

PRESENCE OF *LISTERIA SPP.* IN ICE CREAM AND SEWAGE WATER PARTICULARLY *LISTERIA MONOCYTOGENES* AND ITS PATHOGENECITY

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Abstract

A total of 60 branded Ice cream samples and 90 non branded Ice cream samples were tested for presence of *Listeria spp.*. It was found that both types of samples were positive for *Listeria spp.* and also *L. monocytogenes*. 12% of branded and 9% of non branded ice cream samples were contaminated with *Listeria spp.*. Among positive samples *L. monocytogenes* was detected in 29% and 13% of samples respectively. *Listeria spp.* was much higher in branded ice cream samples than that in non-branded one. Out of a total 20 and 30 sewage water samples collected from industrial and domestic areas 15% and 30% were positive for *Listeria spp.* samples respectively but no sample was positive to *L. monocytogenes*. Pathogenicity was tested in rabbit model and the organisms were re-isolated from test dead rabbit. The histopathological changes find in the experimental rabbits were typical one. It may be concluded from these observations that the zoonotically important as well as highly pathogenic *Listeria* species are present in the sewage water in and around Kolkata, West Bengal, India.

Key Words: *Listeria*, ice cream, sewage water, Kolkata

1. Introduction

Listeria monocytogenes is the most important species in the genus *Listeria* creating human and animal health hazards and having a worldwide distribution with an extensive host range. *Listeria spp.* can grow well in complex media at a wide range of temperature (3° to 42°C) (Seeliger & Jones, 1986). It affects primarily pregnant patients, neonates, elderly and immunocompromised individuals. Its significant role as food borne human pathogen became evident only in 1980s (Schlech *et al.* 1983). The manifestations of the disease in animals include localized encephalitis or meningoencephalitis, a generalized septicaemia form of infection with hepatic necrosis, abortion in mammals and myocardial

degeneration in fowls (Hird and Genigeorgis, 1990). Emergence of human food borne listeriosis as a major public health concern is major attributed to intensification in food production, processing and distribution, increased use of refrigeration as a primary means of preservation for food, change in the eating habits of people, particularly towards convenience and ready- to- eat foods (Swaminathan, 2001). Presence of *Listeria spp.* as well as *L. monocytogenes* in beef collected from Kolkata city and surrounding areas was observed by Biswas, 2010. Considering zoonotic importance of *Listeria spp.* particularly *L. monocytogenes* and scanty knowledge on the existence of different *Listeria spp.* in environment in Kolkata, the present study to enumerate the prevalence of *Listeria spp.* in ice cream and city sewages

was undertaken.

2. Materials and Methods

About 25 gm Ice cream samples (60 and 90 samples from different branded and non branded producer's respectively) were collected from local open markets and 25 ml of Sewage water samples (20 from industrial 30 from domestic area of Kolkata) were collected in sterile container. The sewage water samples were cold enriched up to five weeks. Adult healthy rabbits of about 1.5-2.0 kg body weights were used for pathogenecity test. Adult healthy male sheep were used as source of blood. *Staphylococcus aureus* (β -haemolytic strain) was obtained from the Deptt. of Microbiology, All India Institute of Hygiene & Public Health, Kolkata-700 073. Reference strain of *Listeria monocytogenes* was obtained from Microbial Type Culture Collection & Gene Bank, Institute of Microbial Technology, (CSIR), Chandigarh. PALCAM *Listeria* Selective Enrichment Broth and Agar [*Listeria* Identification Broth (PALCAM)] were from Hi Media. Methyl Red test, Voges-Proskauer Test, Catalase test, Oxidase test, Sugar Fermentation test, Haemolysis test and CAMP test were performed as per OIE 2000. Different species of *Listeria* were identified by biochemical tests (Recourt and Remount, 1983). Lethality of the test strains were assessed by intraperitoneal inoculation of *L. monocytogenes* isolates into healthy rabbit as per FDA protocol (Federal Register, 1988). The rabbit were inoculated with 2ml of tryptose broth with 10^9 cfu/ml *L. monocytogenes* intraperitoneally keeping control and observed for seven days. Histopathological examination of brain and liver was done

using Haematoxylin and Eosin staining method (Moshtaghi *et al.* 2002).

3. Results and Discussion

A total of 60 branded Ice cream samples and 90 non branded Ice cream samples were tested for presence of *Listeria* spp.. It was found that both types of samples were positive for *Listeria* spp. and also *L. monocytogenes*. 12% of branded and 9% of non branded ice cream samples were contaminated with *Listeria* spp.. Among positive samples *L. monocytogenes* was detected in 29% and 13% of samples respectively. These reports were identical with the surveillance reports of the FDA Enforcement (Anonymous, 1998). *Listeria* spp. was much higher in branded ice cream samples than that in non-branded one. This might happen from cross contamination in processing plant as organisms can settled in the factory environment. Another source of contamination might be milk itself. The organism has been detected in raw milk as well as in pasteurized milk (Roy, 1992).

Out of 20 samples from industrial 30 samples from domestic sewages 15% and 30% of samples were positive for *Listeria* spp. respectively. No sample was positive to *L. monocytogenes*. Giridhar and Garg (2002) reported the presence of *L. monocytogenes* and other *Listeria* spp. from these sources and also from farm environments. Probably these workers collected the water samples from farm sewage, which might be contaminated with faeces from diseased, or carrier animals. But in this study the sewage water samples were collected from the industrial areas, where different chemicals were drained

out through sewage and samples from the sewage of domestic areas also were full of detergent, phenyl and bleaching powder. So, the environment might not be suitable for the growth of these organisms. Species wise distribution of *Listeria* organism in ice cream and sewage water samples shown in the Table no.I.

The pathogenicity of *L. monocytogenes* isolates was tested in rabbit. Six male healthy rabbits of about six months of age were used for each isolate keeping six rabbits as control in separate pens. Maximum number of mortality occurred within 72-96 hours of inoculation. Only one isolate showed 100% mortality in rabbit and some isolates showed no mortality at all within a period of one-week of inoculation. The organisms were re-isolated from dead rabbit the findings are correlated with the findings of Kimberling, 1988.

There was remarkable histopathological changes observed in the brain liver of dead test rabbits. Presence of micro abscesses and degenerative changes of brain cells along with neuronal loss were observed. Chromatolysis and haemorrhages in perineural area were observed. Perivascular cuffing was observed along with congestion of cerebral blood vessels. There were also focal accumulations of satellite cells (astrocytosis). Congestion of hepatic portal vein was seen. Albuminous exudates were found inside the bile canaliculi. The endothelial vessels showed hyperplasia in the tunica adventitia. Besides, there were massive hemorrhages in the hepatic parenchyma. Some of the hepatocytes showed necrosis. Micro abscess were also found around the blood vessels along with infiltration of neutrophil and macrophages. These findings were in concurrence to the observations of Moshtaghi (2002).

Table-I: Species wise distribution of *Listeria* organism ice cream and sewage water samples.

Name of samples	Type of sample	No. of samples tested	No of samples (%) positive						Total (%)
			<i>L. monocytogenes</i> (%)	<i>L. ivanovii</i> (%)	<i>L. innocua</i> (%)	<i>L. welshimeri</i> (%)	<i>L. seeligeri</i> (%)	<i>L. grayii</i> (%)	
Ice cream	Branded	60	2(29)	0(0)	1(1)	0(0)	3(43)	1(14)	7 (12)
	Non-branded	90	1(12)	2(25)	0(0)	0(0)	2(25)	3(38)	8(9)
Sewage water	Industrial	20	0(0)	0(0)	0(0)	0(0)	1(33)	2(67)	3 (15)
	Local	30	0(0)	0(0)	2(22)	0(0)	4(45)	3(33)	9 (30)

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