

Original Research Article

Prevalence of *Listeria* spp. in soils of central Kerala, India

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A total of 100 soil samples, from three districts of Kerala viz., Ernakulam, Thrissur and Palakkad were screened to determine the prevalence of *Listeria* species in Central Kerala. Out of these, two samples were found positive for *Listeria* spp. The isolates were confirmed by biochemical tests as well as by molecular methods. Biochemical tests and molecular methods revealed isolates to be *L. innocua*. Antibiotic sensitivity study indicated high susceptibility to cotrimoxazole, chloramphenicol, gentamicin, streptomycin, tetracycline and vancomycin whereas high resistance was observed against cefixime, cefuroxime.

Introduction

Listeriosis is an important emerging food borne zoonotic disease caused by pathogenic strains of *Listeria* spp. particularly *L. monocytogenes* and *L. ivanovii*. The infection has been reported in countries over six continents and the public health significance of the pathogen lies in its ubiquitous nature, wide host range which includes 40 mammals, 20 birds, crustaceans, ticks and fishes and ability to persist for year in the environment.

Listeria monocytogenes is gram positive, facultative intracellular bacterial pathogen of humans and animals. The genus *Listeria* includes 11 species; *L. monocytogenes*, *L. ivanovii*, *L. grayi*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. marthii*, and *L. rocourtiae* including three recently identified species,

L. maris, *L. fleischmannii*, and *L. weihenstephanensis* (Halter *et al.*, 2013). Out of these, *L. monocytogenes* and *L. ivanovii* are considered as pathogens of humans and animals.

Listeria monocytogenes is widely distributed in nature including vegetation, water, sediment and soil. Although *L. monocytogenes* is ubiquitous in the environment, human and animals are likely to be an important reservoir. The organism has been isolated from livestock, domestic and wild animals in both infections and latent stages; in animal feces and in the close environment of animals.

Farm environments are potential sources of *L. monocytogenes* and may contribute to the

contamination of vegetables at the preharvest stage. *Listeria monocytogenes* is frequently isolated from a large variety of vegetables collected in farms. One of the first potential sources of vegetable contamination at the preharvest stage (in the field) is soil when seeds are sown. In addition, some agricultural practices such as recycling animal feces as crop fertilizers or irrigation with contaminated water may increase the risk of soil and vegetable contamination. Soil fertilized with sludge cake can contaminate parsley seeds with *L. monocytogenes* which can be detected until plant harvesting. Direct transfer of *L. monocytogenes* from amended soil carrots, lettuce, radish, spinach and tomato has been described by various scientists. Endophytic migration of *Listeria* spp. in green gram and coriander plants was reported by Sunil *et al.* (2012).

Materials and Methods

Sample collection

A total of 100 samples including 40, 30 and 30 soil samples were collected from three districts viz., Ernakulam, Thrissur and Palakkad of central Kerala. Mostly surface soil samples were collected from fields, near sewages and from farm surroundings. The samples were collected in sterile containers, transferred to laboratory on thermocool boxes and processed for isolation of *Listeria* spp.

Isolation of *Listeria* Species

Isolation of *Listeria* spp. was attempted as per USDA–FSIS method (USDA, 2013). The samples (soil) were inoculated (approx. 5 ml/5 g) into 45 ml of University of Vermont Medium (UVM)–1 supplemented with acriflavin and nalidixic acid, and incubated at 37°C for 18–24 h. Vaginal and

floor swabs were directly inoculated in 10 ml UVM broth. Further enrichment of the samples was carried by inoculating 0.1 ml of UVM–1 to 10 ml of UVM–2 broth. Inoculated UVM–2 broth was incubated further for 24 h at 37°C. A loopful of enriched UVM–2 broth was streaked directly on PALCAM agar for selective isolation of *Listeria* colonies. The inoculated agar plates were incubated at 37°C for 48 h. The isolated pinpoint grayish–green colonies surrounded by black zone of aesculin hydrolysis were presumed as *Listeria*. These colonies were further purified on PALCAM agar and stored in refrigerated conditions in BHI broth.

Biochemical characterization and identification of isolates

A single isolated colony from PALCAM agar was inoculated in fresh BHI broth and incubated at 37°C for 18 h. The freshly grown culture was then studied for their morphological and biochemical characters. Morphology was observed under light microscope while, *Listeria* specific biochemical tests such as catalase, oxidase, characteristics tumbling motility at 20–25°C and fermentation of sugars (rhamnose, xylose and mannitol) were performed. The isolates were compared with standard *Listeria* spp. for identification. Isolates suspected as *L. monocytogenes* from biochemical tests were further subjected to specific tests such as hemolysis on sheep blood agar (SBA) and Christie, Atkins, Munch–Petersen (CAMP) test.

Result and Discussion

Out of 100 samples collected, two samples were found positive for *Listeria* spp. The positive samples were from Ernakulam and Thrissur districts. No *Listeria* spp. could be isolated from Palakkad district. The isolates

were then subjected to the carbohydrate utilization tests for the identification of the organisms at species level. All isolates showed positive to dextrose and rhamnose (Table 1). Further confirmation of the isolates was carried out by hemolysis on blood agar. The two isolates were non hemolytic and confirmed as *L. innocua*.

The standardized PCR allowed the amplification of virulence associated gene of *L. innocua* to their respective base pair, 870 bp. The PCR product was represented by a single band in the corresponding region of the DNA marker ladder (Fig. 1).

The antibiotic resistance pattern of *Listeria* isolates from soil

The antibiotic resistance profile patterns of *L. innocua* isolates were sensitive to cotrimoxazole, chloramphenicol, gentamicin, streptomycin, tetracycline and vancomycin. All the isolates showed resistance against cefixime, cefuroxime. Fifty per cent of the isolates showed resistance against rifampicin and fifty per cent was sensitive to rifampicin. Whereas all the isolates showed intermediate resistance towards ampicillin

Following the initial description in 1926 *L. monocytogenes* has been shown to be of worldwide prevalence and is associated with serious disease in a wide variety of animals, including man. It is an exquisitely adaptable environmental bacterium, capable of existing both as an animal pathogen and plant saprophyte with a powerful array of regulated virulence factors. Even though different studies have provided existence that *Listeria* spp. are broadly distributed through the natural environment, our understanding of the ecology and reserves of *Listeria* species and *L. monocytogenes* is fairly limited.

Prevalence of *Listeria* in soil

L. monocytogenes, has been isolated from a diverse range of environmental sources like vegetation, soil, sewage, river water, waste water effluent, manure and excreta of man and animals (Moshtaghi *et al.*, 2003). Use of sewage sludge and organic fertilizers of animal origin is likely to contaminate soil with *L. monocytogenes*. Of the total 100 samples screened, a prevalence of two per cent was obtained for *Listeria* spp. None of the sample found positive for *L. monocytogenes*. Two isolates of *L. innocua* were obtained.

Higher prevalence of the organism was obtained while screening the samples from soil in earlier investigations. When Sarangi *et al.* (2011) screened 20 soil samples, *L. monocytogenes* was isolated from five per cent of samples.

The overall prevalence of 2 percent in the present study for *Listeria* spp. is less compared to the findings of Moshtaghi *et al.* (2003) who reported the *Listeria* organisms in 17.7 percent of the agricultural field samples from Hisar, India. They reported a prevalence of 5.4 per cent for *L. monocytogenes*. Mohammed *et al.* (2009) reported an overall prevalence of 5.3 percent for *L. monocytogenes* in 1104 soil samples from beef cattle operations. Locatelli *et al.* (2013) isolated *L. monocytogenes* from cow pasture soils but not from cultivated soils, meadows or forest soils. Jeyaletchumi *et al.* (2011) recorded a prevalence of 47.6 percent and 38.1 per cent for *Listeria* spp. and *L. monocytogenes* respectively. They collected soil samples from vegetable farms in Cameron Highlands.

Multiple studies have reported *L. monocytogenes* contamination of soil. Weis and Seeliger (1975) reported *L.*

monocytogenes contamination in 8.7-51.4 per cent of surface samples of soil and 3.2 - 33.3 per cent in soil collected at a depth of 10 cm, Fenlon and Shepherd (2000) proposed that soil is not a general reservoir in which *L. monocytogenes* multiplies, but the presence of *L. monocytogenes* in the soil is probably due to contamination by decaying plant and fecal materials, which together with damp surface soil, provide a cool moist protective environment for *L. monocytogenes* to survive.

Most of the researches who attempted the isolation of the organism reported the presence of organism in soil whereas van Renterghem *et al.* (1991) could not isolate Listeria organisms from manured soil samples. Szymezak *et al.* (2014) screened 173 organically fertilized soil samples and 120 waste land samples for *Listeria* spp. and *L. monocytogenes*.

A prevalence of two per cent in the present study is very low. The lower prevalence may be due to the absence of saprophytic conditions or due to large dilution of the organism in soil. The lower prevalence in soil can be also due to low prevalence in animals also. Result similar to the present study was reported by Nightingale *et al.* (2004) after screening soil samples from pristine environment. The study reported a prevalence of 1.3 per cent. The study showed a prevalence of one percent for *L. monocytogenes* from the areas fertilized organically and no isolates of *Listeria* spp. were found in artificially fertilized areas and wastelands.

In the present study most of the soil samples were collected from areas where animal activity was more like nearby cattle sheds,

farms, market places and agricultural fields. Two soil samples were found positive for *L. innocua* one each from Thrissur (3 per cent) and Ernakulam districts (2.5 per cent). The positive samples were collected from the waste disposal area of poultry farm in Thrissur and premises of a fish market in Ernakulam. The moisture content and humid organic matter of these areas might have favoured the survival of the organism. Other reasons for less prevalence in soil might be attributed to soil chemical nature, changes in the nutrient content of the soil and presence of competing bacteria.

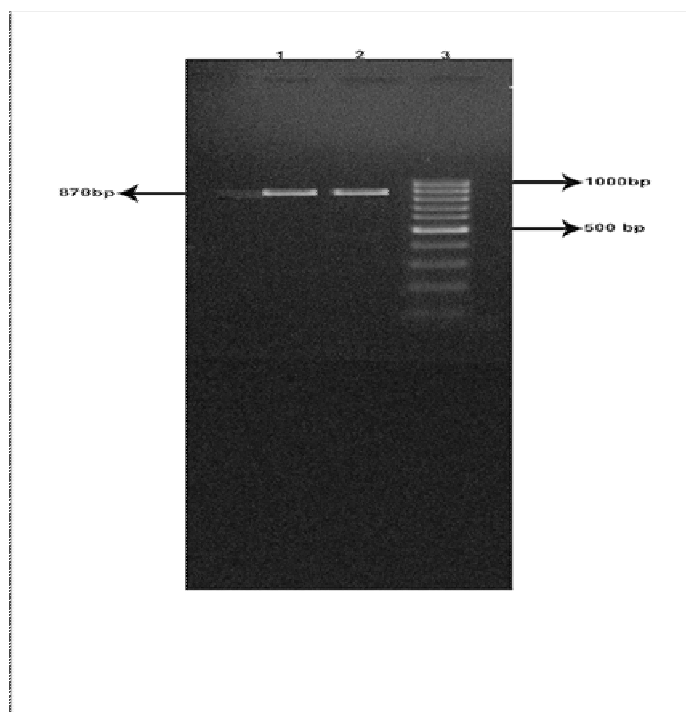
Antibiogram study indicated that in the treatment of Listeria infection cotrimoxazole, vancomycin and streptomycin can be used as primary choice of therapy but not cefixime, ceftriaxone, cefuroxime, gentamicin which are commonly used in field conditions.

Survival and multiplication of the organism in the soil have been documented. Soil contaminated with Listeria may lead to contamination of vegetation and plants which in turn may act as a source of infection for animals and human beings. Presence of *Listeria* spp. in animal associated environment may cause infection to animals being reared in that area. The result of prevalence in soil points to the hazards that may arise due to use of untreated animal excreta as soil fertility enhancers. Considering the zoonotic potential of the organism, there is an urgent need of hygienic measures to be adopted at farms and public awareness must be raised regarding the proper disposal of sewage, proper washing of vegetables before use and avoiding the entry of Listeria into the food chain.

Table.1 Carbohydrate utilisation pattern of the isolates

Sl. No.	Listeria isolates	Acid from carbohydrates				Probable species of Listeria
		D-Mannitol	L-rhamnose	D-xylose	Dextrose	
1	L1	-	+	-	+	<i>L. innocua</i>
2	L2	-	+	-	+	<i>L. innocua</i>

Fig.1 PCR profile of *Listeria* isolates, Lane 1, 2. – *L. innocua* isolates, Lane 3 – 100bp ladder



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