Microbial Load and the Prevalence of *Escherichia coli, Salmonella* spp. and *Listeria* spp. in Ready-to-Eat Products in Trinidad

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Abstract: This study was designed to determine the prevalence and microbial load of *Listeria* spp., *Escherichia coli* O157 and *Salmonella* spp. in ready-to-eat products in supermarkets across Trinidad. The microbial load was assessed using the total aerobic plate count (TAPC) per g/ml of foods and prevalence of *Escherichia coli* O157 and *Salmonella* spp. determined using conventional methods. For *Listeria monocytogenes*, immunomagnetic separation (IMS), TECRA (enzyme-linked immunosorbent assay, ELISA) and conventional methods were used. The $\log_{10}$ mean ± sd TAPC per g or ml was highest for vegetables (11.0±11.6), and lowest for seafood (5.2±5.7) ($p < 0.05$). The prevalence of *L. monocytogenes* was 1.7 %. Sixteen (4.5%) of 153 samples yielded *E. coli* but all samples were negative for *Salmonella* spp. and *E. coli* O157. The high microbial load and isolation of *L. monocytogenes* and *E. coli* from popular RTE foods could pose a health risk to consumers in the country.

1. INTRODUCTION

Ready-to-eat (RTE) foods pose a health threat to consumers, since any microorganisms present will be consumed as compared to food that is cooked before eating which should kill most of the microorganisms. These foods are usually refrigerated until consumed, which would normally inhibit the growth of bacteria, but *Listeria monocytogenes* will continue to grow at this temperature (Huss *et al*. 2000) [1].

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers (Rosmini *et al*. 2002) [2].

Listeria monocytogenes, Escherichia coli O157:H7 and *Salmonella* spp. are among the most dangerous food borne bacterial pathogens in terms of human health and disease (Olsen *et al*. 2000) [3].

Ready-to-eat foods have been implicated in several outbreaks of listeriosis (CDC,2002) [4]. The largest outbreaks of human listeriosis have involved dairy products (Harvey and Gilmour 1993) [5]. *Escherichia coli*, particularly serotype O157:H7 has become an important food borne pathogen responsible for gastroenteritis epidemics in North America, Europe, Asia and Africa and the most frequently implicated foods have been undercooked, contaminated ground beef, raw milk, unpasteurised cider and apple juice, bean sprouts or fresh leafy vegetables such as lettuce and spinach (Altekruse *et al.* 1997) [6]. Various serotypes of *Salmonella* spp., including *S*. Enteritidis, have been reportedly responsible for food borne epidemics in various countries (Todd 1997) [7], emphasizing the importance of the pathogen as a food safety concern.

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In Trinidad and Tobago, *L. monocytogenes* has been isolated from bulk milk (1.7%), raw meat (1.9%), seafood (5.8%) and chicken frankfurters 4/11(36.4%) (Adesiyun 1993 [8]; Adesiyun *et al.* 1996 [9]; Adesiyun and Krishnan 1995 [10]; Gibbons *et al.* 2006 [11]; Adesiyun *et al.* 2007 [12]), but there is a dearth of information on ready-to-eat foods sold across supermarkets in the country. *E. coli* O157 has also been isolated from the water supply to rural communities (2.0%) (Welch *et al*. 2000) [13], local delicacies such as oysters (2%) (Rampersad *et al.* 1999) [14] and 'black pudding' (13.6%) (Adesiyun and Balbirsingh 1996 [9]) and from pre-processed cows' milk (0.8%) (Adesiyun *et al*. 2007) [12]. The first case of neonatal septicaemia and meningitis due to *L. monocytogenes* was reported in Trinidad in 1998, (Ashiru and Bratt 1998) [15] when symptoms appeared three days after a blood transfusion. Information on the frequency of abortion and neonatal illness due to *Listeria* spp. in Trinidad is unavailable, as these cases are often treated symptomatically. In Trinidad and Tobago, *Salmonella* spp. have been recovered from oysters, 2.3% (Rampersad *et al.* 1999) [14], black pudding, 8% (Adesiyun and Balbirsingh 1996) [9] and table eggs, 13.0% (Adesiyun *et al*. 2005) [16].

This study was therefore carried out since there is currently no report on a comprehensive assessment of the bacterial health risk posed to consumers of ready-to-eat food products sold across the country.

2. MATERIALS AND METHODOLOGY

2.1. Selection of Supermarkets

Supermarkets were sampled across the country and grouped based on the number of cash registers present (Meldrum *et al.* 2006) [17]. Stores with < 3 cash registers were classified as small, 3- 7 as medium and > 7 as large.

2.2. Selection of Products to Sample

Five groups of commonly consumed ready-to-eat foods, namely vegetables, pasteurised milk, cheese, deli meats and seafood from supermarkets were selected (Table **1**), based on

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the findings of the questionnaire. Overall, a total of three hundred and fifty three (353) samples of ready-to-eat foods were collected comprising vegetables (71), pasteurised milk (70), deli meats (70), seafood (70) and cheeses (72).

Table 1. Types of Products Sampled for Testing

Products	Type
Vegetables	Lettuce, Cucumber, Cabbage, Sweet-pepper, Carrots
Pasteurised milk	Peanut Punch, Sea-moss, Linseed and Seamoss and Yogurt Drink, Pasteurised milk.
Deli meats	Sliced Bologna, Chicken Bologna, Turkey Breast Loaf, Chicken Franks, Turkey Pack, Cooked Ham
Seafood	Imitation Crab Meat, Imitation Lobster Meat, Prawns, Smoked Salmon, Smoked herring, Shrimp, Crayfish
Cheese	Pre-packaged cheddar, supermarket packaged cheddar

2.3. Processing of Samples

Samples were purchased as regular customers and transported to the laboratory ice-cooled and processed within 24 h. Representative sections of the sample were taken using sterile utensils and placed in sterile plastic containers. Ten grams each of sample were added to 90 mls lactose broth (Oxoid Ltd., Hampshire, England) and 90 mls *Listeria* Enrichment broth (LEB) (Oxoid Ltd., Hampshire, England) in stomacher bags. The samples were emulsified separately in a Seward 400 Lab Stomacher (London, U.K.) at high speed for 120 seconds.

The lactose broth emulsion was then used to determine the total aerobic plate count (TAPC), by making serial dilutions using sterile saline and surface plating on nutrient agar plates and eosin methylene blue (EMB) agar (Oxoid Ltd., Hampshire, England) in duplicate. Inoculated plates were incubated aerobically at 37^{o}C for 48 h after which the colonies were enumerated using a Quebec Darkfield Colony Counter (Cambridge Instruments Inc., Buffalo, USA). The mean count of colonies on duplicate plates were determined. Subsequently, the lactose broth emulsion was incubated at 37^{o}C for 24 h to enrich for *Salmonella* spp.

2.4. Detection of *Listeria* spp.

The LEB suspension was incubated at 30^{o}C for 24 h after which one ml was put into 9 mls of Fraser broth and incubated at 30^{o}C for 24 h. A loopful was plated on *Listeria* selective agar (LSA) as the conventional method. One ml of the Fraser broth suspension was used for Immunomagnetic separation (Slade 1992) [18] using the Dyna Bead Automated Immunomagnetic Separation (IMS) machine (Thermo Electron Corporation, Finland) to isolate *L. monocytogenes*. LSA plates were also inoculated with processed samples from IMS and incubated at 37^{o}C for 48 h. Characteristic dark-brown or black colonies were then selected and inoculated onto blood agar plates (BAP) which were incubated at 30^{o}C for 24 h. Presence of haemolysis was noted and pure cultures were used to inoculate motility medium which was incubated at room temperature for 48 h. Isolates with 'umbrella motility' were subjected to standard biochemical tests (Macfaddin 2000) [19]. Isolates showing reactions characteristic of *L. monocytogenes* were then subjected to the slide agglutination test using specific *L. monocytogenes* antiserum (Difco Laboratories, Detroit, Michigan, USA).

To detect *L. monocytogenes* using an immunologic method, TECRA, one ml of inoculated Fraser broth suspension was added to 50 *u*ls sample additive in a tube and heated for fifteen minutes in a boiling water bath. Two hundred *u*ls of the suspension was then used to inoculate one well of the TECRA test kits per sample (Slade 1992) [18]. The optical density after incubation was determined by spectrophotometry using an ELISA plate reader (Labsystems, Finland) and the results interpreted as recommended by the kit manufacturer.

2.5. Isolation of *E. coli*

Incubated lactose broth suspension was plated on eosin methylene blue (EMB) and sorbitol McConkey (sMAC) agar and incubated overnight at 37^{o}C. Characteristic metallic green colonies from EMB were plated for isolation on blood agar plates (BAP) and incubated at 37^{o}C for 24 h, and non-sorbitol fermenting (NSF) and sorbito-fermenting (SF) colonies on sMAC were identified as *E. coli* using standard methods (Macfaddin 2000) [19]. NSF colonies were screened for the O157 strain using *E. coli* O157 antiserum (Oxoid Ltd., Basingstoke, U.K.) by slide agglutination test.

2.6. Isolation of *Salmonella* spp.

One millilitre each of the lactose broth suspension incubated at 37^{o}C for 24 h was inoculated into 9 mls of selenite cysteine (SC) and 9 mls of tetrathionate (TT) and 200 *u*ls of iodine broth and incubated overnight at 42^{o}C and 37^{o}C respectively. Growths in SC and TT broths were then inoculated and plated for isolation in bismuth sulphite (BS) and xylose lysine desoxycholate (XLD) agar and incubated at 37^{o}C for 24 h. Isolates with characteristic appearance on BS and XLD plates were identified using standard methods (Macfaddin 2000) [19]. Isolates that were biochemically identified as *Salmonella* spp. were confirmed serologically using the Polyvalent *Salmonella* (A-E & Vi) antisera (Benex Ltd., Shannon, Ireland).

2.7. Statistical Analysis

Data were analyzed to determine if there was a relationship between food group and presence of *L. monocytogenes*, *E. coli*, *E. coli* O157, other coliforms and *Salmonella* spp. The possible association of supermarket size, food group, the type of food, microbial load of the products and the type of bacteria were also analysed. The Statistical Package for Social Sciences (SPSS) (Version 13) was used to process the data and analysis was by the chi-square test with level of significance set at $\alpha = 0.05$.

3. RESULTS

Table **2** shows the log10 mean total aerobic plate count and SD per g / ml of the different food groups tested. Vegetables had the highest TAPC, followed by pasteurised milk,

cheese, deli meats and lowest in seafood. The difference was statistically significant (p=0.00).

Table 2. Mean Log + SD TAPC for Each Product Group Tested

Product	No. of Samples	log Mean ± SD TAPC per g or ml[a]
Vegetables	71	11.0±11.6
Pasteurised milk	70	8.6±9.3
Cheese	72	7.0±7.8
Deli meats	70	6.1±6.4
Seafood	70	5.2±5.7
Total	**353**	**10.4±11.3**

[a]Log_{10} mean of 212 samples.

Forty-one (58.6%) of seventy (70) deli meat samples and forty (57.1%) of seventy (70) seafood samples had TAPC counts $<10^2$ cfu per g of sample, which was within acceptable levels. Samples from large supermarkets had significantly higher TAPCs, followed by those from medium supermarkets and least contaminated from small supermarkets (p = 0.00).

The frequency of detection of the pathogens in the food products is shown in Table **3**. Pasteurised milk and milk products had the highest prevalence of *E. coli*, coliforms and *L. monocytogenes*. The difference in prevalence across food products was statistically significant (p = 0.00). Coliforms were detected at a significantly higher frequency in pasteurised milk and vegetables (p = 0.00), *E. coli* was detected in significantly higher frequency in pasteurised milk and milk products than in the other groups (p = 0.004). No relationship was detected between groups of foods and frequency of detection of *Listeria monocytogenes* (P > 0.05).

L. monocytogenes was isolated from pasteurised milk, as well as from a beverage containing pasteurised milk, linseed, pumpkin and carrot. It was also isolated from lettuce, cooked shrimp and smoked herring. The characteristics of the *E. coli* isolates are shown in Table **4**. None of the *E. coli* isolates was an O157 serotype.

Table **5** shows the number of samples positive for *L. monocytogenes* by the three methods used for detection. Forty-nine (13.9%) of the three hundred and fifty three (353) samples tested positive for *L. monocytogenes* using a combination of the three assay methods but *L. monocytogenes* was only isolated from six (1.7 %) samples.

4. DISCUSSION

The prevalence of *L. monocytogenes* in RTE products found in this study (1.7%) is lower than that found in Bel-

Table 3. Frequency of Isolation of the Microorganisms from the Product Groups Tested

		No. (%) positive for:			
		Coliforms			
Product	**No. of samples tested**	***E. coli***	**Others**	***Listeria monocytogenes***	***Salmonella* spp.**
Vegetables	71	1 (1.4)	12 (16.9)	1 (1.4)	0 (0.0)
Deli meats	70	2 (2.9)	4 (5.7)	1 (1.4)	0 (0.0)
Pasteurised milk	70	9 (12.9)	20 (28.6)	2 (2.9)	0 (0.0)
Cheese	72	2 (2.9)	6 (8.3)	0 (0.0)	0 (0.0)
Seafood	70	2 (2.9)	3 (4.3)	2 (2.9)	0 (0.0)
Total	**353**	**16 (4.5)**	**45 (12.7)**	**6 (1.7)**	**0(0.0)**

Table 4. Characteristics of *E. coli* Isolated in the Study

	No. *E. coli* isolated	No. (%) of *E. coli* that were:			
Product		**Haemolytic**	**Mucoid**	**NSF**	**O157**
Vegetables	1	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
Pasteurised Milk	9	4 (50.0)	9 (100.0)	1 (12.5)	0 (0.0)
Deli meats	2	2 (100.0)	2 (100.0)	1 (100.0)	0 (0.0)
Seafood	2	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)
Cheese	2	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)
Total	**16**	**8 (50.0)**	**16 (100.0)**	**6 (40.0)**	**0 (0.0)**

Table 5. Comparing the Methods of Detection Used for *L. monocytogenes*

Isolation procedure	No. of samples tested	No. (%) positive for *L. monocytogenes*
Conventional	353	3 (0.9)
IMS	353	4 (1.1)
TECRA	353	42 (11.9)
TECRA + IMS	353	0 (0.0)
TECRA + Conventional	353	1 (0.3)
Conventional + IMS	353	1 (0.3)

gium (23.4%) (Van Coillie *et al.* 2004) [20], Japan (3.3%) (Inoue *et al*. 2000) [21] and Italy (12.8%) (Gianfranceschi *et al*. 2004) [22]. In a study conducted on a variety of ready to eat foods namely pate, salad vegetables, smoked salmon, prepared salads and cooked/cured meats in Northern Ireland (Harvey and Gilmour 1993) [5], *L. monocytogenes* was isolated from salad vegetables (7/85) and smoked salmon (1/16). A higher prevalence of *L. monocytogenes* was found by (Harvey and Gilmour 1993) [5] in vegetables compared to our study where only one vegetable sample yielded a *L. monocytogenes* isolate.

Of all the vegetables tested in the current study, only one lettuce sample was positive for *L. monocytogenes*. In a study performed by (Heisick *et al.* 1989) [23] in Minnesota, *L. monocytogenes* was isolated from cabbage, cucumbers, potatoes, and radishes but only *L. innocua* was isolated from lettuce.

Failure to isolate *Salmonella* spp. in ready-to-eat foods in the current study is similar to findings reported elsewhere in Wales (Meldrum *et al.* 2006) [17] and Nigeria (Umoh and Odoba 1999) [24] but at variance with the prevalence of 17% reported in Malaysia (Arumugaswamy *et al.* 1995) [25].

The high prevalence of coliforms in RTE foods could be due to, amongst other factors, contaminated water used to clean equipment and cutting/slicing machines leading to cross-contamination especially if used with raw foods, handlers not practising proper sanitation and faulty pasteurisation equipment and monitoring devices. In Europe, it has been reported that 25% of food borne outbreaks could be traced back to recontamination (WHO 1995) [26].

The isolation of coliforms from vegetables was not unexpected since water used to irrigate vegetable crops and the manure used as fertiliser are reported to contain coliforms and other enteric bacteria (Gagliardi and Karns 2000) [27]. The high prevalence and counts of coliforms found in the vegetables studied may, also be explained by the fact that the vegetable samples were not washed before processing. This was because the study was aimed at determining the risk associated with consuming these products as purchased, bearing in mind that sanitary practices post-purchase and prior to consumption may vary across households in the country, in that a number of individuals consume these products as purchased.

Vegetables tested in the current study were found to have the highest log mean TAPC (11.0±11.6 CFU per g) in comparison to the other food groups tested. A study done by (Albrecht *et al.* 1995) [28] reported the log mean TAPC of vegetables in Nebraska to range from 5.51 to 6.63 log CFU/g. In Taiwan the log mean TAPC of vegetables ranged from 3.30 – 8.64 log CFU/g with similar counts in meat and seafood. Seafoods sampled in the current study were found to have a lower TAPC in comparison to the other food groups tested, however counts obtained (5.2+5.7) were within the range of the counts found in the study in Taiwan (Fang *et al*. 2003) [29]. Average aerobic plate counts for salad vegetables in India were greater than 10^{10} cfu/g (Viswanathan and Kaur 2001) [30]. In the Republic of Cyprus, high ($>10^4$ CFU/g) TAPC's were found in 23.5% of ready to eat foods (Eleftheriadou *et al.* 2002) [31].

The process of pasteurisation is aimed at killing all of the pathogenic organisms in milk, and also to reduce the natural microbial flora of milk (Altekruse *et al.* 1998) [32]. Outbreaks of disease in humans have been traced both to the consumption of unpasteurised and pasteurised milk. Milk borne pathogens may enter *via* contaminated raw milk into dairy food processing plants and these pathogens may persist in bio-films, subsequently contaminating processed milk products and leading to the exposure of consumers to pathogenic bacteria. Furthermore, pathogens such as *L. monocytogenes* have been reported to be capable of surviving and thriving in post-pasteurisation processing environments thus leading to recontamination of dairy products (Harvey and Gilmour 1993) [5]. These pathways pose a risk to the consumer from direct exposure to food borne pathogens present in un-pasteurised dairy products as well as dairy products that become re-contaminated after pasteurisation (Oliver *et al.* 2005) [33].

In this study, pasteurised milk was found to be most contaminated with coliforms. The frequency of detection of coliforms, as well as *L. monocytogenes* and *E. coli* indicates a faulty pasteurisation process, post-pasteurisation contamination, or improper storage post-pasteurisation. *Listeria* spp. has also been detected in pasteurised milk in Brazil (0.9%) (Moura *et al*. 1993) [34], England and Wales (1.1%) (Greenwood *et al.* 1991) [35] and Egypt (4%) (Ahmed and Hussein 2005) [36].

Coliforms have been detected in pasteurised milk worldwide, including Australia (Jensen *et al.* 2001) [37], Bulgaria (Kaloianov and Gogov 1977) [38], Brazil (Da Silva *et al*. 2001) [39], and Scandinavia (Persson *et al*. 1980) [40], all findings which concur with the current study.

The public health significance of detecting pathogens in the samples of pasteurised milk and other milk products studied cannot be ignored. This is because it has been estimated that of diseases due to milk and milk products among food-borne diseases recorded in France and in other countries since 1980, a total of 17,405 cases of disease due to *L. monocytogenes*, *Salmonella* spp., *E. coli* or *S. aureus* were linked to pasteurised milk with 22 deaths (De Buyser *et al*. 2001) [41]. Outbreaks of listeriosis linked to pasteurised milk have been reported in Massachusetts (Fleming *et al*. 1985) [42] and Illinois (Dalton *et al*. 1997) [43]. Pasteurised milk has also been linked to outbreaks of disease due to *E. coli* in North Cumbria (Goh *et al*. 2002) [44]. TECRA was found to be highly sensitive but not specific in comparison to IMS and conventional methods. This finding was expected because TECRA is an immunological assay which requires the presence of the microorganism as an antigen, dead or alive. Other studies comparing the sensitivity and specificity of ELISA to bacteriological methods have however reported a good correlation between the two methods. Using a different ELISA (Utyyendaele *et al*. 1995) [45] obtained no false positives or negatives while the Modified FDA method yielded some false-negatives. Other research by (Noah *et al.* 1991) [46] compared TECRA with another ELISA *Listeria*-Tek and a bacteriological analytical manual (BAM) method and reported that TECRA was the most sensitive test detecting 40 confirmed samples as having *L. monocytogenes* while *Listeria* -Tek detected 37 positives and the BAM detected 38 of a total of 178 samples. Differences in results of the ELISAs compared with those of the BAM method were not statistically significant, however, differences between results of the two ELISAs were significant.

E. coli O157 was not isolated from any of the samples in the current study, a finding similar to the reports of (Mankee *et al.* 2005) [47] on 'doubles' in Trinidad. The organism has however been isolated from bulk milk in Trinidad where three (0.8%) out of three-hundred and eighty-six (386) samples were positive for *E. coli* O157 (Adesiyun *et al*. 2007) [48].

It was concluded that the high microbial load, coupled with the isolation of *Listeria monocytogenes*, *Salmonella* spp. and *E. coli* in popular RTE foods in Trinidad could pose a health risk to consumers.

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