

Full Length Research Paper

# Prevalence, antimicrobial resistance profiles of *Listeria monocytogenes* from various foods in Gaborone, Botswana

Morobe, I. C.<sup>1,3</sup>, Obi, C. L.<sup>2\*</sup>, Nyila, M. A.<sup>1</sup>, Gashe, B. A.<sup>3</sup> and Matsheka, M. I.<sup>3</sup>

<sup>1</sup>School of Agriculture and Life Sciences, Department of Life and consumer Sciences, University of South Africa, Pretoria, South Africa.

<sup>2</sup>Academic and Research Directorate, Walter Sisulu University, Nelson Mandela Drive Mthata, Eastern Cape, South Africa.

<sup>3</sup>Department of Biological Sciences, University of Botswana, Mabutu Drive, Private Bag UB00704 Gaborone, Botswana.

Accepted 10 April, 2008

*Listeria monocytogenes* is known to cause epidemic and sporadic cases of listeriosis. The present study investigated the occurrence, antibiograms and molecular serotypes of the organism in various retail outlets in Gaborone, Botswana. Food samples were obtained randomly from selected supermarkets and street vendors in 5 geographical areas of Gaborone from May, 2007 to September 2007. *L. monocytogenes* was isolated and positively identified by using morphological and biochemical tests. From a total of 1324 food samples tested 57(4.3 %) were positive for *L. monocytogenes*. Out of the 57 isolates of *L. monocytogenes* 7 (12.3%), 3 (5.3%), 0 (0%), 27 (47.4%) and 20 (35.1%) were isolated from cheese, raw milk, meat (biltong), frozen cabbage and salad (coleslaw), respectively. From the 5 geographical areas selected for sampling in this study, Gaborone South recorded the highest number 19 (33.3%) of *L. monocytogenes* isolates while Gaborone West recorded the least, 7 (12.3%). The findings in this study reveal the presence of *L. monocytogenes* serotypes 1/2a and 1/2b in ready to eat food and highlight the need for education and training programmes in food safety in Gaborone, Botswana.

**Key words:** *Listeria monocytogenes*, antibiotic resistance, molecular serotyping, food, Botswana.

## INTRODUCTION

*Listeria monocytogenes* causes a very serious illness known as listeriosis. Individuals who are particularly susceptible to this condition are those who are immunocompromised (as in HIV/AIDS infection), pregnant women, newborn babies, and the elderly (Farber and Peterkin, 1991; McLauchlin et al., 2004). Although the incidence of listeriosis is low, what is significant is that very high fatalities ranging from 20 to 30% have been reported (Mead et al., 1999).

*L. monocytogenes* is widely distributed in nature and has been isolated from a wide array of food products. The organism is considered hazardous in the food indus-

try due to its ability to grow in gas or vacuum-packaged products at refrigeration temperatures (Duffy et al., 1994), low water activity (Nolan et al., 1992) as well as low pH (Buchanan et al., 1993) and all these measures are important in the control of food pathogens.

*L. monocytogenes* is also problematic due to its resistance to antibiotics. The first multiresistant strain of *L. monocytogenes* was isolated in France in 1988 (Poyart - Salmeron et al., 1990), thereafter *L. monocytogenes* strains resistant to one or more antibiotics have since been isolated (Franco Albuin et al., 1994; Charpentier et al., 1995).

It has been shown by various studies that listeriosis is a food-mediated illness (Embarek, 1994; Slutsker and Schuchat, 1999). A wide range of foods such as salads, seafoods, meat, and dairy have been implicated in liste-

\*Corresponding author. E-mail: [lobi@wsu.ac.za](mailto:lobi@wsu.ac.za).

riosis (Bell and Kyriakides, 1998; Schlech, 2000), which follows the oral ingestion of the contaminated food (Finlay, 2001). Studies have shown that the number of *L. monocytogenes* cells can rise following refrigeration from fewer than 100 cells per gram, and this is the dose that is generally accepted for healthy people (Huss et al., 2000; Buchanan et al., 2000).

Serotyping has been used extensively to characterize *L. monocytogenes* (Wieldmann, 2002; Wagner and Allerberger, 2003). Thirteen *L. monocytogenes* serotypes (serovars) have been characterized in this species by using specific and standardized sera (Seeliger and Langer, 1979). Although most clinical isolates belong to serovars 1/2a, 1/2b, and 4b are the majority of strains which have caused large outbreaks were serovar 4b (Kathariou, 2000), and serovar 1/2a (Jacquet et al., 2002; Zhang and Knabel, 2005). Serovar identification by serological tests has remained popular. However, numerous molecular biology methods such as multiplex PCR (Doumith et al., 2004) have come to the fore in the characterization of *L. monocytogenes* serotypes. Even though a recent study (Manani et al., 2006) reported the occurrence of *L. monocytogenes* in frozen vegetables in this country, there is little data on the occurrence of this pathogen in foods in Botswana. This investigation was carried out to unravel the prevalence and scope of antimicrobial resistance profiles of *L. monocytogenes* serovars isolated from various retail foods in Gaborone, Botswana.

## MATERIALS AND METHODS

### Sampling

Samples were obtained randomly from selected supermarkets and street vendors in 5 geographical areas of Gaborone (east, west, north, south, and central). Samples collected were; raw vegetables (cabbage), salads, raw milk, cheese and meat (biltong). In this study, 250 - 300 samples per product were obtained. The samples were put in separate properly labeled sterile specimen bags and placed into a cooler box containing ice packs. Gloves were worn to avoid cross-contamination between samples from different supermarkets and street vendors. Aseptic technique was followed to avoid contamination during transport of the samples from the supermarkets to the laboratory.

### Enrichment, culturing, morphological and biochemical identification

On arrival at the laboratory, the samples were transferred to properly labeled stomacher bags and then homogenised with the Stomacher (Seward 400, Tekmar, and Cincinnati Ohio, USA) set at medium speed. The homogenised samples were enriched by placing 25 g of the sample into 225 ml enrichment broth (Mast Diagnostics, Merseyside, UK) and incubated at 30°C for 48 h on Innova 4000 New Brunswick Scientific incubator shaker. A loop full of culture was sub-cultured on Modified Listeria Selective Agar (Oxoid, Basingstoke, UK) supplemented with Listeria Selective Enrichment Supplement (Oxoid) and then incubated at 37°C for 24 h. Dark brown colonies with black zones characteristic of *Listeria*

**Table 1.** Incidence of *Listeria monocytogenes* in various food products.

| Food product      | No. of isolates | % of positive isolates |
|-------------------|-----------------|------------------------|
| Cheese            | 7               | 2.75                   |
| Raw milk          | 3               | 1.08                   |
| Biltong           | 0               | 0.00                   |
| Frozen cabbage    | 27              | 10.11                  |
| Salads (coleslaw) | 20              | 7.41                   |

were sub-cultured on nutrient agar. A Gram stain was performed on suspected colonies and Gram positive short rods colonies were sub-cultured onto tryptose soy agar (Merck, Darmstadt, Germany) slants and these were maintained at 4°C. Following this, the catalase test was performed on all the isolates and catalase positive isolates were identified to the species level by API Listeria (Oxoid). Isolates that were confirmed as *L. monocytogenes* were preserved in a solution containing 80% tryptose soy broth (Oxoid) and 20% glycerol at -80°C for use in the steps that followed.

### Antibiotic susceptibility testing

Isolates that were confirmed as *L. monocytogenes* were inoculated on Mueller-Hinton broth (Oxoid, Basingstoke, Hampshire, England). The flasks were incubated at 37°C on a Gallenkamp shaker (200 rpm) for 24 h. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standard. One milliliter of the cell suspension was then transferred onto the surface of Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England) and then spread evenly. The susceptibilities of all isolates to different antimicrobial agents were tested by the disk-agar method as standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 1998). The following panel of antimicrobial disks and concentrations were used; chloramphenicol (25 µg), erythromycin (5 µg), fusidic acid (10 µg), methicillin (10 µg), novobicin (5 µg), penicillin G (1 U), streptomycin (10 µg), tetracycline (25 µg) (Mast Diagnostics, Merseyside, UK) as well as ampicillin (25 µg), cephalothin (30 µg), sulphamethaxazole/trimethoprim (25 µg), gentamicin (30 µg), and nitrofurantoin (10 µg) (Oxoid). They were obtained from the South African Bureau of Standards (Pretoria, South Africa) *L. monocytogenes* ATCC 19115 was used as the reference strain.

## RESULTS

### Prevalence of *L. monocytogenes*

*L. monocytogenes* was found in all food products except biltong. Table 1 shows that among the food products, the organism was frequently isolated from frozen cabbage (10.11%), while raw milk recorded the least number of isolates (1.08%). *L. monocytogenes* was not isolated at all from biltong.

The geographical areas from which *L. monocytogenes* was sampled seemed to affect the incidence of the organism (Table 2). Of the geographical zones sampled in this study, Gaborone South recorded the most number (33.33%) whilst Gaborone West recorded the least

**Table 2.** Prevalence of *L. monocytogenes* in different localities in Gaborone.

| Geographical area | No. of positive isolates | % of positive isolates |
|-------------------|--------------------------|------------------------|
| Gaborone East     | 9                        | 15.79                  |
| Gaborone West     | 7                        | 12.28                  |
| Gaborone North    | 9                        | 15.79                  |
| Gaborone South    | 19                       | 33.33                  |
| Gaborone Central  | 13                       | 22.81                  |

(12.28%) with Gaborone East and North recording slightly higher than Gaborone West.

### Antimicrobial susceptibility testing of *L. monocytogenes*

Antimicrobial susceptibility testing was performed on all the 57 confirmed *L. monocytogenes* isolates. Of these isolates, 31 (54.39%) were found to be resistant to one or more antibiotic. Resistant rates to penicillin G, sulphamethoxazole/trimethoprim, chloramphenicol, and tetracycline were encountered in 42.11, 29.82, 28.30, and 22.81%, respectively (Table 3). Antibiotic resistance was not encountered for fusidic acid, erythromycin, methicillin, ampicillin and cephalothin. In all, 15 different resistance patterns were observed. Of the food products tested, frozen cabbage and salads recorded the highest diversity of resistance patterns. From the resistance patterns, only one pattern (penicillin G and tetracycline) was common among all the food products that tested positive for *L. monocytogenes* (Table 4). Otherwise, the rest of the resistant patterns were unique or peculiar to the different food products that were tested.

## DISCUSSION

In the present study contamination rates of food products with *L. monocytogenes* were 2.75, 1.08, 0.00, 10.11, and 7.41% for cheese, raw milk, biltong, frozen cabbage, and salads (coleslaw), respectively. *L. monocytogenes* is known to contaminate milk and milk products such as cheese because of the complex nature of these products (Cimmons, 2001). The non-occurrence of *L. monocytogenes* in biltong is significant in public health because the product is a ready-to-eat (RTE) and dry meat. Most reports on the occurrence of this food borne pathogen in meats have tended to concentrate on raw meat, meat mixed with salads and poultry. Low water activity had been shown to profoundly limit the growth and multiplication of the pathogen in some conditions (Vermeulen et al., 2007). Biltong from this country is exported to the EU markets and the absence of the organism may be due to the zero tolerance limits in the processing of the meat.

The incidence rate (2.75%) of *L. monocytogenes* in

cheese was less than the 8.2% reported in the United Kingdom (Greenwood et al., 1991) and the 6.4% reported in Germany (Rudolph and Scherer, 2001). In a previous study in Iran (Moshtaghi and Mohamadpour, 2007), the incidence of *L. monocytogenes* in raw milk was found to be 1.6%, and is similar to the 1.08% in the present study. The prevalence of the organism in cheese is hazardous to consumers because cheese is a ready to eat (RTE) food. Although raw milk is heat treated (pasteurization) before consumption, where the raw milk is processed to dairy products before pasteurization it poses a health risk to the consuming population.

Of public health concern is the occurrence of the microorganism in frozen cabbage (10.11%) and salads (7.41%). A recent study (Little et al., 2007) found a lower proportion (6.0%) of salads to be contaminated with *L. monocytogenes* while another study conducted in the United States of America (Prazak et al. 2002) found an even lower number (2.34%) of cabbage samples to be contaminated. The high incidence of the microorganism in salads and cabbage are alarming because the salads (coleslaw) are ready to eat and the cabbage is sometimes eaten raw when it is mixed with other vegetables to make salads. According to Little et al. (2007), the occurrence of *L. monocytogenes* in pre-packaged mixed salads could result from the original contamination of raw material, cross contamination during processing, packaging or at retail. In chilled foods such as coleslaw and frozen cabbage, temperature is the principal controlling factor for their safety. However, *L. monocytogenes* has been shown to thrive under refrigeration temperatures (Duffy et al., 1994).

Among the five geographical areas sampled in the present study, the highest prevalence rate was recorded in Gaborone south (33.33%) whereas Gaborone west recorded the least (12.28%). It is important to note that Gaborone South is characterized by overcrowding and the inhabitants are generally of low economic and educational status. An interesting point to note from this study is that there were no significant differences in the prevalence of *L. monocytogenes* in retail supermarkets and street vendors. A previous study in South Africa (Lues et al., 2006) found the microbiological quality of foods served by street vendors to be within acceptable safety limits. In the same study the occurrence of specific microorganisms was thought to be indicative of a degree of ignorance on the part of the food handlers towards proper hygienic practices.

This study found resistance to penicillin G, sulphamethoxazole/trimethoprim, chloramphenicol, and tetracycline to be 42.11, 29.82, 28.30 and 22.81%, respectively. However, no isolate was resistant to fusidic acid, erythromycin, methicillin, ampicillin and cephalothin. In contrast to the present study, Dhanashree et al. (2003) found no strain that was resistant to chloramphenicol in a study in India as did Facinelli et al. (1991) in a survey of Italian meat and dairy products. Tetracycline resistance

**Table 3.** Susceptibility of *L. monocytogenes* to 13 antimicrobial agents.

| Antibiotic                     | Sensitive (%) | Intermediate (%) | Resistant (%) |
|--------------------------------|---------------|------------------|---------------|
| Chloramphenicol                | 71.70         | -                | 28.30         |
| Fusidic acid                   | 100           | -                | -             |
| Erythromycin                   | 100           | -                | -             |
| Methicillin                    | 100           | -                | -             |
| Novobicin                      | 85.96         | -                | 14.04         |
| Penicillin G                   | 54.79         | 3.10             | 42.11         |
| Streptomycin                   | 80.70         | -                | 19.30         |
| Tetracycline                   | 67.84         | 9.35             | 22.81         |
| Ampicillin                     | 100           | -                | -             |
| Cephalothin                    | 100           | -                | -             |
| Sulphamethaxazole/trimethoprim | 70.18         | -                | 29.82         |
| Gentamicin                     | 84.21         | -                | 15.79         |
| Nitrofurantoin                 | 92.98         | -                | 7.02          |

**Table 4.** Resistance patterns of *L. monocytogenes* isolates from food sources.

| Food source | No. of isolates | Resistant pattern   | No. of resistant strains | % Resistant |
|-------------|-----------------|---------------------|--------------------------|-------------|
| Cheese      | 7               | PG, T               | 1                        | 14.29       |
|             |                 | C, PG, NO, S        | 1                        | 14.29       |
|             |                 | C, PG, NO, S, T     | 1                        | 14.29       |
| Raw Milk    | 3               | PG, T               | 1                        | 33.33       |
| Cabbage     | 27              | PG, T               | 2                        | 7.41        |
|             |                 | S, SXT, T           | 2                        | 7.41        |
|             |                 | C, PG, SXT          | 3                        | 11.11       |
|             |                 | C, PG, NO, T        | 1                        | 3.70        |
|             |                 | C, CN, PG, SXT      | 5                        | 18.51       |
|             |                 | CN, PG, NO, SXT     | 1                        | 3.70        |
|             |                 | C, NI, PG, NO, S, T | 1                        | 3.70        |
| Salad       | 20              | PG, T               | 1                        | 5.00        |
|             |                 | NO,PG,T             | 1                        | 5.00        |
|             |                 | PG, S, SXT          | 3                        | 15.00       |
|             |                 | CN,PG,SXT           | 3                        | 15.00       |
|             |                 | C, PG, S, T         | 1                        | 5.00        |
|             |                 | C, NI, PG, T        | 1                        | 5.00        |
|             |                 | C, NI, PG, NO, S    | 2                        | 10.00       |

The antibiotics are Chloramphenicol (C), Gentamycin (CN), Novomycin (NO), Nitrofurantoin (NI), Penicillin G (PG), Streptomycin (S), Sulphamethoxazole/Trimethoprim (SXT), Tetracyclin (T).

has been the most frequently observed phenotype among *L. monocytogenes* strains (Charpentier et al., 1995). Tetracycline resistance in this study was noted to be much higher than the 8.4% reported USA (Zhang et al., 2007). Tetracycline resistance is thought to originate from the use of antibiotic in animal production (Schroeder et al., 2002). Antibigram profiles point to the high diversity of the microorganism in the food products tested. Since the first isolation of a multiresistant strain of *L. monocytogenes* in France in 1998 (Poyart -Salmeron et

al., 1990) multi-resistant strains have been extensively isolated.

The findings clearly highlight the occurrence of *L. monocytogenes* (serotypes 1/2a and 1/2b) among retailers and street vendors in the 5 geographical areas of Gaborone. The presence of this human pathogen in ready-to-eat foods should be considered as having significant public health implications, particularly among the immunocompromised and HIV/AIDS persons who are at greater risk.

It was evident that *L. monocytogenes* was resistant to chloramphenicol, penicillin G, sulphamethazole/trimethoprim and tetracycline, suggesting that the incorrect use of these antimicrobial agents for therapeutic purposes in animals and humans may lead to the development of antibiotic resistance.

We recommend education of stakeholders such as product suppliers, supermarket management, cleaning staff and hygiene specialists on the biology of food borne infections and the intricate need to maintain the cold chain order to prevent food borne outbreaks.

## ACKNOWLEDGEMENTS

ICM wishes to convey sincere gratitude and thanks to the University of Botswana for awarding a sponsorship for this research work. We are indebted to Mr Daniel Loeto for the great technical assistance provided during this work. Special thanks go to Dr M. Dithlogo, Head of Biological Sciences Department and Mrs K. Modisanyane-Kelaeng, Chief technician for their unique roles. Finally, appreciation is extended to supermarket managers and street vendors for affording us the opportunity to collect the various samples.

## REFERENCES

- Buchanan RL, Smith JL, Long W (2000). Microbial risk assessment: dose-response relations and risk characterization. *Int. J. Food Microbiol.* 58: 159-172.
- Buchanan RL, Golden MH, Whiting RC (1993). Differentiation of the effects of pH and lactic acid or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. *J. Food Prot.* 56: 474-478.
- Charpentier E, Gerbaud G, Jacquet C, Rocourt J, Courvalin P (1995). Incidence of antibiotic resistance in *Listeria* species. *J. Infect. Dis.* 172: 277-281.
- Cimmons M (2001). Food safety concerns drive FDA review of fine cheeses. *ASM News* 67: 1-6.
- Dhanashree D, Otta SK, Karunasagar I, Goebel W, Karunasagar (2003). Incidence of *Listeria spp.* in clinical and food samples in Mangalore, Ind. *Food Microbiol.* 20: 447-453.
- Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P (2004). Differentiation of major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42: 3819-3822.
- Duffy LL, Vanderlinde PB, Grau FH (1994). Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of low pH, aw, nitrite and ascorbate. *Int. J. Food Microbiol.* 23: 377-390.
- Embarek PKB (1994). Presence, detection and growth of *Listeria monocytogenes* in seafood: a review. *Int. J. Food Microbiol.* 23: 17-34.
- Farber JM, Peterkin PI (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55: 476-511.
- Facinelli B, Giovanetti E, Varaldo PE, Casolari P, Fabio U (1991). Antibiotic resistance in foodborne *Listeria*. *Lancet* 338: 1272.
- Finlay BB (2001). Cracking *Listeria's* password. *Science* 292: 1665-1667.
- Franco ACM, Quinto FEJ, Fente SC, Rodriguez Ot JL, Dominguez RL, CSA (1994). Susceptibilities of *Listeria* species isolated from food to nine antimicrobial agents. *Antimicrob. Agents Chemother.* 38: 1655-1657.
- Greenwood MH, Roberts D, Burden P (1991). The occurrence of *Listeria* species in milk and dairy products: a national survey in England and Wales. *Int. J. Food Microbiol.* 12: 197-206.
- Huss HH, Jorgenssen LV, Vogel BF (2000). Control options for *Listeria monocytogenes* in seafood. *Int. J. Food Microbiol.* 62: 267-274.
- Jacquet C, Gouin E, Jeannel D, Cossart P, Rocourt J (2002). Expression of ActA, Ami, InlB, and listeriolysin O in *Listeria monocytogenes* of human and food origin. *Appl. Environ. Microbiol.* 68: 616-622.
- Kathariou S (2000). Pathogenesis determinants of *Listeria monocytogenes*. In Cary JW, Linz JE, Bhatnagar D, Lancaster PA (ed.), Technomics Publishing Co., Inc., *Microbial Foodborne Dis.* pp. 295-314.
- Little CL, Taylor FC, Sagoo SK, Gillespie LA, Grant K, McLauchlin J (2007). Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in retail pre-packaged mixed vegetable salads in the UK. *Food Microbiol.* 24: 711-717.
- Lues JF, Rasephei MR, Venter P, Theron MM (2006). Assessing food safety and associated food handling practices in street food vending. *Int. J. Environ. Health Res.* 16: 319-28.
- Manani TA, Collison EK, Mpuchane S (2006). Microflora of minimally processed frozen vegetables sold in Gaborone, Botswana. *J. Food Prot.* 69: 2581-2586.
- McLauchlin J, Mitchel RT, Smerdon WJ, Jewell K (2004). *Listeria monocytogenes* and listeriosis. A review of hazard characterization for use in microbial risk assessment of foods. *Int. J. Food Microbiol.* 92: 15-33.
- Mead PS, Slutsker L, Dietz V, McCraig LF, Breese JS, Shapiro C, Griffin PM, Tauxe RV (1999). Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5: 607-623.
- Moshtaghi H, Mohamadpour AA (2007). Incidence of *Listeria spp.* in raw milk in Shahrekord, Iran. *Foodborne Pathog. Dis.* 4: 107-10.
- National Committee for Clinical Laboratory Standards (NCCLS) (1998). Performance testing for antimicrobial Susceptibility Testing, 8<sup>th</sup> information supplement. NCCLS, Wayne, Pa. Vol. 18.
- Nolan DA, Chamblin DC, Troller JA (1992). Minimal water activity levels for *Listeria monocytogenes* and *Listeria innocua*. *Int. J. Food Microbiol.* 16: 323-335.
- Seeliger HPR, Langer B (1979). Serotyping of *Listeria monocytogenes* and related species. *Methods Microbiol.* 13: 31-49.
- Prazak AM, Murano EA, Mercado I, Acuff GR (2002). Prevalence of *Listeria monocytogenes* during production and postharvest processing of cabbage. *J. Food Prot.* 65: 1728-1734.
- Schroeder M, Zhao C, DebRoy C, Torcolini J, Zhao S, White DG, Wagner DD, McDermott PF, Walker RD, Meng J (2002). Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. *Appl. Environ. Microbiol.* 68: 576-581.
- Slutsker L, Schuchat A (1999). Listeriosis in humans. In: Ryser ET, Marth EH (Eds.), *Listeria, Listeriosis and Food Safety*, 2<sup>nd</sup> Edition. Marcel Dekker, New York, pp. 75-96.
- Vermeulen A, Gysemans KPM, Benaerts K, Geeraerd AH, Van Impe JF, Debevere J, Devlieghere F (2007). Influence of pH, water activity and acetic acid concentration on *Listeria monocytogenes* at 7°C: Data collection for the development of a growth/no growth model. *Int. J. Food Microbiol.* 114: 332-341.
- Wagner M, Allerberger F (2003). Characterization of *Listeria monocytogenes* recovered from 41 cases of sporadic cases in Austria by serotyping and pulsed-field gel electrophoresis. *FEMS Immunol. Med. Microbiol.* 35: 227-234.
- Wiedmann M (2002). Molecular subtyping methods for *Listeria monocytogenes*. *J. AOAC Int.* 85: 524-531.
- Zhang W, Knabel SJ (2005). Multiplex PCR assay simplifies serotyping and sequence typing of *Listeria monocytogenes* associated with human outbreaks. *J. Food Prot.* 68: 1907-1910.
- Zhang Y, Yeh E, Hall G, Cripe J, Bhagwat AA (2007). Characterization of *Listeria monocytogenes* isolated from retail foods. *Int. J. Food Microbiol.* 113: 47-53.