**Original Research Article**

**Phylogeny of the *Listeria* species obtained from dairy environmental samples**

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| **ABSTRACT:**  **Aims:** To establish the phylogeny of the species of *Listeria* isolated from dairy environmental samples  **Study design:** Isolates of *Listeria* were isolated from dairy environmental samples and subjected for pheno and genotypic identity. Based on genotypic identification, phylogeny of the species of *Listeria* was created based on neighbour joining method.  **Place and Duration of Study:** The study was taken up at the Department of Dairy Microbiology, Dairy Science College, KVAFSU, Bengaluru-85, Karnataka between June 2020 and July 2021.  **Methodology:** Isolation of *Listeria* species were carried out on PALCAM agar. Isolates were maintained on the same agar slants and working cultures were prepared in the broth medium. The isolates were identified through various biochemical tests and confirmed using rRNA sequencing. The neighbour joining method was adopted to establish the phylogeny of all the *Listeria* species obtained from dairy environmental samples  **Results:** A total of 18 numbers of isolates of Listeria were obtained from the dairy  environmental samples such as about 3 isolates from soil, dung, chilled milk each followed by 2 from fodder; feed; swab of udder; can milk and one from urine. The pheno and genotypic identity of *Listeria* revealed as *Listeria ivanovii* (6 nos; 50.0%), followed by  Listeria grayi (5 nos;41.6 %) and Listeria seeligeri (1 no; 8.40%). Phylogenetic analysis through Neighbour joining method revealed that *L. ivanovii* L18, L8, L16 and *L. grayi* L9, L10, L12 were having relatedness about 87% while *L. ivanovii* L15, L7, L17, *L. grayi* L11, L13 and *Listeria seeligeri* L14 had about 92% homology. Blasting the obtained sequences in  NCBI of the *Listeria* isolates revealed that there was a significant genetic correlation between *Listeria* isolate L7, L14, L15, L16 and L17 with NewYork isolate of *isteria*, isolates  L8 and L12 with Nigerian isolate, L9 and L11 with American isolate, L10 with German isolate, L13 with Himachal Pradesh isolate and L18 with Greece isolate.  **Conclusion:** Genetic correlation among *Listeria* isolates were noticed with isolates of NewYork, Nigeria, Germany, Himachal Pradesh and Greece, indicating their sources of origin. |

*Keywords: Listeria spp.; rRNA sequencing; Phylogeny; Neighbour joining method; Dairy environmental samples*

1. INTRODUCTION

The genus Listeria currently includes six species: L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, and L. grayi. Two of these species, L. monocytogenes and L. ivanovii, are potentially pathogenic. The infectious disease caused by these bacteria is known as listeriosis. L. monocytogenes causes serious localized and generalized infections in humans and a variety of other vertebrates, including domesticated and wild birds and mammals. They are widely distributed in nature, psychrotrophs present in vegetation, sewage, human and animal carrier and infect mainly warm-blooded ruminants, causing economic loss. The pathogens produce toxin called Listeriolysin O (LLO), responsible for the cause of disease coded by the gene *hly A* (Chen *et al*., 2017). Listeriosis has emerged as the typical foodborne disease of major public health concern that predominantly affect pregnant women, neonates, elderly or immunocompromised people. It manifests as abortion, septicaemia, meningitis and meningoencephalitis and potentially life threatening because of the mortality rate (20 – 30 per cent) and hospitalization (91 per cent) following infection. The epidemiological data on listeriosis in India available to date, are not adequate for assessing the extent of disease. The disease largely remains undiagnosed because of the lack of a suitable and rapid detection test (Karus et al., 2024)

The isolated typical colonies of *L. monocytogenes* isolated from food products were identified by performing Gram’s staining, catalase reaction, oxidase reaction, tumbling motility at 20–25 °C, methyl red-Voges Proskauer (MR-VP) reactions, CAMP test with *Staphylococcus aureus* in which *Listeria* isolate was streaked perpendicular to a streak of S. aureus on sheep blood agar. Gram positive motile short rods with catalase positive and oxidase negative, MR-VP tests positive and a positive reaction that appeared as an arrow head zone of haemolysis adjacent to the place where the two streak lines come into proximity, haemolysis on sheep blood agar, nitrate reduction and fermentation of sugars (rhamnose, xylose) confirmed the presence of *Listeria* spp. (Mary and Shrinithivihahshini, 2017; Shamloo *et al.* 2019; Sushmita et al., 2023 and Chaithra et al., 2024).

Evolutionary relatedness of species of *Listeria* may help in finding the lineage and source which is possible through construction of dendrograms of phylogenetic tree for them.  In molecular [phylogenetic](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/phylogeny) analysis, the sequence of a common gene or protein can be used to assess the evolutionary relationship of species. By establishing phylogenesis of *Listeria*, the evolutionary relationship of *Listeria* species with another species can be known from different sources. Phylogenesis of *Listeria* was first constructed by Weidmann in 2002. Many phylogenetic works had been carried out on *Listeria* species isolated from different food and environmental samples by using MEGA software and most of the research on phylogeny was carried out for *Listeria monocytogenes*. (Jarvis *et al.,* 2017). All the *L. monocytogenes* isolates from different environmental, food and clinical samples showed similarity with the isolates from the milk product (KF894986.1), in India and sludge and waste water (AJ535697.1) in France. Additionally, most of the isolates from vegetables also showed similarity with those from food (KF588562.1), in Chile and majority of the isolates from water and human clinical samples showed similarity with the isolates obtained from intestine (HM007564.1) in Germany and chicken (KF956739.1) in Turkey. The amplified 16S rDNA fragment for the isolates of *L. monocytogenes* obtained from different samples like humans, vegetable, soil, water and cow milk in Varanasi was through PCR and phylogenetic relatedness was estimated using the neighbour-joining method. (Soni and Dubey 2014). The typical isolates (23) were biochemically confirmed by subjecting to PCR using primers Forward 5′- CTGCTTGAG CGTTCATGTCTCATCCCCC-3 and Reverse 5′ CATGGGTTTCACTCTCCTTCTAC-3. Out of 23 isolates, 14 were confirmed as *Listeria monocytogenes* while nine isolates showed negative reaction. Phylogeny for 14 isolates of *Listeria monocytogenes* obtained was constructed by using UPGMA (unweighted pair group method with arithmetic mean) method in MEGA 6.0 software that indicated *Listeria monocytogenes* from raw milk showed relatedness with *Listeria ivanovii* and *Listeria innocua*. (Mary and Shrinithivihahshini 2017)

The results of amplified 16S rRNA of 13 isolates of *Listeria monocytogenes* obtained from clinical and food samples in Iraqconcluded that the Iraqi isolates of *Listeria monocytogenes* showed that there was a significant genetic correlation and high homology between isolate No.1 with Indian isolate (HM589603.1), isolate No.2 with Indian isolate (HM589597.1), isolate No. 3 with US isolate (M24199.1), isolate No.4 with Indian isolate (HM589599.1), and isolate No.5 with Italian isolate (AF253320.1). (Yousif and Alshamari 2018)

2. material and methods

**2.1 Phenotypic identification of *Listeria* isolates**

Isolates of *Listeria* obtained from dairy environmental samples were propagated in sterile nutrient broth as working cultures and maintained on PALCAM agar slants. The working cultures of the isolates were prepared fresh (18-hour old) whenever required for biochemical tests while stock cultures were subcultured once in 15 days and after growth stored in the refrigerator at 5oC All the isolates were subjected for preliminary identification tests like Gram’s staining, spore staining, catalase test, oxidase test, motility test, methyl red test and nitrate reduction test as per the procedure given by Harrigan (1998). The final species level identification was possible only after conducting specific tests that included acid from ribose and rhamnose, coagulase test, Christie Atkin Munch Peterson (CAMP) test, lecithinase activity, growth in peptone water with 10 per cent NaCl and at 4 °C. The results of biochemical tests obtained for the *Listeria* isolates were compared with identification key developed from Volume 3 of Bergey’s manual of systematic bacteriology (II edition) to place them under the species.

**2.2 Key for the identification of *Listeria* species**

The identification key for *Listeria* species was prepared by referring to Bergey’s manual of systematic bacteriology (II edition) Volume 3 - the firmicutes edited by Ludwig *et al.* (2009).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Biochemical tests** | ***L. monocytogenes*** | ***L. ivanovii*** | ***L. innocua*** | ***L. seeligeri*** | ***L. welshimeri*** | ***L. grayi*** |
| **Specific Tests** | | | | | | |
| Acid from ribose | - | + | - | - | - | + |
| Acid from L-Rhamnose | + | - | d | - | d | + |
| β-haemolysis | + | + | - | + | - | - |
| CAMP test | + | - | - | + | - | - |
| Coagulase test | + | + | + | + | + | + |
| Lecithinase activity | + | + | - | D | - | - |
| Growth in peptone water with 10 per cent NaCl | + | + | + | + | + | + |
| Growth at 4 °C | + | + | + | + | + | + |

**Note:**

\* d – variable

* Among the preliminary tests, all the *Listeria* species were Gram positive motile short non-spore forming rods with catalase and methyl red tests positive while oxidase negative, nitrate reduced by all species except *L.grayi*

**2.3 Genotyping of obtained *Listeria* isolates**

All the obtained isolates of *Listeria* spp. were genotyped. The DNA extraction of the isolates were carried out by using centrifugation and further subjected to PCR for 16s rRNA sequencing as per the procedure prescribed by Wachiralurpan *et al.* (2016). Polymerase Chain Reaction was done for amplification of DNA copies. Forward and reverse primers of about 5 µl was diluted 10 times by using 45 µl biological grade water. All the ingredients like sterile milliQ water (12.0 µl), 2X PCR master mix (12.5 µl), forward primer (0.5 µl) 27F AGAGTTTTGATCCTGGCTCAG and reverse primer (0.5 µl) 1492R GGTTACCTTGTTACGACTT were mixed together in the above order in an eppendorf tube using a vertex mixer. Accurately 1.0 µl of DNA was added to the above 25.5 µl mixture and transferred to thermocycler to perform PCR and set up the PCR program

|  |  |  |
| --- | --- | --- |
| **Step** | **Temperature (°C)** | **Time** |
| Denaturation | 95 | 1 min |
| Annealing | 58.5 | 30 sec |
| Extension | 72 | 30 sec |

After completion of PCR, to confirm the amplification of DNA, agarose gel electrophoresis was performed by using 2 per cent agarose gel at 80 V for 1 h as per the standard procedure. After obtaining PCR product, it was outsourced for gene sequencing at Barcode scientific, Whitefield, Bengaluru. The gene sequence obtained was blasted in NCBI (http://www.ncbi.nlm. nih.gov) and final identity was obtained. The identified culture was submitted for accession number in NCBI website (http://www.ncbi.nlm. nih.gov).

**2.4 Establishment of phylogeny of obtained *Listeria* isolates**

Phylogeny was constructed to know the evolutionary development of the organism by using the obtained gene sequence of the isolate after outsourcing of DNA sequence. MEGA 10X software was used to construct phylogenesis by using Neighbour joining method and Unweighted Pair Group Method with Arithmetic mean (UPGMA) and the obtained tree was analyzed. Relatedness among the strains of *Listeria* was given by the per cent. (

[www.megasoftware.net).](http://www.megasoftware.net).)

3. results and discussion

Both pheno and genotypic characterization were carried out for 12 Listeria isolates as well based on genotypic identity of the isolates, phylogeny was determined

**3.1 Phenotypic characterization of *Listeria* isolates**

A total of 12 *Listeria* isolates were obtained from various dairy environmental samples comprosed 3 isolates from chilled milk; 2 from fodder; feed; swab of udder; can milk and one from urine through pour plate technique using PALCAM agar medium (Table 1). These isolates, when subjected for preliminary tests revealed as Gram positive, catalase positive, oxidase negative, methyl red positive motile rods. Among them 6 *Listeria* isolates (L7, L8, L15, L16, L17, L18) reduced nitrate, produced acid from ribose, no acid from rhamnose, haemolysin positive, lecithinase positive, coagulase positive, CAMP test negative with *Staphylococcus aureus* and showed growth at 4°C and in 10 percent NaCl, L14 was positive for CAMP test with *Staphylococcus aureus* whereas 5 isolates of *Listeria* (L9, L10, L11, L12, L13 ) did not reduce the nitrate, produced acid from ribose as well from rhmnose, haemolysin negative, lecithinase negative, coagulase positive, CAMP test negative with *Staphylococcus aureus* and showed growth at 4ºC and in 10 percent NaCl. Based on the results obtained, after comparing with identification key (2.2), the species identified were 6 isolates as *Listeria ivanovii* at 50% predomination followed by 5 isolates as *Listeria grayi* (41.60%) and 1 isolate as *Listeria seeligeri* (8.40%) obtained from dairy environmental samples (Table 2).

**Table 1: Number of *Listeria* isolates obtained from dairy environmental samples**

|  |  |  |
| --- | --- | --- |
| **Name of the Dairy environmental sample** | **Codes of *Listeria* isolates** | **Number of isolates obtained** |
| Fodder | L7, L8 | 2 |
| Feed | L9, L10 | 2 |
| Urine | L11 | 1 |
| Swab of udder | L12, L13 | 2 |
| Can milk | L14, L15 | 2 |
| Chilled milk | L16, L17, L18 | 3 |
| Total | | 12 |

**Table 2: Phenotypic identification of *Listeria* spp. isolated from dairy environmental samples**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Codes of *Listeria* isolates**  **(Total number of isolates)** | **Specific biochemical tests carried out** | | | | | | | | | **Identity** |
| **Nitrate reduction** | **Acid from ribose** | **Acid from**  **L-Rhamnose** | **Haemolysis** | **Lecithinase activity** | **Coagulase test** | **CAMP test** | **Growth** | |
| **4** **ºC** | **10 % NaCl** |
| L7, L8, L15, L16, L17, L18  (6 nos.) | - | + |  | + | + | + | - | + | + | *Listeria ivanovii* |
| L14  (1 no.) | - | + |  | + | + | + | + | + | + | *L. seeligeri* |
| L9, L10, L11, L12, L13  (5 Nos.) | + | + |  | - | - | + | - | + | + | *L. grayi* |

**Note:**

* The results mentioned in the table are confirmed after 3 replications
* All the 12 isolates were Gram positive, non-spore forming rods, motile, catalase positive, oxidase negative and methyl red positive

Nayak *et al.* (2015) found that out of 200, 18 samples of milk and milk products from Navsari, Gujrat were found positive for *Listeria* spp*.* which were identified as *Listeria   
seeligeri* (6, 33.3 percent), *Listeria innocua* (5, 27.7 percent), *Listeria welshimeri* (4, 22.2 percent), and *L. monocytogenes* (3, 16.6 percent). Matto *et al.* (2018) collected dairy farm samples like soil, dung, water, bulk milk to study the prevalence of *Listeria* species. They isolated only one isolate of *L. monocytogenes* from pasture. No isolation of *Listeria* spp. was retrieved from the bulk tank milk or drinking water from of the farms. *Listeria innocua* was detected in 13 feedstuffs and seven samples of soil from the entry and exit points of the milking parlour. EL-Naenaeey *et al.* (2019) determined the overall isolation rate of *Listeria* spp. as 16 per cent in feaces of dairy cows; 8 per cent *Listeria monocytogenes* from normal milk and mastitis milk; 4 percent and 2 percent in feaces of dairy cows, normal milk and mastitis milk, respectively. The prevalence of *L. ivanovii* and *L. welshimeri* in milk of dairy cows were 6 percent and 4 percent, respectively. Among dairy environmental samples, soil, fodder, dung, feed while swab of udder, urine of milch animal, can milk and chilled milk showed the counts of listeria onPALCAM agar. A total of 18 number of isolates of listeria were obtained from the dairy environmental samples of KVAFSU, Bengaluru. Sushmita et al., 2023

The phenotypic identity of the 23 isolates of *Listeria* obtained from environmental samples of university dairy farm in Bengaluru was declared as *Listeria monocytogenes* (56.50%) followed by*L. innocua* (17.39%**)**, *L. ivanovii* (8.70%); *L. seeligeri*  (4.35%) and 13.06 % of unidentified  *Listeria* spp. Chaitra et al (2024)

**3.2 Genotypic characterization of *Listeria* isolates**

The DNA was extracted from allthe isolates of *Listeria* grown in PALCAM broth and further amplified by PCR. The PCR product was outsourced for sequencing. Based on the gene sequence obtained through nucleotide blast at the website www.ncbi.com (Fig 1 to Fig 12) the isolates were confirmed as *Listeria ivanovii* (L7, L8, L15, L16, L17, L18), *Listeria seeligeri* (L14) and *Listeria grayi* (L9, L10, L11, L12, L13) which was matching with phenotypic identity. After confirmation of genotypic identification, the accession numbers were obtained from NCBI. The accession numbers obtained for *Listeria ivanovii* were L7 - KP01000451.1, L8 – MW386242.1, L15 - KP01000447.1, L16 - KP01000445.1, L17 - KP01000434.1 and L18 - MW386243.1 respectively and MW466720 for *Listeria seeligeri* L14 while *Listeria grayi* accession numbers were L9 - AY643840.1, L10 - M80352.1, L11 – AY643839.1, L12 - MW020239.1 and L13 – MW386232.1 respectively (Table 3).

**Base Pairs: 1 – 809 F** CTGCAGATAAAATGATTTCTCACTTTGTCTACAGTCTGACAGCTACTAAGATTAGTAGCTGTTCGAAAAGCCAAAGTAGAAAGTTGCTTTGGTTTTTTATTTGCTTAAGAAAGCAGCCATTCGGTCTAGTGCTTCTGCTAGTTTATTAAAAGAAGTGGCATAAGAAAGTCGGAAATAACGGTCGCCTTTTTCTGAAAAAGCATTGCCTGGAACTACTGCAACTTTTGCTTCTTCTGCAAGCTTGACAGCCCAGTCAAAAGAATTTTCGGTAATCTCATCTGGCAGTTTTACGAAGAAATAAAAAGCGCCATCAGGTGGAACAACCGTGAATCCCATTTTTTCTAAACGATCTTGTGTGAAGTTTGCTCTAGTTTTATATTCGGTACGCATTTGAAAAGCATCATCCTTGCCATTTGTGATGGCTTCTAAAGCTGCTTTTTGAGAAATAGAGCTCGCGCAAGTAACGGAATATTGATGGATTTTCAACATTTGTTTGGTGATTATTTCAGGTGCAAGCAGAAAACCAATTCGCCAGCCAATCATCGCGTGGGATTTGGATAGGCCGTTAATCACGATTGTTTGTTCTCGAAGCATAGGAGCGATACTAGCATGTTCTTCGTGATAGATTAATTCGCTATAGATTTCATCAGCAATAACAAAAATACCAGCTTCTCTTAAAACATTTGCTAAATCCACCAGTTCTTCTTTTGTTAAACTAACACCAGTTGGATTGGAAGGGTAAGGAATAATAAGCGCTTTTGTTTTAGGTGTAATGTGTTTCCGTAATTGCGTGGGGGTTAATTTGAAAT

**Fig 1: 16S rRNA sequence of *Listeria* *ivanovii* L7 (ACNO - KP01000451.1)**

**Base Pairs: 1 – 698 F** ACAGAGTGCTAGTGTTGGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGATAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGAAA

**Fig 2: 16S rRNA sequence of *Listeria* *ivanovii* L8 (ACNO - MW386242.1)**

**Base Pairs: 1 – 766 F**

CATTGTAAACGCCATCCACATTGTTCTTCGCCATTAAAATCACGTCTGCTTCAATTTCTGCTGCCCGAAGCAGCTGCAGTTGTATCTGTCGAGAAATATGGATTTCCCGTACCACCAGCGAAGATTACGACACGTCCTTTTTCCAAGTGTCTGATTGCTTTCCGGCGAATATAAGGTTCAGCGATTTGGCGCATATCGATAGAAGTTTGTACACGTGTTGCTACCCCAATATTTTCCAAGGAATCTTGAAGAGATAAGGAATTCATGACCGTTGCAAGCATTCCCATATAATCTGCTGCTGCACGATCAAGGTCAATCTTACCGGTCAGTTCTTAGGTCTTAAATATGTGCTACCTATTTTAACGAAACAAGGAAATGGAAGCGTTATCAACACGGCTTCTGTGGCCGGACTTGATGGCAGTTCCTTTTTAGCGCCATATGTGGCTTCAAAACACGGCGTCAGTGGTCTGACAAAAGTCCGCAGCACTAGAAGTAGCGGATAAAGGTGTTCGGGTCAACTCCGTCCATCCATCACCAGTCAATACCCGGATGATGCGATCGATCGAAAAGAATCTCAACCCAGACGATGCGGAAAAAGCAAAAGAAGAATTTACAAAAGATATTCCAGTCGGAAGATATGCAGAAGCCAGCGATGTCGCGAAACTTGTCTTATTCCTAGCCTCGGACGATAGCAAATTTATCACTGGTGCGCAATACGGGTAGATGGCGGTATGGGGGCTACACAATAAAATTAAAAATAAAGATC

**Fig 3: 16S rRNA sequence of *Listeria* *grayi* L9 (ACNO - AY643840.1)**

**Base Pairs: 1 – 773 F**

AAAAAAGCAACGATCGTTTCCGCTGCTGGAATAGCTGTCACAGCATTCGCTGCTCCATCGGTTGTCTCAGCAAATACAGTGGTTGTCGCATCTGGTGATACACTTTGGGGGATCGCTTCCAAAACTGGTACTACCGTTGACCAACTAAAACAACTCAATAAACTTGACTCTGATAGAATCGTACCGGGACAAAAATTAACAATCAAAGAAGTAGCTGCTCAAAAAGTAGAAAAATCTGTTAGTGCAACATGGCTGAATGTTCGTCATGCTCCTGACGCAAATGAAAAAATACTTACTTCCCTAAAAGGCCGTACAGTTGTCAAAGTAGAAAGTTCTGAAGCTAATGGTTGGAACAAGATCTCTTTTGACAATGGTAAAACTGGTTATGTAAACGGAAAATACTTATCTGACGCAAAAGTCGCTGCACCTGTCGTTACGAAAGCAGTGACCCACAAAGCAGAAGCAAAAGTCGCTGCTACTTCGACACACGCAGTTAAAGTGGATACGAACGCTTCTACTTACAAAGTAAAAAGCGGTGATACGATCTGGGCTTTATCTGTCAAATATGGCGTTCCTGTTCAAAAATTGATCGAATGGAATAATCTTTCTTCTTCTTCGATCTATGTTGGTCAAACGATCGCAGTAAAAGAAGCGGCTGCTAAAGCTGCTCCAACAACTGTAAAACAAGCCGCTCCTGCTAAAGTCGCTCCGAAACAAGAAGTGAAGCAAACAGCACCGGCTAAACAAGAACAAGCAAAACCAGCTGCTAAA

**Fig 4: 16S rRNA sequence of *Listeria* *grayi* L10 (ACNO - M80352.1)**

**Base Pairs: 1 – 762 F**

TTGTAAACGCCATCCACATTGTTCTTCGCCATTAAAATCACGTCTGCTTCAATTTCTGCTGCCCGAAGCAGCTGCAGTTGTATCTGTCGAGAAATATGGATTTCCCGTACCACCAGCGAAGATTACGACACGTCCTTTTTCCAAGTGTCTGATTGCTTTCCGGCGAATATAAGGTTCAGCGATTTGGCGCATATCGATAGAAGTTTGTACACGTGTTGCTACCCCAATATTTTCCAAGGAATCTTGAAGAGATAAGGAATTCATGACCGTTGCAAGCATTCCCATATAATCTGCTGCTGCACGATCAAGGTCAATCTTACCGGTCAGTTCTTAGGTCTTAAATATGTGCTACCTATTTTAACGAAACAAGGAAATGGAAGCGTTATCAACACGGCTTCTGTGGCCGGACTTGATGGCAGTTCCTTTTTAGCGCCATATGTGGCTTCAAAACACGGCGTCAGTGGTCTGACAAAAGTCCGCAGCACTAGAAGTAGCGGATAAAGGTGTTCGGGTCAACTCCGTCCATCCATCACCAGTCAATACCCGGATGATGCGATCGATCGAAAAGAATCTCAACCCAGACGATGCGGAAAAAGCAAAAGAAGAATTTACAAAAGATATTCCAGTCGGAAGATATGCAGAAGCCAGCGATGTCGCGAAACTTGTCTTATTCCTAGCCTCGGACGATAGCAAATTTATCACTGGTGCGCAATACGGGTAGATGGCGGTATGGGGGCTACACAATAAAATTAAAAATAAAGA

**Fig 5: 16S rRNA sequence of *Listeria* *grayi* L11 (ACNO - AY643839.1)**

**Base Pairs: 1 – 719 F**

TAAAGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAATGACCTTAGGAGCTTGCTCCTTTGGTCGTTAGTGGCGGACGGGTGAGTAACACGTGGCAACCTGCCTGTAAGATTGGGATAACTCCGGGAAACCGGAGCTAATACCGAATAATAATCACTCCGCATGGAGCAGGTTTGAAAGGCGGCTTCGGCTGTCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGTGTGAAGAAGGTTTTCGGATCGTAAAGCACTGTTGTTAGAGAAGAACAAGGATAAGAGTAACTGCTTGTCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCCGGTAATACGTAGGTGGCAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTTAACCGGGGGGTCATTGGAAACTGGGAGACTTAGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGA

**Fig 6: 16S rRNA sequence of *Listeria* *grayi* L12 (ACNO - MW020239.1)**

**Base Pairs: 1 – 701 F**

AAAAGAATGCTAAGTGTTGGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA

GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGATAGATGATTGGGGTGAAGTCGTAACAAGGGTAACCAGAA

**Fig 7: 16S rRNA sequence of *Listeria* *grayi* L13 (MW386232.1)**

**Base Pairs: 1-881 F**

GTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACGGAGGAAGAGCTTGCTCTTCCAAAGTTAGTGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGTTGGGGATAACTCCGGGAAACCGGGGCTAATACCGAATGATAAGGAGTGACGCATGTCACTGCTTTGAAAGATGGTTTCGGCTATCGCTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGTATGAAGAAGGTTTTCGGATCGTAAAGTACTGTTGTTAGAGAAGAACAAGGATAAGAGTAACTGCTTGTCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGCGCGCAGGCGGTCTTTTAAGTCTGATGTGAAAGCCCCCGGCTTAACCGGGGAGGGTCATTGGAAACTGGAAGACTGGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGATATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCC

**Fig 8: 16S rRNA sequence of *Listeria* *seeligeri* L14 (ACNO – MW466720)**

**Base Pairs: 1- 812 F**

TGTTTAACCTTTGAGAACTTCCGTTTTTCTACAGTCTGAGACGTTTTCTAGTTAGCCTAGAAAACGTCTTTTGTTATCATTGAGTGATAGTGGGTCTTTTTTTCACACAATTATACAATTCTTTATTATGAATAACCCAACAATTATCCACTTTCTCGATTTTACCTTGCTCTGTTAAAATCGCAAGTTGTTTATAAAAACTACTCTTGGAAAGACCACTATATGCAGCGATAATTTTTGTCCGTATTTCTTTAGGAAGTATCACGCTATTTGTACCATCATAGTTCCGTGGAATTTCGCAATATTAGCAAAAGAAAGCTCTAATTTACCAGCGGGAATACAATCACTAAGCAGACTTTTGAAAAAAGAATGCCTCGTTAGTTCCTTCATTATGAAAAATTGAAAACCGAAATTTTCAGGAAACATGGAAAGGAAATACTCTAAATCTTTAAAGGAATATTTATAAACCACGCATTCGGAAATAGTTTTGAAAGTTAATTTTTGCATATTTTCTTCTAATAAACTGTAATAATTAACAAATAAGCCTTTACCTAAAATAGTATAAATATTAGGTGAATCTGGACTTAACGATGTGGAAACATATCCATCAATAATTAGATAAACATGTTCTTTGGTAAGCCCTTCTTCATCAATTAATTGTGTACGGTTAGGAATAGTAATTTTTTCAAAGCTAATATTACCTTTTTGAGAGAGTTGGATGAATTCCTGATAATTAAATATCGTATTCATTTCTGAGGAATCCTTGCATTTTTATTTGTCTTACTAAGAATGTGTAACAAGTTTCCCTTCTC

**Fig 9: 16S rRNA sequence of *Listeria* *ivanovii* L15 (ACNO - KP01000447.1)**

**Base Pairs: 1- 799 F**

AGGGGGGAGAATGAAAATGTTTTGCTTGTTGTTAGCTTATGGTTATATATTAACCAACTAAAGAAAGTTTCCCCTGTGCATTTGATTAGAAAATAGTAAGAAATCTATTTATTTCTTTTAATAAATAAAAAAAGCAACATGCCTAGGATTTCCCCAAAACACATCGCTTCGCCTCATTAATCTTCAAAATTATAAAGCGGTGTACTTAAGTAGCGTTCACCATTACTTGCAACGATAGCTAGAACTTTTTTTCCTGCTCCAAGTTCTTTAGCTAAATCAAGTGCAGCTTTAATTGTTGCTCCTGAAGAAATACCAACCAGAATACCTTCTTTTTTGGCTACTTCACGCGCTGTTTCTAAAGCATCTTCACTAGAAACTTTTAAAATACCATCATAGACTTTTGTATCTAGTGTATCCGGGACAAAGCCAGCACCAATTCCTTGGATTTTATGTGGGGATGGGGATCCACCACTAAGTACTGGAGATTCTTCCGGCTCTAGTGCGTAAATTTTTACATCAGGATAATTTTTCTTAAGTACATGTCCTACACCGGTTACTGTTCCACCGGTTCCAACGCCTGCGATAAAAGCATCCAGACCATCTTTACCGAACGCTTCGACGATTTCTGGACCAGTTGTTTCTTCATGAACAGCTGGATTTGCTGGATTATGGAATTGTTGTGGAACAAAATAATTATTTTCTTTCGCTAACTCCTCTGCCTTGGCAATAGCGCCTTTCATACCGTCTGGTCCTGGCGTTAATACTAATTTCGCGCCATATGCTTGAAGTAATTTGCGAC

**Fig 10: 16S rRNA sequence of *Listeria* *ivanovii* L16 (ACNO - KP01000445.1)**

**Base Pairs: 1- 802 F**

AGATAATAATTACTCCGATGCTACCATATGTTGCGGAGTAATTAGCGAAATTATTGACATAGAAAGCAAATCCGGCTGATGCACTGGTCCAACCCACTGTTGAAAATATCGCACCTGGAAGTACACTTATTAATGTACTGCGCCGATTTGGAGCCACCCAATATAAAAAAGTAAATACGACAAAAATAACAATCAATGTAACCGTCCACCGAAGATTATTCCAAAAGCCCAGAAAATCTTCTGAGAAATTCAAATGGTTAGTTAAAAATAAGCCAATTTGTTGTCCAAAAACAATTAAGAGCAACGTTACTCCAACAGTTGCGAGCATGGCAATCGTAAAAAGCATTGATAATAAGCGCTGCATCACATAGTTGCGTTTATTCGTTACACCATAGGCTTTATTGAGCGATTTCATAACGGCATTCATACCATTAGATGCTGACCATAAAGTCGCAATGATCCCGATAGATAACAATCCTCCATTTTTCTGGGTTAAAAGCGTGTTTAAATTTTCTTCTAAAAAGTCTAAAATCTGTTCTGGAGCAAACTCTTTTAGCATATTAAAAACAGAATCTTTATCAATATGCATGTAAGCAAGTAGTGTTGCTGCAATTAACATCATCGGAAATATCGAAAACAACATATAATAGGCTAATTGTGCTGCGTTTCCAGATACATCATTTCGGCCGATTCTCCCGCTTACCGTTTGGCCAACTTGAAAAACACTACTATGCTTTACATATTTTAATGCTTTTTTCCACACGTATACTGCCCCTTTCCTAGTCATTCGTCTGGAAGTTTT

**Fig 11: 16S rRNA sequence of *Listeria* *ivanovii* L17 (ACNO - KP01000434.1)**

**Base Pairs: 1-699 F**

ACAGAATGCTAGTGTTGGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGATAGATGATTGGGGTGAAGTCGTAACAAGGGTAACCAGAC

**Fig 12: 16S rRNA sequence of *Listeria* *ivanovii* L18 (ACNO - MW386243.1)**

**Table 3: Genotypic identification and Accession numbers of *Listeria* isolated from**

**dairy environmental samples**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No** | **Code of isolate** | **Genotypic identity** | **Source** | **Accession number** | **Data base**  **(Type)** |
| 1 | L7 | *Listeria ivanovii* | Fodder | KP01000451.1 | GenBank  (Direct submissions) |
| 2 | L8 | *Listeria ivanovii* | MW386242.1 |
| 3 | L9 | *Listeria grayi* | Feed | AY643840.1 | EMBL  (Patents - Nucleotide) |
| 4 | L10 | *Listeria grayi* | M80352.1 | GenBank  (GSDB Direct submissions) |
| 5 | L11 | *Listeria grayi* | Urine | AY643839.1 | EMBL  (Patents - Nucleotide) |
| 6 | L12 | *Listeria grayi* | Swab of udder | MW020239.1 | GenBank  (Direct submissions) |
| 7 | L13 | *Listeria grayi* | MW386232.1 |
| 8 | L14 | *Listeria seeligeri* | Can milk | MW466720 |
| 9 | L15 | *Listeria ivanovii* | KP01000447.1 |
| 10 | L16 | *Listeria ivanovii* | Chilled milk | KP01000445.1 |
| 11 | L17 | *Listeria ivanovii* | KP01000434.1 |
| 12 | L18 | *Listeria ivanovii* | MW386243.1 |

On par to the present study, Soni and Dubey (2014) who also confirmed 16S rDNA fragment for 80 isolates of *Listeria* spp. obtained from different samples like humans, vegetable, soil, water and cow milk in Varanasi by performing PCR and used bacterial universal primers 27F (50 AGAGTTTGATCMTGGCTCAG-30) and 1492R (50-GGTTA CCTTGTTAC GACTT-30) and identified them as *Listeria monocytogenes*. Six isolates of *Listeria* spp.isolated from meat samples in Egypt were characterized genotypically by performing PCR to amplify the 16S rDNA fragmentto detect *hlyA* gene encoding listeriolysin O (LLO) by using universal bacterial primers LMA: CGGAGGT TCCGCAAAAGATG and LMB: CCTCCAGAGTGATCGATGTT and confirmed them as *Listeria monocytogenes* (Mohamed *et al.,* 2016). Mary and Shrinithivihahshini (2017) characterized 23 typical isolates of *Listeria* spp. identified from milk and milk products in Tamil Nadu. These typical isolates were biochemically confirmed by subjecting to PCR using primers Forward 5′- CTGCTTGAG CGTTCATGTCTCATCCCCC-3 and Reverse 5′ CATGGGTTTCACTCTCCTTCTAC-3. Out of 23 isolates, 14 were confirmed as *Listeria monocytogenes* while nine isolates showed negative reaction. 13 isolates of *Listeria* spp. were genotyped obtained from different food samples chicken, meat and cheese and clinical samples in Iraq by using primers F AGGGGTGGCA AACGGTATTT and R CATCCGCGTGTTTCTTTTCGA encoding *hlyA* gene and identified them as *Listeria monocytogenes* (Yousif and Alshamari, 2018).

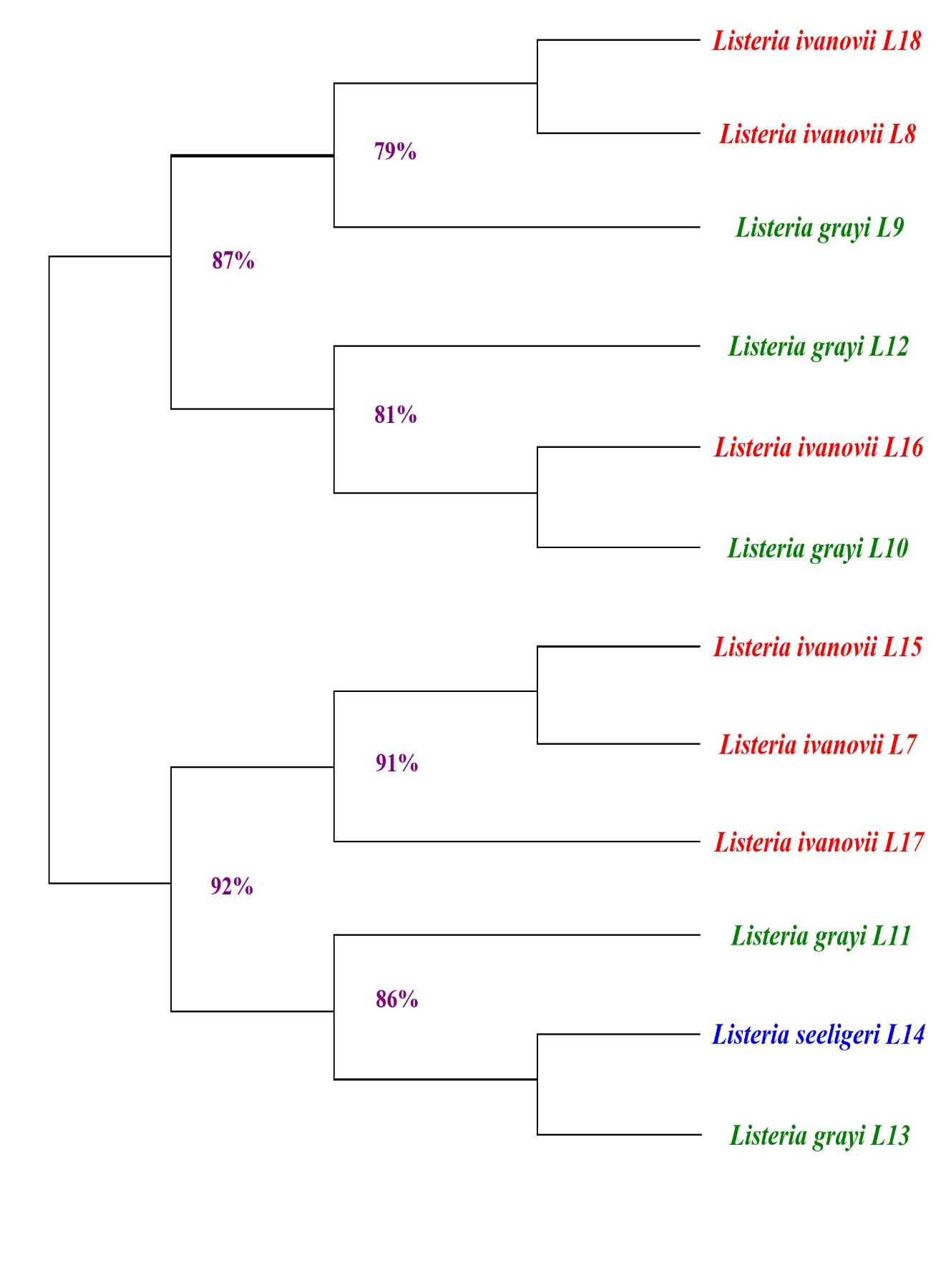
**3.3 Phylogeny of the isolates of *Listeria* obtained from dairy environmental samples**

The DNA sequences obtained for 12 *Listeria* isolates were used to construct phylogeny based on Neighbour joining as well UPGMA (unweighted pair group method with arithmetic mean)methods in MEGA 10X software (

[www.megasoftware.net).](http://www.megasoftware.net).)

The phylogenetic analysis with Neighbour joining method revealed that *Listeria ivanovii* L18, L8 and *Listeria grayi* L9 had about 79 per cent homology and correlation with each other whereas *Listeria grayi* L12, *Listeria ivanovii* L16 and *Listeria grayi* L10 revealed about 81 per cent correlation. All the above six isolates had about 87 per cent relatedness with each other. The relatedness among *Listeria ivanovii* L15, L7, L17 was 91 per cent while *Listeria grayi* L11, *Listeria seeligeri* L14 and *Listeria grayi* L13 related to each other with 86 per cent and all six isolates related with 92 per cent homology. In UPGMA method, the genetic correlation and homology was about 79 per cent for *Listeria ivanovii* L18, L8 and *Listeria grayi* L9 while *Listeria ivanovii* L17, *Listeria seeligeri* L14 and *Listeria grayi* L13 had about 91 per cent correlation, all six isolates were related with 87 per cent homology. The homology among *Listeria ivanovii* L16; *Listeria grayi* L12 and L10 was 88 per cent while *Listeria grayi* L11, *Listeria ivanovii* L15, L7 were 86 per cent correlated to each other. All the 12 isolates were related with 91 per cent homology and relatedness (Fig 13).

Blasting the sequences with those in database in NCBI of the *Listeria* isolates obtained from university dairy farm, Bengaluru showed that there was a significant genetic correlation between *Listeria* isolate L7, L14, L15, L16 and L17 with NewYork isolate of *Listeria*, isolates L8 and L12 with Nigerian isolate, L9 and L11 with American isolate, L10 with German isolate, L13 with Himachal Pradesh isolate and L18 with Greece isolate. Few strains of *Listeria* showed variation which was not comparable with both the methods used in phylogeny, only relatedness among the species of *Listeria* and their strains was depicted for neighbour joining method (Table 4).

**Fig 13: Phylogenetic tree of *Listeria* isolates obtained from dairy environmental**

**samples using Neighbour joining m**

**Table 4: *Listeria* isolates obtained in the present study with the available ones in Gene**

**Bank from different country**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No** | **Code of isolate** | **Genotypic identity** | **Accession number** | **% relatedness based on Neighbour joining method** | **Country** |
| 1 | L18 | *Listeria ivanovii* | MW386243.1 | 79 | Greece |
| 2 | L8 | *Listeria ivanovii* | MW386242.1 | Nigeria |
| 3 | L9 | *Listeria grayi* | AY643840.1 | USA |
| 4 | L12 | *Listeria grayi* | MW020239.1 | 81 | Nigeria |
| 5 | L16 | *Listeria ivanovii* | KP01000445.1 | NewYork |
| 6 | L10 | *Listeria grayi* | M80352.1 | Germany |
| 7 | L15 | *Listeria ivanovii* | KP01000447.1 | 91 | NewYork |
| 8 | L7 | *Listeria ivanovii* | KP01000451.1 | NewYork |
| 9 | L17 | *Listeria ivanovii* | KP01000434.1 | NewYork |
| 10 | L11 | *Listeria grayi* | AY643839.1 | 86 | USA |
| 11 | L14 | *Listeria seeligeri* | MW466720 | NewYork |
| 12 | L13 | *Listeria grayi* | MW386232.1 | Himachal Pradesh |

Normally researchers used either neighbour joining or UPGMA methods to determine the relatedness but in the present study in order to compare, 2 methods had been used, that indicated the distribution of strains of *Listeria* showed little variation with respect to relatedness but on the whole neighbour joining method showed 91 per cent relatedness followed by 89 per cent in UPGMA method for the species of *Listeria* and their strains. Though the strains were obtained from the same source, the relatedness varied indicating the contamination from one source to another source.

Many researchers carried out phylogeny for *Listeria monocytogenes* hence those studies have been quoted here.On par to the present study,Soni and Dubey (2014) also constructed phylogeny for 80 isolates of *Listeria* spp. using MEGA 4.0isolated from humans, vegetable, soil, water and cow milk samples in Varanasi by using neighbour joining method. The data revealed that the isolates recovered showed high level of 16S rRNA sequences relatedness (97–99 per cent) with *L. monocytogenes.*

Phylogeny of 6 *Listeria monocytogenes* isolates obtained from meat samples in Egypt was constructed by using neighbour joining method in MEGA 6.0 software. Phylogenetic analysis showed that all the six Egyptian isolates have high homology with Colombian isolate, except one Egyptian isolate which showed high homology with Indian isolate of *Listeria monocytogenes*. This may be due to the importation of animals and raw meat from the Latin America and India (Mohamed *et al.,* 2016).

Mary and Shrinithivihahshini (2017) performed phylogeny for 14 isolates of *Listeria monocytogenes* obtained from milk and milk products in Tamil Nadu by using UPGMA (unweighted pair group method with arithmetic mean) method in MEGA 6.0 software. They concluded that *Listeria monocytogenes* from raw milk showed relatedness with *Listeria ivanovii* and *Listeria innocua*.

Phylogenetic analysis was conducted for 5 *Listeria monocytogenes* isolated fromchickens, meat, cheeseand clinical samples in Iraq using MEGA 6.0 software by UPGMA tree analysis (unweighted pair group method with arithmetic mean). Phylogeny based on the *hlyA* gene of the Iraqi isolates showed that there is a significant genetic correlation and high homology between isolate No.1 with Indian isolate, No.2 with Indian isolate, No. 3 with US isolate, No.4 with Indian isolate, and isolate No.5 with Italian isolate. The genetic analysis for the 5 *L. monocytogenes* isolates in this study showed a genetic affinity in the isolates of *L. monocytogenes* isolated from chickens, meat and cheese by 70 per cent, while there is a genetic difference in the isolates of *L. monocytogenes* isolated from the clinical samples (Yousif and Alshamari, 2018).All isolates obtained from milk vending machine (2), tank milk(2) and single cow milk samples(5), udder swab (2) and clinical samples (2) of Piacenza Province, Italy belonged to lineage II, serogroup IIa, sequence type 37, clonal complex 37 and harboured some virulence determinants. This case showed that, although relatively rare, prolonged milk contamination by *L. monocytogenes* can originate from subclinical and persistently infected cows, posing a health risk to consumers. (Ricchi et al., 2019) Out of 220 *Listeria* isolates obtained from dairy processing facilities of Vienna, Austria originating from different cheese types, product-associated liquids, product-associated samples, raw material, food contact surfaces, non-food contact surfaces and environmental liquid samples (139 *L. innocua* and 81 *L. monocytogenes*), *L. innocua* ST1597 and ST603 and *L. monocytogenes* ST121 and ST14 were the most abundant genotypes (Kaszoni-Rückerl, 2020). The highest prevalence of *L. monocytogenes* was found in the dairy environment—soil samples near to manure storage (93%), mixed feed from the feeding trough and hay (29%), water samples from farms drinking trough (28%) and cattle feces (28%). Clonal complexes (CC) of CC37 (30%), CC11 (20%) and CC18 (17%) (all IIa serogroup) were predominant *L. monocytogenes* clones (Terentjeva et al., 2021)

The majority of samples (91%, n = 387) were positive for Listeria spp., of which 54% (n = 211) were positive for L. monocytogenes. Each of the 14 farms yielded at least one positive L. monocytogenes sample. Environmental samples obtained from sites around the farms related to feces (soil, slurry spreader and slurry drain) and fecal samples (stored manure and fresh feces) showed a higher percentage of L. monocytogenes and Listeria spp. in general in comparison with the feed samples and the raw dairy samples of dairy cattle farms in the Cantabria region (Northern Spain, Atlantic Coast). Taking into account the prevalence of lineage I isolates can be an additional indicator that the presence of L. monocytogenes in the farm environment is due to fecal contamination. (Varasaki et al., 2022).

Overall, detection of Listeria spp. was higher in samples collected from the dairy farm environmental samples (75%) compared with samples collected from the dairy processing samples (24%). Whole-genome sequencing performed on select isolates collected from the dairy processing samples and dairy farm environmental samples supported the identification of 6 clusters (range of 3 to 15 isolates per cluster) that showed ≤ 50 high-quality single nucleotide polymorphism differences. Of these 6 clusters, 3 (i.e., clusters 2, 4, and 5) contained isolates that were collected from both the dairy processing samples and dairy farm environmental samples, suggesting that transmission between these 2 environments was likely. (Bolten et al., 2024)

**3.4** **Occurrence of *Listeria* spp. in dairy environmental samples**

Among 12 isolates of *Listeria* obtained from dairy environmental samples, the species identified after phenotypic and genotypic characterization were*Listeria ivanovii* (6), *Listeria seeligeri* (1)and *Listeria grayi* (5). Among the species of *Listeria*, *Listeria ivanovii* (50.0 per cent) predominated followed by*Listeria grayi* (41.60 per cent) and *Listeria seeligeri* (8.40 per cent) (Fig. 13).

**Fig. 14: Occurrence of *Listeria* spp. in dairy environmental samples**

Nayak *et al.* (2015) found that out of 200, 18 samples of milk and milk products from Navsari, Gujrat were found positive for *Listeria* spp*.* which were identified as *Listeria seeligeri* (6, 33.3 per cent), *Listeria innocua* (5, 27.7 per cent), *Listeria welshimeri* (4, 22.2 per cent), and *L. monocytogenes* (3, 16.6 per cent). Matto *et al.* (2018) collected dairy farm samples like soil, dung, water, bulk milk to study the prevalence of *Listeria* species. They isolated only one isolate of *L. monocytogenes* from pasture. No isolation of *Listeria* spp. was retrieved from the bulk tank milk or drinking water from of the farms. *Listeria innocua* was detected in 13 feedstuffs and seven samples of soil from the entry and exit points of the milking parlour. EL-Naenaeey *et al.* (2019) determined the overall isolation rate of *Listeria* spp. as 16 per cent in feaces of dairy cows; 8 per cent *Listeria monocytogenes* from normal milk and mastitis milk; 4 per cent and 2 per cent in feaces of dairy cows, normal milk and mastitis milk, respectively. The prevalence of *L. ivanovii* and *L. welshimeri* in milk of dairy cows were 6 per cent and 4 per cent, respectively.

*Listeria monocytogenes* (13, 56.50 per cent) predominated followed by  *Listeria innocua* (4, 17.39 per cent)*, Listeria ivanovii* (2, 8.70 per cent)*, Listeria seeligeri* (1, 4.35 per cent) and three as unidentified *Listeria* species (13.06 per cent) out of 23 isolates from the dairy environmental samples of university dairy farm in Bengaluru. Chaitra (2020).

4. Conclusion

Among the 18 dairy environmental *Listeria* isolates, *Listeria ivanovii* was predominant (50.0%), followed by Listeria grayi (41.6 %) and Listeria seeligeri (8.40%). The neighbour joining method of phylogenetic analysis of these isolates depicted genetic correlation with *Listeria* isolates of NewYork, Nigeria, Germany, Himachal Pradesh and Greece, indicating their sources of origin.

References

# Bolten, S., Ralyea, R.D., Lott, T.T., Orsi, R.H., Martin, N..H, Wiedmann, M., and Trmcic A. (2024). Utilizing whole-genome sequencing to characterize Listeria spp. persistence and transmission patterns in a farmstead dairy processing facility and its associated farm environment. J Dairy Sci., 107(11), 9036-9053.

* Chaithra, S., Kempanna, C., and Prabha. R. (2024). Characterizing *Listeria* Species of Dairy Environments: Phenotypic Insights. Microbiology Research Journal International 34 (12), 42-54. <https://doi.org/10.9734/mrji/2024/v34i121510>.
* Chen, J. Q., Regan, P., Laksanalamai, P., Healey, S., and Zonglin, H. U. (2017). Prevalence and Methodologies for Detection, Characterization and Subtyping of *Listeria monocytogenes* and *L. ivanovii* in Foods and Environmental Sources, Food Science and Human Wellness, pp: 1-58.
* El-Naenaeey, E. Y. M., Wahab. A. M. O. A., Merwad. A. M. A. and Abdou, H. M. A. (2019). Prevalence of *Listeria* species in dairy cows and pregnant women with reference to virulotyping of Listeria monocytogenes in Egypt. Zagazig Vet. J., 47(3):248-258.
* Harrigan, W. (1998). Laboratory methods in food and dairy microbiology. Academic Press Inc. (London) Ltd. UK.
* Hilly, M., Adams, M. L., and Nelson, S. C. (2002). A study of digit fusion in the mouse embryo. Clinical and Experimental Allergy, 32(4), 489-498.
* http://www.ncbi.nlm. nih.gov
* Jarvis, P. D., Holland, B. R., and SUMNER. J. G. (2017). Phylogenetic Invariants and Markov Invariants. Elsevier. pp: 1-3.
* Kaszoni-Rückerl, I., Mustedanagic, A., Muri-Klinger, S., Brugger, K., Wagner, K.H., Wagner, M., Stessl, B. (2020). Predominance of distinct *Listeria innocua* and *Listeria monocytogenes* in recurrent contamination events at dairy processing facilities

Microorganisms, 8:234. <https://doi.org/10.3390/microorganisms8020234>

* Kraus, V., Čižmárová, B., Birková, A. (2024). *Listeria* in Pregnancy—The Forgotten Culprit. Microorganisms, 12(10), 2102. <https://doi.org/10.3390/microorganisms12102102>
* Ludwig, W., Schleifer, K. and Whitman, W. B. (2009). Bergey’s Manual of Systematic Bacteriology, The Firmicutes. Springer. 2(3), 244–256.
* Mary, S. M. and Shrinithivihahshini, N. D. (2017). Pervasiveness of *Listeria* *monocytogenes* in Milk and Dairy Products. J. Food. Microbiol. Saf. Hyg*.* 2(3), 1-5.
* Matto, C., Varela, G., Braga, V., Vico, V., Gianneechini, R. E. and Rivero, R. (2018). Detection of *Listeria* spp. in cattle and environment of pasture-based dairy farms. Brazilina J. Vet. Res. 38(9), 1736-1741.
* Mohamed, Y., Reda, W. W., Moein, K. A., Razik, K. A., Barakat, A. M. A., Fadaly, H. A., Hassanain, N. A. A. and Hegazi, A. G. (2016). Prevalence and phylogenetic characterization of *Listeria monocytogenes* isolated from processed meat marketed in Egypt. J. Genetic Eng. Biotechnol.,14,119-123.
* Nayak, D. N., Savalia, C. V., Kalyani, I. H., Kumar, R., and Kshirsagar, D. P. (2015). Isolation, identification, and characterization of *Listeria* spp. from various animal origin foods. Vet. World*.,* 8(6): 695-701.
* Ricchi, M., Scaltriti, E., Cammi, G., Garbarino, C., Arrigoni, N. M., Morganti, M., Pongolini, S, (2019). *Short communication:* Persistent contamination by *Listeria monocytogenes* of bovine raw milk investigated by whole-genome sequencing. J. Dairy Sci., 102, 6032-6036.
* Shamloo, E., Jalali. M., Mirlohi, M., Madani, G., Metcalf, D., and Merasi, M. R. (2019). Prevalence of *Listeria* species in raw milk and traditional dairy products in Isfahan. Iran. Int. J. Env. Health. Eng. 3(3),1-5.
* Soni. D. K., and Dubey, S. K. (2014). Phylogenetic analysis of the *Listeria* *monocytogenes* based on sequencing of 16S rRNA and hlyA genes. Mol. Biol Rep. Springer. pp: 1- 11.
* Sushmitha, P., Ramachandra, B., Sachindra Babu, A. and Prabha, R. (2023). Enumeration and isolation of *Listeria* species from dairy environmental samples. biological forum – An International Journal, 15(12), 149-153.
* Terentjeva, M., Šteingolde, Z., Meistere, I., Elferts, D., Avsejenko, J., Streikiša, M., Gradovska, S., Alksne, L., Ķibilds, J., Bērziņš A. (2021). Prevalence, genetic diversity and factors associated with distribution of *Listeria monocytogenes* and other *Listeria* spp. in cattle farms in Latvia. Pathogens, 10, 851 <https://doi.org/10.3390/pathogens10070851>

# Varsaki, A., Ortiz, S., Santorum, P., López, P., López-Alonso, V., Hernández, M., Abad, D., Rodríguez-Grande, J., Ocampo-Sosa, A.A., Martínez-Suárez, J.V. (2022). Prevalence and Population Diversity of *Listeria monocytogenes* Isolated from Dairy Cattle Farms in the Cantabria Region of Spain. Animals (Basel), 19, 12(18):2477. doi: 10.3390/ani12182477. PMID: 36139336; PMCID: PMC9495194.

* Wachiralurpan, S., Sriyapai, T., Areekit, S., Kaewphinit, T., Sriyapai, P., Santiwatanakul, S. and Chansiri, K. (2016). One-Step DNA Extraction for Detection of *Listeria monocytogenes*, Int. J. Adv. Sci. Eng. Technol.,3: 1-2.
* www.megasoftware.net
* Yousif, M. G. and Alshamari, A. K. (2018). Phylogenetic Characterization of *Listeria monocytogenes* Isolated from Different Sources in Iraq, Asian. J. Pharm. Clin. Res., 11(2), 289-292.