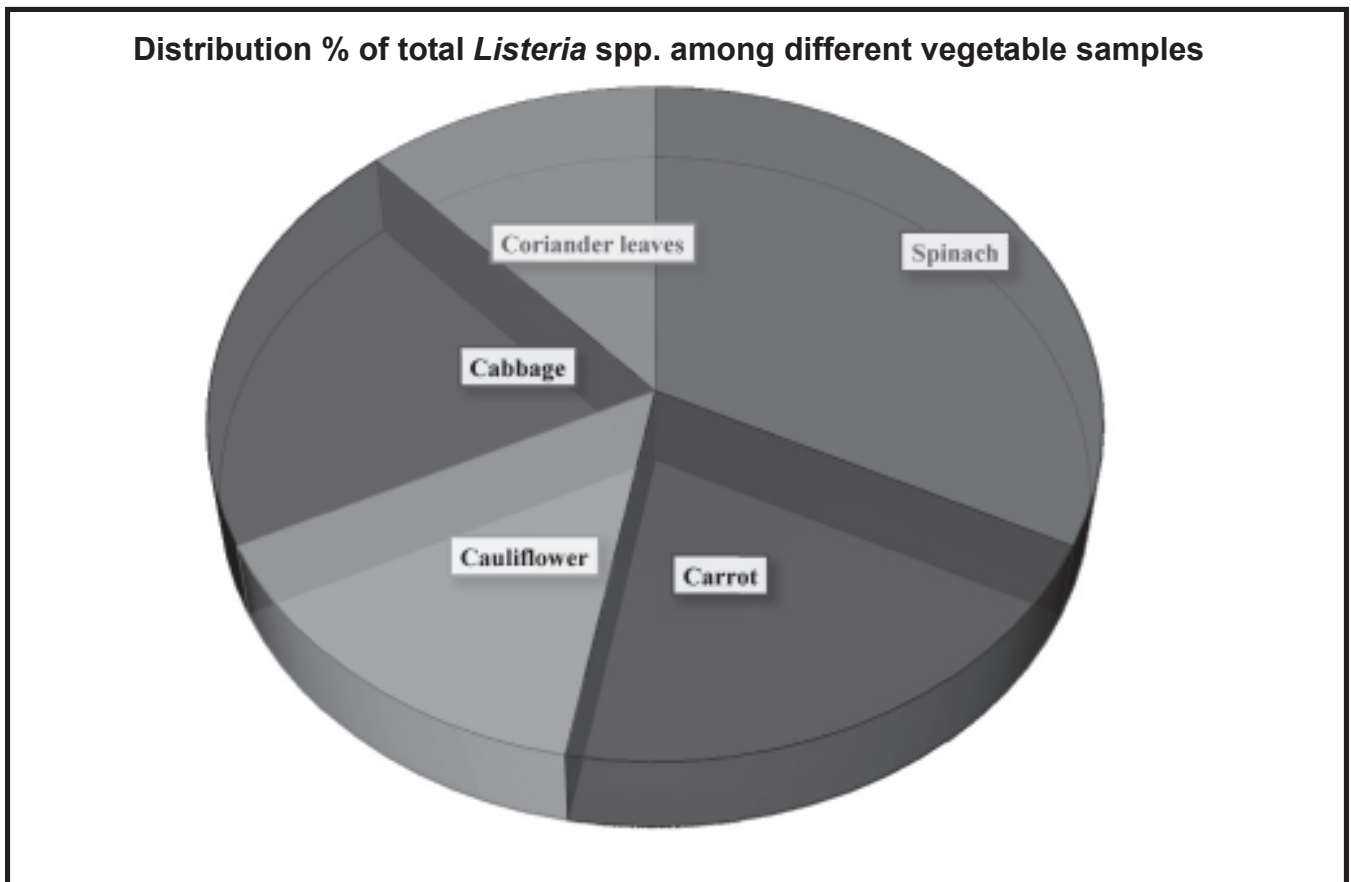


Fig. 2 : Distribution % of total *Listeria* spp. among different vegetable samples

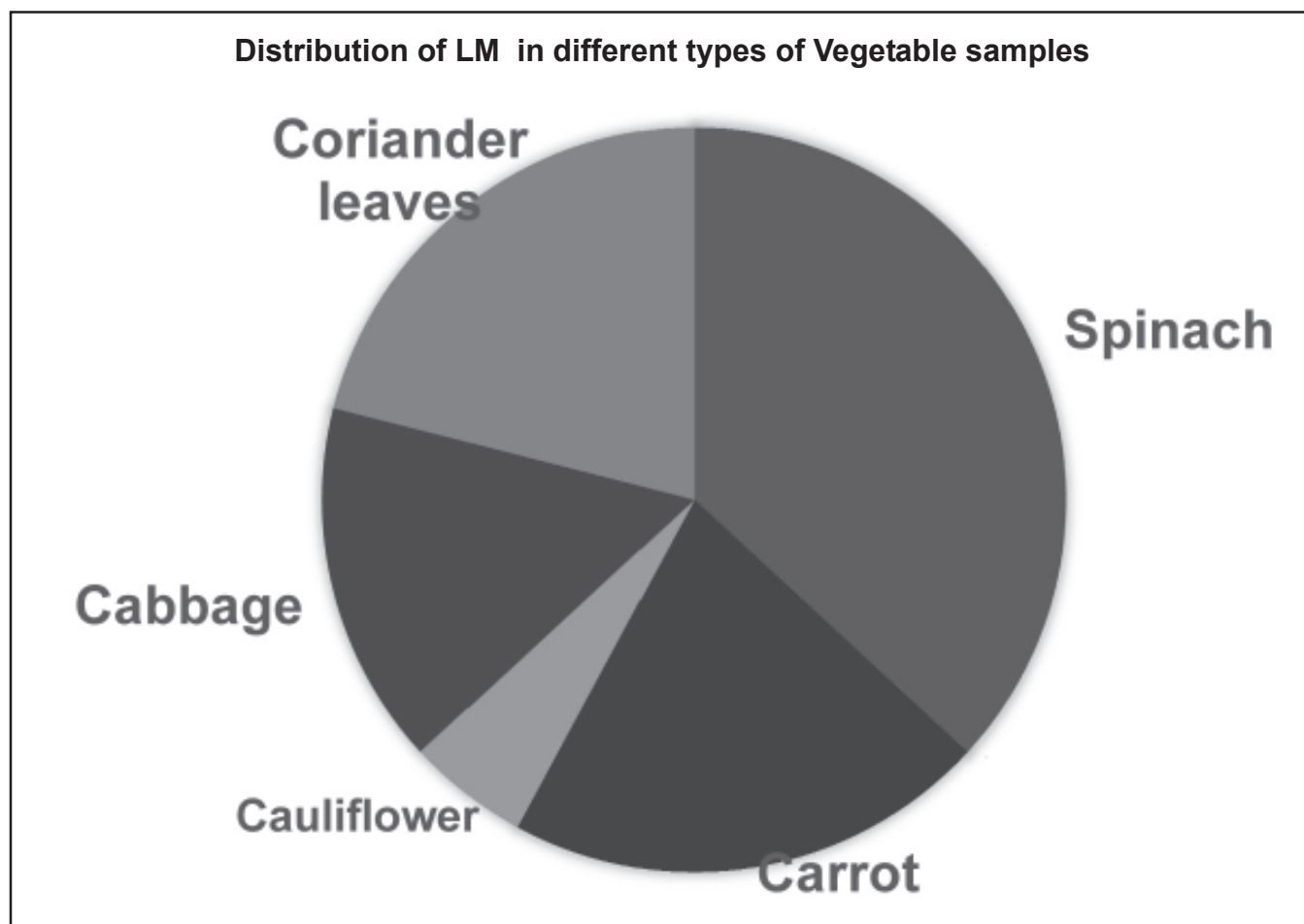


Fig. 3 : Distribution pattern of *Listeria monocytogenes* among vegetable samples

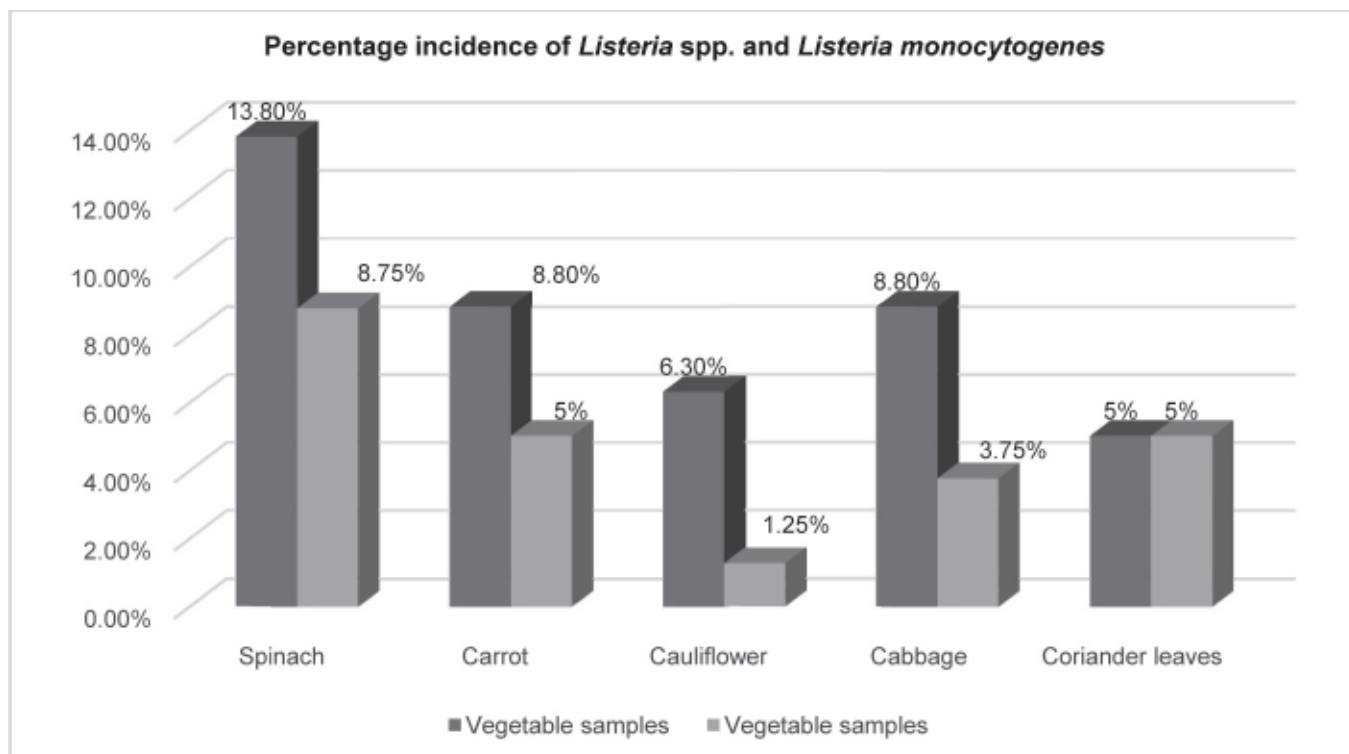


Fig. 4 : Distribution percentage of *Listeria* spp. and *Listeria monocytogenes* among different vegetable samples

vegetables sourced from commercial farms and 7.3% of vegetables sourced from local small-scale farms has been reported<sup>16,28</sup>. An Indian report has recorded a higher incidence of *L. monocytogenes* (10%) from vegetables in Varanasi, India<sup>27</sup>. In a contradictory report<sup>26</sup> a high incidence of *L. monocytogenes* in coriander leaves, cabbage, and spinach 50%, 25%, and 50% respectively, and no incidence in carrots, was recorded in India, as compared to the present findings. In comparison to the present work, a comparable occurrence of *Listeria* spp. (7.7%) among the salad vegetables samples was recorded, and a contrary high frequency of *Listeria monocytogenes* 31% was noted<sup>24</sup>. Survival of *L. monocytogenes* is influenced by the external environment, including contaminated soil and water qualities<sup>13,31</sup>. It is established that the presence of *L.*

*monocytogenes* in vegetable produce was contaminated due to the use of untreated water that may be used for washing, sprinkling fresh produce during hot and dry seasons, its storage in unhygienic areas or conditions, and transportation processes that may attribute it to be prime reasons for the increased level of contamination<sup>2,21</sup>.

From the above study it is concluded that the spinach was found to be highly contaminated (*Listeria* species, and *L. monocytogenes*) followed by cabbage, carrot, coriander and least was in cauliflower, thus the scope of research posing several health concerns to the consumers. These findings are of public health significance based on the fact that *Listeria monocytogenes* is the cause of listeriosis in animals and humans.

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