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Prevalence of *Listeria monocytogenes* and Indicator Bacteria in Selected Food from Local Yemeni Markets

N. S. Al-Zoreky
Department of Food Science, Faculty of Agriculture, Sana'a University
Sana'a, Republic of Yemen

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Abstract. Seventy-five samples of milk (raw and pasteurized), raw tuna (uncooked) and lettuce were examined for occurrence of *Listeria monocytogenes*, Coliforms (total), *Staphylococcus aureus* and fecal enterococci. *Listeria monocytogenes* (one isolate) was detected only in 5.3 % of tuna samples; other *Listeria spp.* were not isolated from examined food, except raw tuna which was contaminated with *L. ivanovii and L. murrayi*. Various microflora isolated from listerial enrichment broths of tuna and lettuce exhibited some characteristics of *Listeria spp.* but were differentiated from listeriae by their alkaline reactions in bromocresol purple carbohydrate broths. Average coliform counts ranged from 6.1×10^2 to 7.6×10^4 cfu g^{-1} (or ml^{-1}) indicating significant differences ($P < 0.05$) in coliform numbers among analyzed foods. Fecal coliforms and *Enterococcus spp.* were among the natural microflora of tuna whereas the other foods did not harbor fecal-contamination indicators.

Introduction

Many foodborne pathogens are normal inhabitants of the intestinal tracts of humans and animals. It is well documented that disease causing microorganisms could be easily transmitted via food cultivated in polluted areas and handled under unsanitary conditions [1; 2]. Unfortuanately, raw wastewater has been used in irrigation of agricultural crops in various countries [1; 3]. Furthermore, drug resistant enterics *e. g. Salmonella spp.* have been isolated from municipal wastewater and other sources [1; 3]. In this regard, food poisoning incidences were linked to food grown in contaminated areas with human filth or manure [1; 2; 4]. For many years, coliform bacteria have been used as indicators of fecal contamination in food and water [2] and special emphasis has been given to products commonly consumed as raw *e.g.* vegetables. Based on that, several enterics have been detected in a wide range of food indicating fecal contamination [2; 4-6].

Listeria monocytogenes, a newly-emerging pathogen, has been incriminated in food outbreaks associated mainly with serotypes 1/2a, 1/2b and 4b of *L. monocytogenes* [4; 7]. The organism is widespread in nature and capable of growth under refrigeration. As a matter of fact, listeriosis could be fatal for the sensitive groups of the population, *e.g.* the infants and immunocompromized patients. Consequently, concerns regarding listerial risk have led to worldwide investigations for isolation of *L. monocytogenes* and other pathogens from food products [2; 5-7]. However, information is little or lacking on listeriae prevalence in food of many developing countries such as Yemen.

Accordingly, the present study assessed prevalence of *L. monocytogenes* and related species in food commonly consumed in Yemen; milk, fish and leafy vegetables. Another objective was screening products for coliforms, *Staphylococcus aureus* and fecal enterococci.

Materials and Methods

Sample collection and preparation

Fresh raw milk was obtained from the Instructional Farm, Faculty of Agriculture, Sana'a University, Yemen. Meanwhile, pasteurized milk (*Rosabah*), raw tuna slices (locally known as *Thamed*) and fresh lettuce were collected from retail markets in Sana'a. Microbial analyses were conducted within 1 h of sample arrival or after storage (5°C) for $\leq$ 16 h. Representative subsamples were prepared by aseptically mixing 500 g (or ml) from food samples. Twenty-five ml or g (duplicates) were aseptically taken and blended for 2 min. with 225 ml sterile diluent (0.1% peptone) or listeria enrichment broths [8]. On the other hand, milk was drawn under aseptic conditions and added into diluent bottles. Decimal dilutions were further prepared as needed. For the aerobic plate counts of samples, poured plate count agar was incubated at 32°C (milk) or 35°C (other foods) for 48 h. Unless stated otherwise, microbiological media were Difco products (Detroit, MI, USA).

Isolation and identification of listeriae and other bacteria

The FDA established methods [8] were adopted for isolation of *Listeria spp.* from 10^{-1} diluted samples in listeria enrichment broth (LEB). Selective enrichments for listeriae, LEBs, were statically incubated for 24 h at 30°C. Aliquots (0.1 ml portions) or a loopful were aseptically drawn from LEBs incubated at 30° for 0 h (direct plating) and 24 h (after selective enrichment) followed by spreading (0.1 ml) or streaking (a loopful) onto Listeria Selective agar (Oxoid, UK). For comparative purposes, the other selective agar used for isolation of listeriae from tested foods was the ASLM [9]. Spread or streaked plates of both Listeria Selective agar and ASLM were incubated at 37°C for 48 h. Persumptive, listerial colonies were randomly picked and purified on nutrient agar. Active cultures grown in nutrient broths (+ 0.1 % glucose) were tested for : gram reaction and morphology; catalse; esculin hydrolysis; reduction of litmus milk; TSI slant reactions; motility at 25°C; production of acid from carbohydrates (glucose, mannitol, xylose and rhamnose) and haemolysis in stabbed blood agar plates.

Total and fecal coliforms were enumerated according to procedures of the American Public Health Association [10] using the Violet Red Bile Agar (VRBA) and the EC broths, respectively.

The three-tube MPN (azide broth; 37°C) was done for enumeration of food enterococci. Gram stain and cell morphology, growth at 45°C, esculin hydrolysis and initiation of growth in 6.5 % NaCl broths were the criteia for enterococcal identification. For *Staphylococcus aureus*, the Baird-Parker agar with egg tellurite enrichment was employed. Where appropriate, results were evaluated using the standard statistical methods [11].

Results and Discussion

Table 1 shows prevalence of *L. monocytogenes* in experimental foods. Both raw and pasteurized milk had below-detection limit (<10 cfu ml^{-1}) of listerial species. In this regard, several authors mentioned that natural inhibitors of raw cows' milk *i. e.* lactofferin and lactoperoxidase were antagonistics for *L. monocytogenes* as well as other pathogens [2; 4; 12]. In many parts of the world, raw milk was found contaminated with different levels of *L. monocytogenes* and other *Listeria spp.*[4; 6]. Not long time ago, pasteurized milk was incriminated in a listerial outbreak in the USA [4; 13]. Later on, Lovett *et al.*[13] stated that proper pasteurization of raw milk was detrimental to *L. monocytogenes*. Furthermore, sources of *L. monocytogenes*.in adequately pasteurized milks were related to post-pasteurization contaminations [4; 13].

Table 1. Prevalence of *L. monocytogenes* and other *Listeria spp.* in various foods

Food (no . of samples)	No. of listeriae isolates	
	L. monocytogenes	*Listeria spp.*[a]
Raw milk (24)	0	0
Pasteurized milk (17)	0	0
Raw tuna (19)	1 (5.3 %)[b]	2 (10.6 %)
Lettuce (15)	0	0

[a] *L. ivanovii* and *L. murrayi*

[b] % Positive samples

Even though *L. monocytogenes* was not isolated by direct plating, 5.3 % of raw tuna samples had contaminated *L. monocytogenes* when using selective enrichments; other *Listeria spp.* were also isolated from raw tuna (Table 1). It was surprising that lettuce samples were not contaminated with listeriae (<10 cfu g^{-1}). Al-Mohizea [14] reported considerable variability in listerial contamination of different fresh vegetables. In agreement with previous studies [15-17], outgrowth of natural microflora could possibly interfere with isolation of *L. monocytogenes* from most tuna samples (approx.95 %) and other food analyzed. In fact, average aerobic counts (Table 2) in milk, tuna and lettuce

outgrow *Listeria spp.*.and other pathogens in tested foods. Inhibitory substances for *L. monocytogenes* and other pathogens were identified in certain vegetable [18].

Occasionally, certain microflora in tested samples of lettuce and tuna recovered only on Listeria Selective agar (Oxoid) showed identical morphology, gram and catalse reactions and esculin hydrolysis of *Listeria spp.* However, their alkaline reactions in BCP glucose broths separated those false positive isolates from listerial species. Buchanan *et al.* [19] reported presence of bacteria similar to listeriae which did not utilize glucose.

Table 2. Types and mean counts (cfu g^{-1} or ml^{-1}) of selected microflora in various foods

Organisms/ Food (no . of samples)	Raw milk (24)	Pasteurized milk (17)	Raw tuna (19)	Lettuce (15)	SE [a] x 10^5
Aerobic count	$3.1x10^6$	$1.6x10^5$	$2.4x10^7$	$2.7x10^6$	55
Coliforms (total)	$7.6x10^4$	$6.1x10^2$	$3.9x10^4$	$1.9x10^3$	0.17
Fecal coliforms	-	-	+[b]	-	
S. aureus	$< 10^2$	n. d.*	$< 10^2$	$< 10^2$	
Enterococcus spp. (MPN g^{-1} or ml^{-1})	n.d.	$< 3x10$	$9.3x10^4$	$< 3x10^2$	0.31

[a] Pooled standard errors
[b] present in 61 % of samples
* not done

On the other hand, non-listerial bacteria were extensively inhibited in ASLM. Previous reports indicated that recovery of *L. monocytogenes* from cheeses and other products was unsatisfactory when using Listeria Selective agar [4; 7; 20]. It is worthwhile to mention that both *L. monocytogenes* and *L. murrayi* were isolated from tested tuna using the ASLM. However, 0.2 ml from enriched samples was necessary for better isolation of *Listeria spp.* in ASLM [9; 20]. Unlike Listeria Selective agar, ASLM contained moxalactam a potent inhibitor for most enterococci and staphylococci [4; 7; 17]. Luxurious growth of enterococci contaminated tuna samples was evident by black discolarations (esculin hydrolysis) on Listeria Selective agar.

Lack of isolation of *L. monocytogenes* and fecal enterococci was the trend in lettuce samples containing low coliform count (ave. 1.9×10^3 cfu g^{-1}). This was unexpected, especially when considering unhygienic irrigation, fertilization, handling and marketing practices of leafy vegetables. In other countries, investigators pointed out contamination of fresh produce with *L. monocytogenes* and fecal coliforms [14; 21]. Coliforms recovered from processed foods *viz.* pasteurized milk (Table 2) could probably be attributed to post pasteurization contamination [2; 7; 10]. Food samples analyzed were negative for *Staphylococcus aureus* ($< 10^2$ cfu g^{-1} or ml^{-1}).

It could be concluded that positive identification of *L. monocytogenes* and indicator bacteria in raw tuna and lettuce highlighted the potential health hazard associated with consumption of improperly prepared and handled products. Even though results

presented in this study reflected the microbial status of examined samples, reduction of the risk of enteric pathogens could be achieved by using treated in irrigation and food preparation as well as by avoiding use of manure in soil planted with salad vegetables. Hygienic practices in handling, preparation, processing, storage and marketing of food have also been advised for reduction of public health risk of foodborne pathogens.

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انتشار ليستيريا مونوسيتوجينيس وبكتيريا الدالة على التلوث البرازي في بعض الأغذية من الأسواق المحلية اليمنية

نجيب الزريقي

قسم علوم الأغذية، كلية الزراعة، جامعة صنعاء

صنعاء، الجمهورية اليمنية

(قدم للنشر في ١٤١٩/٢/٧هـ؛ وقبل للنشر في ١٤١٩/١٠/٢٨هـ)

ملخص البحث. تم فحص خمس وسبعين عينة من الحليب (الخام والمبستر) وسمك التونة الطازج والخس للكشف عن وجود ليستيريا مونوسيتو جينيس وبكتيريا القولون (الكلية) والكرويات العنقودية الذهبية وكذلك كرويات القولون البرازية. عزلت ليستيريا مونوسيتو جينيس (عزلة واحدة) من ٥,٣ ٪ من عينات التونة فقط بينما لم تعزل الأنواع الأخرى لليستيريا من الأغذية الأخرى المختبرة باستثناء التونة التي كانت أيضا ملوثة بليستيريا إيفانوفاي وليستيريا ميوراي. ظهرت بعض الصفات المميزة لأنواع الليستيريا بواسطة العديد من الكائنات الدقيقة التي تم عزلها من بيئات الإكثار الاختيارية لليستيريا لعينات التونة والخس، إلا أن هذه الكائنات كانت مختلفة عن أنواع الليستيريا في تفاعلاتها القاعدية في البيئات السائلة لبروموكريزول البنفسجي المحتوية على سكريات. بلغ متوسط بكتيريا القولون في عينات الأغذية المختبرة ٦,١×١٠^{٢} إلى ٧,٦×١٠^{٤} وحدة مكونة للمستعمرة لكل جرام أو مل دالا على اختلافات معنوية (مستوى معنوي ٠,٠٥) في إعداد عصويات القولون الملوثة للأغذية المختبرة. كانت عصويات وكرويات القولون البرازية ضمن الكائنات الدقيقة الطبيعية في التونة، بينما لم تحتو الأغذية الأخرى على هذه البكتيريا الدالة على التلوث البرازي.