Proceeding of 2nd AFSSA Conference on Food Safety and Food Security
August 15 – 17, 2014
Asian Food Safety And Security Association
ISSN: 2306-2150

Prevalence and antimicrobial susceptibility of *Listeria* spp. in dairy food products and water samples in Dhaka, Bangladesh

Rajib Sarker, Sangita Ahmed*

Department of Microbiology, University of Dhaka. Dhaka-1000. Bangladesh.

Email address:
srajib100@yahoo.com (Rajib Sarker)
sangita@du.ac.bd (Sangita Ahmed)

Abstract:

A total of 57 samples, of which 17 were surface water samples and 40 were dairy food samples (raw milk, pasteurized milk, icecream, sweet, milk based drink like matha and borhani) were tested for the isolation of *Listeria* spp. Putative *Listeria* isolates were identified by conventional microbiological tests and Analytical Profile Index. Overall prevalence of *Listeria spp.* in both food and water samples were 8.77%, of which one was (1.75%) *Listeria monocytogenes*, 2 (3.5%) were *Listeria innocua* and 2 were (3.5%) *Listeria welshimeri*. When compared between two types of samples, water samples contained two *Listeria* spp. (11.76%) of which one was pathogenic *Listeria monocytogenes* and the other was *Listeria innocua*. In case of food samples, three *Listeria* spp. (7.5%) were isolated of which one was *Listeria innocua* (icecream sample) and two were *Listeria welshimeri* (icecream sample and raw milk). No *Listeria* was found in pasteurized milk, sweet, matha and borhani. Antibiotic resistance profile of the *Listeria* isolates showed that 60% isolates were resistant to Ampicillin and Erythromycin, 20% isolates were Sulphamethoxazole and Ciprofloxacin resistant. No resistance was observed to Chloramphenicol for any *Listeria* isolates.

Keywords: Prevalence, Antimicrobial susceptibility of *Listeria* spp., Dairy food products, Water.

1. Introduction

Listeria monocytogenes is associated with listeriosis, a foodborne infection which can lead to severe conditions like meningitis, endocarditis and has high mortality rate. It is widespread in the environment and has been isolated from water, soil, dust, plants, animal feed, feces and sewage and has been associated with mammals, birds and possibly fish (Sauders *et al*., 2012). This pathogen has also been isolated from food products like unpasteurized (raw) milk or foods made from unpasteurized milk, red meats, poultry, seafood, vegetables, fruits and ready-to-eat food products (Kasalica *et al*., 2011, Karakolev, 2009, Malek *et al*., 2010) . Of these, milk and other dairy products have been implicated in about half of all the listeriosis outbreaks and in several sporadic cases worldwide (Molla *et al*., 2004; Mahmoodi, 2010). The ability to grow or survive at low temperatures, low pH and low water activities makes *L. monocytogenes* an important hazard in foods (Mahmoodi, 2010). Multi-drug resistance among *Listeria monocytogenes* isolated from food or the environment have also been described, which imposes an additional risk to public health (Coner *et al*., 2009; Jamali *et al*., 2013; Gomez *et al*., 2014). However, in Bangladesh information on the occurrence and distribution of pathogenic *Listeria monocytogenes* as well as other *Listeria* spp. is very limited. This study is aimed to study the prevalence of *Listeria spp.* in dairy food products and water samples.

2. Materials and Methods

2.1. Collection of samples

A total of 40 dairy milk products (such as ice-cream, Sweet, Sondash, Matha, Borhani), raw and pasteurized milk from shops and restaurants and 17 water samples from various ponds around Dhaka city have been collected in sterile container. These samples were collected during the period of April, 2013 to June, 2014. The samples were transferred under aseptic conditions in an ice box to the laboratory at the Department of Microbiology, University of Dhaka.

2.2. Sample Processing and isolation of *Listeria* sp.

Samples were processed according to combined method of United States Department of Agriculture (USDA) and U.S. Food and Drug Administration (FDA). 25 gm or 25 ml food/water sample was mixed with Oxfoid Listeria selective enrichment broth and was incubated at 30°C for 48 hours. In case of solid sample, it was homogenized in 0.1% Peptone Water before inoculating into broth. After 48 hours, from each broth loopful inoculum was taken and was streaked on Oxoid Listeria selective agar followed by incubation at 37°C for 48 hours. Each plate was examined for typical colonies of *Listeria* and colonies with characteristics typical of *Listeria* spp was subcultured on Tryptone soya agar supplemented with 0.6% yeast extract at 37 °C for 24 hours.

2.3. Identification of *Listeria* sp.

To identify *Listeria* species, all isolates were screened for their Gram reaction by Gram staining method. Isolates which appeared as Gram positive, rod shaped organisms were further screened for their biochemical tests (Catalase test, Oxidase test, xylose and mannitol fermentation test, motility test and hemolysis on blood agar) as suggested in the Bergey's Manual of Systematic Bacteriology Volume 2 (2005). Isolates showing typical properties of *Listeria* were identified by Listeria API.

2.4.Determination of Antimicrobial Susceptibility

Susceptibility of the *Listeria* isolates to different antimicrobial agents was measured *in vitro* by employing the modified Kirby-Bauer method (Barry and Thornsberry, 1985). Commercially available antimicrobial discs (Oxoid limited, England) were used for the test. An inoculating needle was used to pick single, isolated colony, and inoculated into 3 ml of Muller-Hinton broth. The broth cultures were then allowed to incubate at 37°C for 4 h to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a McFarland 0.5 standard (3×10^8 CFU/ml). To streak on the surface of agar medium, a sterile, nontoxic cotton swab was dipped into the tube containing young culture and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then streaked evenly in three directions over the entire surface of the agar plate to obtain a uniform inoculum. A final sweep was made around the agar rim with the cotton swab. This plate was then allowed to dry for 3 to 5 minutes, before the discs were applied. Antibiotic impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. All discs were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface. Within 15 min after the discs were applied, the plates were inverted and placed in an incubator at 37°C. After 16 to 18 h of incubation, the plates were examined and zone size was measured.

3. Results and Discussion

3.1. Isolation of *Listeria spp.* from food and water samples

Among 57 collected samples, bacterial isolates showing black halo colonies typical of *Listeria* sp. were obtained from 15 samples: in particular 12 (30%) of 40 food samples and 3 (17.65%) of 17 water samples. No growth was obtained from pasteurized milk and borhani. Further investigation showed that all were Gram positive rod and showed similar morphology as *Listeria spp.* For further identification biochemical tests were performed which included Catalase test, Oxidase test, sugar (xylose and mannitol) fermentation test and motility test. As a positive control, *Listeria monocytogenes* ATCC 43256 strain was included in the test. Results are summarized in Table 1.

Table 1. *Biochemical properties of putative Listeria isolates*

Isolate no	Catalase	Oxidase	Sugar fermentation		Motility	
			Xylose	Mannitol	At 25^0C	At 37^0C
1	+	-	-	-	+	-
2	+	+	+	+	+	+
3	+	-	+	-	+	-
4	+	-	+	-	+	-
5	+	-	+	+	+	+
6	+	-	+	+	+	+

7	+	-	+	+	+	+
8	+	-	+	-	+	-
9	+	-	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+	-	-	-	+	-
13	+	-	-	-	+	-
14	+	+	-	+	+	+
15	+	-	+	+	+	+
Listeria monocytogenes ATCC43256	+	-	-	-	+	-

All *Listeria* sp. are catalase positive, oxidase negative, motile at 30^0C and non-motile at 37^0C. 6 out of 15 isolates showed these properties (isolate no. 1, 3, 4, 8, 12, 13, Table 1). All of these isolates failed to ferment mannitol, while only two could ferment xylose, indicating to differentiation at species level.

3.2. Identification of the *Listeria* isolates by Analytic Profile Index (API)

The six isolates that showed biochemical characteristics typical of *Listeria* sp. were further tested for identification using Listeria Analytical profile Index (API). As a positive control, *Listeria monocytogenes* ATCC 43256 was included in the test. Results from API showed that among the six isolated tested, two showed characters that matched those of *Listeria weilshmeri*, two with *Listeria innocua* and one with *Listeria monocytogenes* (Table 2). Isolate no. 13 did not match with those of *Listeria* sp.

Table 2. *Identification of the Listeria isolates by Analytic Profile Index*

Isolate no	Species
1	*Listeria innocua*
3	*Listeria welshimeri*
4	*Listeria innocua*
8	*Listeria welshimeri*
12	*Listeria monocytogenes*

Following the results of *Listeria* API, it can be concluded that this study isolated 5 (8.77%) *Listeria* sp from 57 samples tested, of which one was (1.75%) *Listeria monocytogenes*, 2 (3.5%) were *Listeria innocua* and 2 (3.5%) were *Listeria welshimeri* (Figure 1).

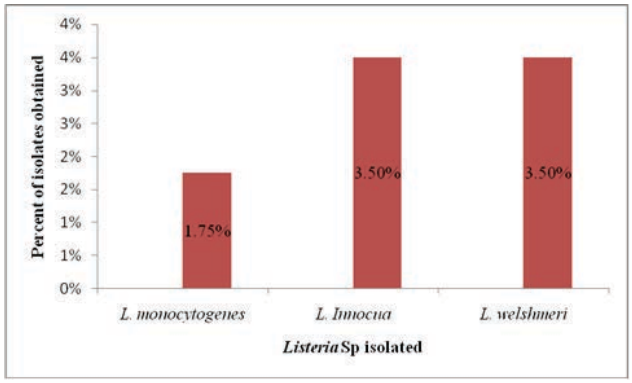


Fig 1*. Prevalence of Listeria sp in total number of samples*

When the prevalence of different species of *Listeria* were compared between the different types of samples it was observed that, among 17 water samples tested, two *Listeria* spp. (11.76%) were found of which one was pathogenic *Listeria monocytogenes* and the other was *Listeria innocua* (Figure 2). In case of 40 food samples tested, three *Listeria* spp. (7.5%) were isolated of which one (2.5%) was *Listeria innocua* (icecream) and two (5%) were *Listeria welshimeri* (raw milk and icecream) (Figure 2).

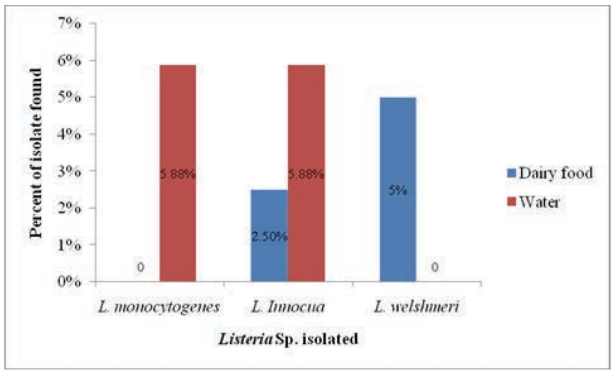


Fig. 2. *Prevalence of Listeria spp. in dairy food and water samples*

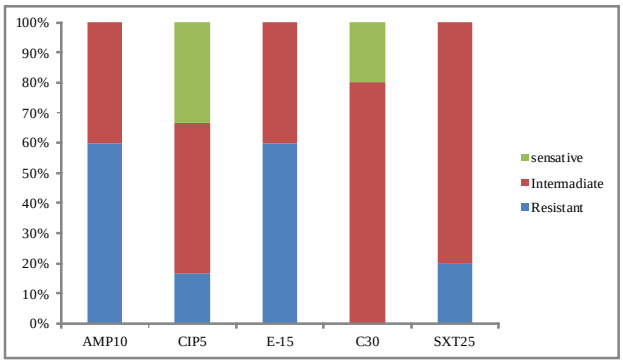
3.3. Antibiotic Susceptibility Pattern of the *Listeria* isolates

Identified *Listeria spp.* were tested for their antibiotic susceptibility against the five (5) commonly used antibiotics, which were Ampicillin (AMP), Chloramphenicol (C), Erythromycin(E), Ciprofloxacin (CIP) and Sulphamethoxazole (SXT) by disc diffusion assay (ref). The pathogenic *Listeria monocytogenes* isolate was resistant to Ampicillin and Erythromycin, while intermediate resistance of this isolate was observed against Chloramphenicol, Ciprofloxacin and Sulphamethoxazole (Table 3). Both *Listeria welshmeri* isolates were Ampicillin and Erythromycin resistant. There was difference between the sensitivity patterns of these isolates to other antibiotics tested. The *Listeria innocua* isolate did not show resistant to any of the antibiotics tested and was sensitive to Ciprofloxacin and, while intermediately resistant to other four antibiotics. When the sensitivity patterns of these isolates to different antibiotics were combined, it was observed that 60% isolates were resistant to Ampicillin and Erythromycin, 20% isolates were Sulphamethoxazole and Ciprofloxacin resistant (Fig. 3). No resistance was observed to Chloramphenicol for any *Listeria* isolates

.

Table 3*. Antibiotic sensitivity pattern of the Listeria isolates*

Isolate	AMP-15	CIP5	E 15	C30	SXT25
Listeria innocua isolate 1	I	S	I	I	I
Listeria welshemeri isolate 1	R	R	R	S	I
Listeria innocua isolate 2	I	S	R	S	S
Listeria welshemeri isolate 2	R	I	R	I	R
Listeria monocytogenes	R	I	R	S	S



S=Sensitive, R=Resistant, I= Intermediate

Fig. 3. *Antibiotic sensitivity pattern of Listeria isolates*

AMP- Ampicillin, C-Chloramphenicol, E-Erythromycin, CIP-Ciprofloxacin and SXT-Sulphamethoxazole.

Among 40 dairy food samples, three samples (7.5%) were positive for *Listeria* spp., two from icecream and one from raw milk. Of these two were *Listeria welshimeri* (5%), one from raw milk and the other from one icecream sample. Another species isolated was *Listeria innocua* (2.5%), which was isolated from another icecream sample. Similar prevalence (6.397%) of *Listeria* sp. in dairy food products and environmental samples has been reported in a study conducted in Istanbul, Turkey (Atil. *et al*, 2011). In another study, the overall prevalence of *Listeria* in dairy food was 7.2%, of which *L. innocua* was the most commonly recovered species (66.6%) (Rahimi *et al.*, 2012).

Among 17 water samples, 2 (11.764%) samples were positive for *Listeria* spp, of which one was *Listeria innocua* and one was *Listeria monocytogenes.* Isolation of *Listeria* in water in low prevalence has been reported by Atil, *et.al*, (2011), who found 1 (0.8%) *Listeria monocytogenes* and 2 (1.5%) *Listeria innocua* from 132 water samples tested. The presence of *Listeria monocytogenes* in water in the current study indicates to contamination of water by fecal materials of animals, birds or human. In Bangladesh, water-borne infection is a major health issue and presence of *Listeria monocytogenes* imposes additional threat to public health. It also suggests that further study of water bodies in Bangladesh is necessary for investigating the prevalence of pathogenic *Listeria monocytogenes.*

Resistance of pathogenic as well as non-pathogenic bacteria to antibiotics has become a concern for last few decades and this problem is intensifying worldwide. Several studies report the isolation of multi-drug resistant pathogenic and non-pathogenic *Listeria* sp. (Conter *et al*., 2009; Jamali *et al*., 2013; Gomez *et al*., 2014). Antibiotic resistance not only interferes with effective treatment measure, the antibiotic resistance gene pool in bacteria facilitates horizontal transfer of these genes among different bacterial strain that poses a huge threat to humankind. It was observed that the pathogenic *Listeria monocytogenes* was resistant against ampicillin and erythromycin and had intermediate susceptibility to Ciprofloxacin. Arslan and O¨ zdemir (2008) isolated *L. monocytogenes* that was resistant to 2 antimicrobials among 11 tested. In another study, 89% of *L. monocytogenes* isolates were sensitive to 14 different antibiotics. Resistance to ampicillin is significant, as it is a first choice of antibiotic in

listeriosis treatment for humans (Conter *et al*., 2009). 100% susceptibility of *Listeria* sp. to chloramphenicol has also been reported by Marian *et al*., 2012.

Listeria welshimeri isolate obtained in this study was most resistant against antibiotics, while *Listeria innocua* was not resistant against any tested antibiotics. McDowell *et al*., (2000) observed resistance to one or two antibiotic tested among 0.6% *Listeria monocytogenes*, while no resistance was observed among *Listeria seeligeri or Listeria welshimeri.* Therefore it seems like antibiotic resistance has not become such big threat in *Listeria* sp, though much more investigation is needed to draw any conclusion.

4. Conclusion

Outbreaks of Listeriosis in some countries, caused by consumption of milk and dairy products contaminated with *Listeria monocytogenes*, indicates the risk and danger to consumer health. The purpose of the present study was to find out the prevalence of *Listeria spp.*, in particular the pathogenic *Listeria monocytogenes* from dairy food products and water samples. Detection of *Listeria* sp in food samples indicate the overall standard of sanitation and hygiene in food processing in Bangladesh is very poor. Presence of *Listeria monocytogenes* in water sample is of particular concern for public health and suggests for further detailed investigation of food products as well as environmental samples in Bangladesh.

References

[1] Brian, D. Sauders, Overdevest, J., Fortes, E., Windham K., Schukken Y., Lembo A., & Wiedmanna M. 2012. Diversity of *Listeria* Species in Urban and Natural Environments. Applied Environmental Microbiology, 78(12), 4420-4433.

[2] Rumen Karakolev. 2009. Incidence of *Listeria monocytogenes* in beef, pork, raw-dried and raw-smoked sausages in Bulgaria. Food Control, 20(10), 953–955.

[3] Kasalica, V., Vuković, A., Vranješ, N., & Memiši, N. 2011. *Listeria monocytogenes* in milk and dairy products. Biotechnology in Animal Husbandry, 27(3), 1067-1082.