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Occurrence of *Listeria* in food chilling facilities of the different campuses in Haramaya University, Ethiopia☆

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ABSTRACT

Ready-to-eat food could act as sources of *Listeria* posing foodborne listeriosis. This study was aimed to assess *Listeria* along selected institutes food chilling, Ethiopia. A total of 230 samples (115 from refrigerator and 115 from the food items within the respective refrigerator) were tested. An overall 32.4% ranging from 7.8 to 45.0% *Listeria* were observed in total samples. *Listeria* was high in Campus-A (OR = 9.6, 95%OR CI = 3.2–31.5), Campus-B (OR = 8.6, 95%OR CI = 3.2–26.4) and Campus-D (OR = 7.1, 95%OR CI = 2.2–26.3) than in Campus-C (7.8%) were observed ($p = 0.00$). Of the total 115 samples from the refrigerator, 38 (33.0%) were positive for *Listeria* with significantly lowers (9.4%) in Campus-C than in others (35.0%–45.8%) ($\chi 2 = 11.87$; $p = 0.008$). High (31.3%) food items were *Listeria* positive from Campus-C than in others (29.2%–55.0%) ($\chi 2 = 16.33$; $p = 0.001$). Of 115 paired samples, food alone (11.3%), refrigerator alone (13.0%) and co-contamination (20.0%) were observed. The refrigerator used for beef, milk and poultry were found positive for *Listeria* at 33.0%, 44.4% and 33.3% with the food themselves too, at rate of 32.0%, 22.2% and 50.0%, respectively. Refrigerator used for tomato and butter storage and the respective food items were *Listeria* negative. Co-contamination of both the refrigerator and the food indicated the need raw food safety.

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Introduction

Listeria has a potential to grow at low temperatures (0–45°C) and become challenging microorganism in chilled/refrigerated ready-to-eat food [1–3]. Consumption of such food could potential risk for food-borne listeriosis outbreaks [1,2,4,5]. Although all individuals are susceptible to the diseases [6], immunocompromised AIDS patients, pregnant women, new born babies and elderly people are high risk groups [7,8]. The pathogenic strain, *Listeria monocytogenes,* has been reported from different food items [2–4,5–10] at 10%–80% in poultry, red meat, meat products [9–15], dairy products, processed vegetables, fish and seafood [16–18]. These food items, particularly the processed products, were frequently kept in refrigerator (the low marginal thermal) where *Listeria* preferred [11,19]. Occurrence and prevalence of the *Listeria* in food

☆ *Listeria* along selected institute food chilling in Ethiopia

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at different parts of Ethiopia has been also reported. Prevalence of 32.6% in retail meat and milk products [20], 26.6% in ready-to-eat (RTE) foods and raw meat products [18], 26.1% in foods of animal origin [9], 27.45% in retail meat and dairy products [21], 24.2% in raw milk and dairy products [22], 28.4% in raw milk and milk products [23] and 25.0% in RTE foods of animal origin [24] were reported in Ethiopia. Although it was known that *Listeria* survive in cold chain, including in the refrigerator [11,25–27], survey on its occurrence in refrigerator has not yet carried out in Ethiopia. Thus, the aim of this study is to assess the occurrence of *Listeria* in refrigerator and in the chilled food items within the respective refrigerator at Haramaya University Campuses, Ethiopia.

Materials and methods

Study institution

Study was conducted at Haramaya University four different campuses. The campuses provide cafeteria service for served community working within the university. Due to ethical purpose and confidentiality, the names of the campuses were coded as Campus-A, Campus-B, Campus-C and Campus-D. They are found in separated locations.

Study design

Simple random sampling technique was applied. While doing, the Campus, refrigerator, and the storage use of refrigerators and the stored food items were considered as variables of study. The temperature adjustments of each refrigerator were immediately registered before any sample collection. During sampling, all refrigerators were at +4 °C. Parallel to the sample collection from the refrigerator, sample of food were also collected from the respective refrigerator. Samples from the refrigerator and from the food items within respective refrigerators were collected aseptically.

Sample size calculation

The sample size was calculated for the refrigerator and the food within the refrigerator. The desired sample size was calculated by using the formula given by Arsham [28] which is:

$N = 0.25/SE^2$, *where: N= sample size, SE (standard error) = 5%)*

Thus, the sample size required becomes was 100. Thus, a minimum sample size was 100 each from refrigerator and the food within the refrigerator collected in paired forms. For this study, 115 each samples from both with total of 230 samples were collected.

Sample collection techniques and transportation

Using sterile wet swabs, swab sampling was done gently (without being touching the food item) from the bottom of the interior part of the refrigerator. Swabs sampling was also gently (without being touching the refrigerator) applied on the food item which we encountered (the beef, butter, poultry and tomato) within respective refrigerator. All swabs were placed in 9 ml of pre-sterile *Listeria* enrichment broth (EB) following Food and Drug Administration procedures [26,29–31]. About 10 ml of the available milk sample was also taken from the refrigerator used for milk storage. All samples were immediately labeled with specific identification number and transported to Veterinary Microbiology Laboratory College of Veterinary Medicine, Haramaya University ice box at +4 °C.

Isolation of Listeria

Primary isolation: All tubes containing the samples (primary enrichment) were incubated aerobically at 30 °C for 24 h. One milliliter of milk was transferred in to pre-sterile *Listeria* enrichment broth (EB) followed by secondary enrichment procedures [26,29].

Secondary enrichment: After very well mixing the primary culture, a volume of 0.1 ml was transferred to 9 ml EB containing tubes (secondary enrichment). The tubes were incubated at 30 °C for 24 h.

Streaking out on selective agar: A volume 0.01 ml secondary enrichment culture was subculture by streaking onto Lithium chloride-phenylethanol-moxalactam (LPM) plating agar [26,31]. After incubating all secondary EB at 30 °C for 4 h, the culture were kept in a refrigerator at 4 °C for 28 days. Then, a loop full was streaked again on LPM plating agar and incubated at 37 °C for 48 h. The plates were checked after 24 h and 48 h for typical *Listeria* colonies [26,29,31].

Biochemical characterization: Listeria presumptive colony(ies) were sub-cultured for purity on brain heart infusion (BHI) agar (Difco), from which further biochemical characterization was done within a week. All suspected colonies were tested for the presence of characteristics of *Listeria* using Gram positive, catalase positive and rods shape morphology. Motility and carbohydrate utilization test (xylose and Mannitol) utilization test were done. The Christie-Atkins-Munch-Peterson (CAMP) test using ß-haemolytic *Staphylococcus aureu* and *Rhodococcus equi* in single straight lines in parallel, on a sheep blood agar plate or a double-layered agar plate with a very thin blood agar overlay were done. After incubation for 24–8 h at 35–37 °C (12–18 h if using the thin blood agar overlay), a positive reaction consists of an enhanced zone of ß-haemolysis, at the

Table 1
Over all *Listeria* in along food chilling among studied campuses.

Campus code	Status of *Listeria*					
	No. of samples	Positive no. (%)	$\chi 2$	*P* value	OR	OR 95%CI
Campus-A	40	18 (45.0)	24.72	0.000	9.6	3.2–31.5
Campus-B	78	33 (42.3)			8.6	3.2–26.4
Campus-C	64	5 (7.8)			1	0.28–3.6
Campus-D	48	18 (37.5)			7.1	2.2–26.3
Total	230	74 (32.4)				

Table 2
Statues of *Listeria* in refrigerator and the stored food items among studied campuses.

Campus Code	Refrigerator samples				Samples of food items from the refrigerator			
	No. of samples	Positive no. (%)	OR	OR 95%CI	No. of samples	Positive no. (%)	OR	OR 95%CI
Campus-A	20	7 (35.0)	5.2	1.1–27.3	20	11 (55.0)	18.3	3.5–131
Campus-B	39	17 (43.6)	7.5	2.0–34.5	39	16 (41.0)	10.4	2.4–20.8
Campus-C	32	3 (9.4)	1	0.2–6.3	32	2 (6.3)	1	0.1–10.1
Campus-D	24	11 (45.8)	8.2	1.9–40.2	24	7 (29.2)	6.2	1.2–46.1
Total	115	38 (33.0)			115	36 (31.3)		
$\chi 2$		11.87				16.33		
P value		0.008				0.001		

Table 3
Refrigerator and stored food co- contamination with *Listeria* along the studied campuses.

Campus code	No. of paired samples	Positive no. (%)		
		Refrigerator alone	Food alone	Both the refrigerator and food*
Campus-A	20	1 (5.0)	5 (25.0)	6 (30.0)
Campus-B	39	6 (15.4)	5 (12.8)	11 (28.2)
Campus-C	32	3 (9.4)	2 (6.3)	0
Campus-D	24	5 (20.8)	1 (4.2)	6 (25.0)
Total	115	15 (13.0)	13 (11.3)	23 (20.0)

* Note: paired positive. i.e. the refrigerator and the food items were positive for *Listeria*.

intersection of the test/control and indicator strains. *Listeria monocytogenes* is positive with the *S. aureus* streak and negative with *R. equi*, whereas the test with *L ivanovii* gives the reverse reactions [26,31,32]. Further attempt to differentiate *Listeria monocytogenes* and other species were not done in this study.

Data analysis

The data was entered in Microsoft Excel 2013© and computed for descriptive statistics was analyzed using STATA 11 version software. Percentage, 95% Mid-prevalence exact Confidence Interval, $\chi 2$, odds ratio (OR) and OR 95%CI were calculated to assess significant of *Listeria* prevalence at $P < 0.05$.

Results

All of the studied refrigerators were adjusted at 4–5 °C temperature during sample collection. Out of all 230 samples, 32.4% ranging from 7.8–45.0% were positive for *Listeria* (Table 1). *Listeria* was significantly high ($p = 0.00$) in Campus-A (OR = 9.6, 95%OR CI = 3.2–31.5), Campus-B (OR = 8.6, 95%OR CI = 3.2–26.4) and Campus-D (OR = 7.1, 95%OR CI = 2.2–26.3) than in Campus-C (7.8%).

Of the total 115 samples from the refrigerator, 38 (33.0%) were positive for *Listeria* with significantly lowers (9.4%) in samples from refrigerator at Campus-C than in others (35.0%–45.8%) ($\chi 2 = 11.87$; $p = 0.008$). Regarding the food item, a total of 36/115 (31.3%) samples were positive (Table 2). *Listeria* in food sample from Campus-C was significantly lower (6.3%) than in others (29.2%–55.0%) ($\chi 2 = 16.33$; $p = 0.001$).

As shown in Fig. 1, from the paired parallel sample of 115, *Listeria* was observed in refrigerator alone (13.0%), in food alone (11.3%), and both in refrigerator and the food as co-contamination (20.0%)

As shown in Table 3, higher contamination rate of refrigerator alone were observed in Campus-B (15.4%) and Campus-D (20.8%) than Campus-A (5.0%) and Campus-C (9.4%). Co-contamination of both the refrigerator and the food were null in Campus-C but higher in remaining three campuses ranged from 25.0% to 30.0%.

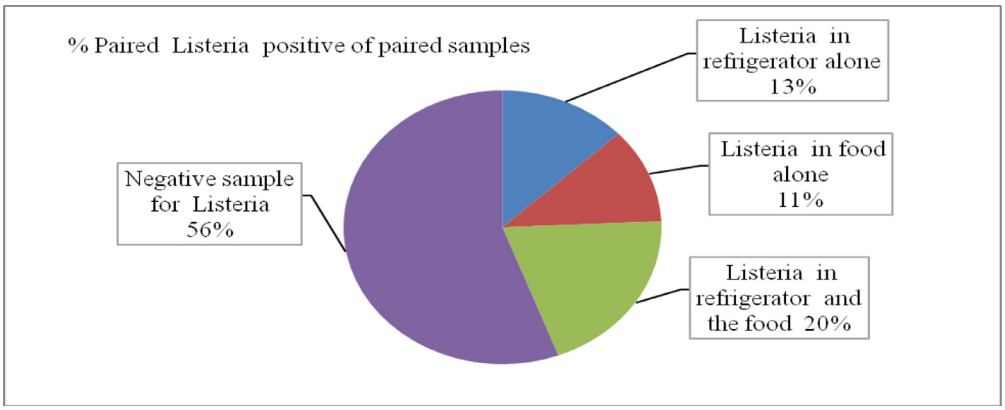

Fig. 1. Co-contamination status of refrigerator and the food items by *Listeria* in pairs of 115 samples.

Table 4
Listeria in refrigerators and in the specific types of chilled food items.

Uses/ functions of refrigerator	Samples source					
	Refrigerator		Food items		Over all *Listeria*	
	No. of samples	Positive no. (%)	No. of samples	Positive no. (%)	Total no. of sample	Total no. (%) positive
Beef storage	97	32 (33.0)	97	31 (32.0)	194	63 (32.5)
Milk storage	9	4 (44.4)	9	2 (22.2)	18	6 (33.3)
Poultry storage	6	2 (33.3)	6	3 (50.0)	12	5 (41.7)
Tomato storage	2	0	2	0	4	0
Butter storage	1	0	1	0	2	0
Total	115	38 (33.0)	115	36 (31.3)	230	74 (32.4)

The refrigerator used for beef, milk and poultry were found positive at 33.0%, 44.4% and 33.3% for *Listeria* with the food themselves too, at rate of 32.0%, 22.2% and 50.0%, respectively (Table 4). Refrigerator used for tomato and butter storage and the respective food items were *Listeria* negative.

Discussion

Foodborne infection and intoxication are getting greater attention due to frequent occurrence of the cases globally. Food can be contaminated with zoonotic pathogens [27,33] including *Listeria*, at any point in time. Consumption of such food with *Listeria* causes Listriosis [1,2,5,15]. Moreover, the contaminated food can further contaminate the working and processing facilities as well as food storage equipment like refrigerator [11,15,26,27]. The present overall 32.4% *Listeria* in the food refrigeration with 31.1% in the refrigerated food and 33.0% in the refrigerators were similar among each other. This indicated the presence of contamination of refrigerator from already contaminated food items remain unclean with subsequent multiplication of the agent acting as cross-contamination. The possible presence of cross-contamination was confirmed by the 20.0% *Listeria* observstion in both the refrigerator and the food from this study. The present findings of *Listeria* in the food refrigeration, in the refrigerator and the food items were comparable with the 32.6% [20], 28.4% [23], 27.45% [21], 26.6% [18], 26.1% [9], 25.0% [24] and 24.2% [22] reports from different food items in Ethiopia. Unlike former [9,18,20–24] whose investigate *Listeria* only from food, the present finding indicated presence of the agent refrigerator sample were positive for *Listeria*. The presence of *Listeria* in 7.8%–45.0% of the studied campus, in the refrigerators at 9.4% to 45.8% and in the refrigerated food items at 6.3%–55.8% within the campus indicated presence of poor hygienic application with wide distribution of *Listeria*. James et al. [25] also indicated the possible contamination of household refrigerators of various brands with zoonotic microbial including *Listeria, Yesinia, Salmonella* and *Staphylococcus auras*. The present findings of *Listeria* in refrigerator used for beef (33.0%), milk (44.4%) and poultry (33.3%) with the food themselves too, at rate of 32.0%, 22.2% and 50.0%, respectively were similar with others [9,18,20–24] reports in Ethiopia. In this finding, *Listeria* was not observed in refrigerator used for tomato and butter storage and the respective food. This could be due to the fewer number of tested samples. But, the possible presence of *Listeria* in various stony fruits such as whole peaches, nectarines, pulums and pluots were reported [7]. Moreover, the acid or osmotic stress tolerances of L. monocytogenes cells in fresh produce tomato at low temperatures were determent [34].

Conclusion

The current finding indicates the presence of *Listeria* species in studied food refrgiratiion which shows possible occurrence of also other foodborne pathogen by implications. High occurancde of *Listeria* species in food (beef, milk and poultry) and in the refrigerator used for respective food storage indicates the contaminated food could act as sources of *Listeria* which further act as sources for subsequent food contamination. *Listeria* Species are psycrophilic and resulted in co-contamination of both the refrigerator and the chilled food indicated the need raw food safety, scheduled cleaning and disinfection with proper heat treatment of the food items before consumption.

Authors' contributions

AH: Study conduction, data collection, analysis, reference search, and manuscript writing; EB: Study conduction, reference search, manuscript writing, and editing. DB: analysis, reference search, and manuscript writing. All authors have read and approved the final manuscript.

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Computing interests

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2019.e00074.

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