African Journal of Microbiology Research Vol. 6(22), pp. 4702-4706, 14 June, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1329
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Prevalence and antibiotic resistance of *Listeria* species in food products in Taipei, Taiwan

Feng-ing Wang[1], Ming-kai Chern[2], Chi-wang Li[3], Min Yan[4,5]* and Yu-hsien Hsieh[3, 6]

[1]Department of Food and Beverage Management, China University of Science and Technology, Taipei, Taiwan.
[2]Department of Chemistry, Tamkang University, 151 Ying-Chuan Road, Tamsui District 25137, New Taipei City, Taiwan.
[3]Department of Water Resources and Environmental Engineering, Tamkang University, 151 Ying-Chuan Road, Tamsui District 25137, New Taipei City, Taiwan.
[4]The Institute of Oral Sciences, Chung Shan Medical University, 110, Sec.1, Jianguo North Road, Taichung City 402, Taiwan.
[5]Department of Dentistry, Chung Shan Medical University, 110, Sec.1, Jianguo North Road, Taichung City 402, Taiwan.
[6]The Center for Environment Protection, Safety and Health, Tamkang University, 151 Ying-Chuan Road, Tamsui District 25137, New Taipei City, Taiwan.

Accepted 16 March, 2012

A total of 400 samples including meat products, dairy and dairy products, fresh vegetables, fresh seafood, and ready-to-eat food products from supermarkets in Taipei area were collected and analyzed for the prevalence of *Listeria* species. The overall occurrence of *Listeria* spp. was 16.5%, and most of them were isolated from meat products and vegetables. *Listeria monocytogenes* was isolated from 22 out of the 400 (5.5%) samples studied. Other species found were *Listeria innocua* (7.5%), *Listeria ivanovii* (1%), *Listeria seeligeri* (0.5%), *Listeria grayi* (0.5%) and *Listeria welshimeri* (1.5%). The possibility of antibiotic resistance of the 66 isolated *List*eria spp. was also examined by the standard disk diffusion method. *L. monocytogenes* strains isolated from food sample were treated with 8 antibiotics currently used in human or domestic animal therapy. Considering the fact that *L. monocytogenes* is slowly becoming antibiotic resistant, a continuous examination of emerging antimicrobial resistance of this pathogen is important to ensure effective treatment of human listeriosis. Overall, *Listeria* spp. was resistant to penicillin (7.58%), chloramphenicol (3.7%) and tetracycline (1.96%), but sensitive to amoxicillin, vancomycin, ampicillin, rifampicin and sulfamethoxazole. The results in this study are helpful in enriching the data on antibiotic resistance of strains isolated from food and in developing effective risk management strategies.

Key words: *Listeria* spp., *Listeria monocytogenes,* moxicillin, vancomycin, ampicillin, rifampicin.

INTRODUCTION

Listeria monocytogenes is a bacterial pathogen that contaminates many ready-to-eat (RTE) food products, of which the list includes but is limited to meat products, dairy and dairy products, fresh vegetables, fresh seafood, and ready-to-eat food. Consumption of foods contaminated with this pathogen can lead to listeriosis (Conter et al., 2009), a disease characterized by symptoms and conditions such as diarrhea, encephalitis, and miscarriage in pregnant women. Since the population of industrial countries was the largest consumers of processed foods, they have a high mortality rate associated with food contamination with *L. monocytogenes*.

L. monocytogenes is a widely recognized food-borne pathogen that can survive under adverse conditions of temperatures, pH, and water activity. This pathogen has been found in a wide range of foods, including meat

*Corresponding author: E-mail: yan@csmu.edu.tw. Tel: 886- 4-24718668, ext. 55523.

products, dairy and dairy products, fresh vegetables, fresh seafood, and ready-to-eat food, milk (Arslan and Ozdemir, 2008; EFSA, 2007; Gianfranceschi et al., 2007; Bell and Kyriakides, 2005; Schlech, 2000), milk products, soymilk (Hsieh et al., 2011; Yan et al., 2011), and fruit (Hsieh et al., 2010). Since 1980's, several outbreaks of listeriosis associated with the consumption of contamination in all five category of food have been reported; this raises a great concern to the dairy industry, because of the increasing number of cases, with an overall mortality rate of about 30% (Griffiths, 1989).

In Taiwan, there is a lack of information on the occurrences of *L. monocytogenes* in food samples, and we have no knowledgeable documentation of listeriosis outbreaks. Therefore, the present study was undertaken to assess the prevalence of *L. monocytogenes* and other *Listeria* spp. in various food samples in Taipei, Taiwan. *Listeria* spp. have been reported as susceptible to antibiotics active against Gram-positive bacteria, but recently cases of antibiotic resistance in *Listeria* spp. have been reported (Walsh et al., 2001; Arslan and Ozdemir, 2008; Conter et al., 2009). As a matter of fact, only a limited number of cases are reported of the incidence of listeriosis in the whole Asia each year. Consequently, more information is required on the patterns of distribution of antibiotic resistance with *Listeria* spp.

This finding would provide information to evaluate health risks for consumers, and to determine the susceptibility of *Listeria* spp. isolated in Taipei, Taiwan to the antimicrobials used in food production.

Objective

This study is aimed at a comprehensive survey of the prevalence and antibiotic resistance of *Listeria* species in food products in Taipei, Taiwan.

MATERIALS AND METHODS

Food samples

In this study, a total of 400 samples including meat products 90 samples, dairy and dairy products 70 samples, fresh vegetables 90 samples, fresh seafood 90 samples, and ready-to-eat food products 60 samples were purchased from local retail supermarkets and traditional markets in Taipei and study for the absence of *Listeria* spp. The samples were transported under insulated cold boxes to the laboratory and were analyzed on the same day.

Isolation and identification of *Listeria* spp.

The isolation and purification procedures were performed in accordance with the method ISO 11290-1; twenty-five grams of a sample was aseptically taken, blended for 2 min in 225 ml of University of Vermont Media (UVM) *Listeria* enrichment broth (UVM I) (Merck, Germany) and incubated at 37℃ for 24 h. One milliliter of primary enrichments were transferred to 9 ml of UVM II (Frazer broth) (Merck, Germany) and incubated at 37℃ for 24 h. Secondly, enrichments were streaked on Oxford agar (Merck, Germany) and PALCAN agar (Merck, Germany) and incubated at 37℃ for 48 h. The plates were examined for typical *Listeria* colonies (black colonies with black sunken center) and at least 3 suspected colonies were sub-cultured on Trypton Soy Agar supplemented with 0.6% of yeast extract (TSAYE) and incubated at 37℃ for 24 h. All the isolates of *Listeria* spp. were subjected to standard biochemical tests such as Gram staining, catalase test, motility at 25 and 37℃, acid production from glucose, manitol, xylose, rhamnose, α-methyl-D-mannoside, and nitrate reduction, esculin hydrolysis test. For the confirmation of *Listeria* spp., other biochemical reactions including ß-haemolytic activity, and CAMP test were performed according to the Bergey's Manual of Systematic Bacteriology (Seeliger and Jones, 1986).

Antimicrobial susceptibility

The antimicrobial susceptibility testing was performed following the standard disk diffusion method on Mueller–Hinton agar (Merck, Germany). Eight common antibiotics were chosen for the study. They were penicillin, 10 mg; ampicillin, 10 mg; amoxicillin, 30 mg; tetracycline, 30 mg; vancomycin, 30 mg; chloramphenicol, 30 mg; rifampicin, 5 mg; and sulfamethoxazole, 25 mg. All antibiotic disks used were obtained from Oxoid (Basingstoke, UK).

RESULTS

The actual situation of listeriosis in Taiwan is still unknown, and little information exists on the occurrence of *L. monocytogenes* in foods consumed in the country. It is also important to note that listeriosis is a disease ignored in Taiwan health system. In addition, there have been no criteria or standard for *L. monocytogenes* in food in the country. The food habit of Taiwan population is also different from other countries. Besides common foods, a significant variety of locally produced and traditional foods are consumed. The first step to persuade regulatory authorities and private manufacturers about the importance of *Listeria* in foods is to provide the data on the prevalence of this bacterium in various foods.

The isolation and detection of *Listeria* spp. were carried out from five categories of foods: Meat and meat products, dairy and dairy products, fresh vegetables, fresh seafood, and ready-to-eat food (Table 1). The samples were collected from supermarkets and traditional markets in Taipei, Taiwan. Among the total of 400 samples of examined, 66 (16.5%) were contaminated with *Listeria* spp. and *L. monocytogenes* was specifically detected in 22 (5.5%). The incidence of *L. monocytogenes* in beef, chicken liver, and pork intestine were 5 (16.67%), 3 (10%) and 8 (26.67%), respectively. Our study shows apparently lower incidence of *L. monocytogenes* contaminated in meat products when compared to some previous studies (Gibbons et al., 2006); probably the risk of infection with *L. monocytogenes* would be lower due to well cooking of food before consumption. Diary productions including

Table 1. Detection of *Listeria* spp. in five categories of food collected from supermarket in Taipei.

Food samples	No. of sample	Detected *Listeria* spp.		Detected *L. monocytogenes*	
		No.	(%)	No.	(%)
Meat and meat productions					
Beef	30	8	26.67	5	16.67
Chicken liver	30	15	50.00	3	10.00
Pork intestine	30	13	43.33	8	26.67
Dairy and dairy productions					
Yogurt	20	0	0.00	-	
Pasteurized milk	30	0	0.00	-	
Soymilk	20	0	0.00	-	
Fresh vegetable					
Celery	30	10	33.33	2	6.67
Lettuce	30	8	26.67	1	3.33
Tomatoes	30	4	13.33	1	3.33
Fresh seafood					
Shrimp	30	2	6.67	0	0.00
Fish	30	1	3.33	0	0.00
Octopus	30	2	6.67	0	0.00
Ready-to-eat food					
Ham salad	30	2	6.67	1	3.33
Tofu	30	1	3.33	1	3.33
Total	400	66	16.50	22	5.50

yogurt, milk and soymilk samples were free of *Listeria* spp. and Jalali and Abedi (2008) had the same result. In this study, *L. monocytogenes* were isolated from raw vegetables, 4 in 90 samples. The incidence of *Listeria* spp. in fresh vegetables has been reported in different studies (Dhanashree et al., 2003; Jalali and Abedi, 2008). In Taiwan, eating fresh vegetables is a common practice; therefore, the role of raw vegetables in the transmission of listeriosis should be taken in concern. The presence of *L. monocytogenes* in one sample (3.3%) of ham salad and one sample (3.3%) of tofu needs to be paid more attention, as unlike other foods, ham salad and tofu are not processed further. This situation may increase the risk of listeriosis posed by the consumption of ready-to-eat food. The fresh seafood also had no *L. monocytgenes* in all the 90 samples tested. Thus, our studies shows meat productions has lower detectable *Listeria* spp. in comparison with other countries including New Zealand (Hudson et al., 1992), Belgium (Uyttendaele et al., 1999), Korea (Baek et al., 2000), and Spain (De Simon et al., 1992). On the other hand, our finding is in agreement with that for Thailand (Stonsaovapak and Boonyaratanakornkit, 2010).

Table 2 lists the *Listeria* spp. isolated from 400 samples of food collected from supermarket in Taipei, Taiwan. Overall, 44 (11%) of food samples examined were positive for *Listeria* spp. other than *L. monocytogenes*; 30 (7.5%) were positive for *L. innocua*; 4 (1%) for *L. ivanovii*; 2 (0.5%) for *L. seeligeri*; 2 (0.5%) for *L. grayi*; and 6 (1.5%) for *L. welshimeri*. The results of our study indicate much lower incidence of *L. monocytogenes* contaminated in meat and meat products when compared to some previous studies (Gibbons et al., 2006).

The antimicrobial susceptibility of the 66 strains of *Listeria* spp. isolated from five categories of food samples was also examined by the standard disk diffusion method. Table 3 summarizes the antimicrobial susceptibility profiles of *Listeria* spp. Resistance to penicillin was the most common, with five isolates showing resistance to this antibiotic. Chloramphenicol was the second common one, with two isolates showing resistance, followed by tetracycline with one isolate. All *Listeria* isolates were sensitive or displayed intermediate susceptibility to ampicillin, amoxicillin and vancomycin.

Table 4 shows the distribution of resistant isolates in *Listeria* spp. against these three antibiotics according to individual species. The level of resistance in *L. monocytogenes* was low (9.09%) compared with *L. innocua* (13.3%), *L. ivanovii* (25.0%) and *L. seeligeri* (50.0%). No resistance was observed in *L. grayi* or *L. welshimeri*. Interestingly, our data for penicillin, tetracycline, and chloramphenicol are in agreement with

Table 2. *Listeria* spp. isolated from 400 samples of food collected from supermarkets in Taipei.

***Listeria* species**	**No. of positive samples**	**(%) of positive samples**
L. monocytogenes	22	5.50
L. innocua	30	7.50
L. ivanovii	4	1.00
L. seeligeri	2	0.50
L. grayi	2	0.50
L. welshimeri	6	1.50
Total	66	16.50

Table 3. *Listeria* spp. isolates susceptible to antimicrobial agents.

Antibiotic	**Dose (mg/disk)**	**Resistant**		**isolates Intermediate**		**Susceptible**	
		No	**%**	**No.**	%	**No.**	%
Penicillin	10	5	7.58	0	0.00	61	92.42
Ampicillin	10	0	0	0	0.00	66	100.00
Amoxicillin	30	0	0	0	0.00	66	100.00
Tetracycline	30	1	1.96	14	21.21	51	77.27
Chloramphenicol	30	2	3.7	10	15.15	54	81.82
Rifampicin	5	0	0	12	18.18	54	81.82
Vancomycin	30	0	0	0	0.00	66	100.00
Sulfamethoxazole	25	0	0	6	9.09	60	90.91

Table 4. Number of individual *Listeria* spp. isolates resistant to antibiotics.

***Listeria* secies**	**No. of isolates**	**Percentage of isolates (%)**	**Penicillin**	**Tetracycline**	**Chloramphenicol**
L. monocytogenes	22	9.09	2	0	0
L. innocua	30	13.33	2	1	1
L. ivanovii	4	25.00	1	0	0
L. seeligeri	2	50.00	0	0	1
L. grayi	2	0.00	0	0	0
L. welshimeri	6	0.00	0	0	0
Total	66	12.12	5	1	2

those reported by Li et al. (2007). Other researchers (Stonsaovapak and Boonyaratanakornkit, 2010) found that most *L. monocytogenes* were resistant to ampicillin and rifampicin. These results were in obvious contrast to those obtained in the present study. Generally, overall incidence of antibiotic resistance in *L. monocytogenes* is relatively low here. However, the study confirms that since 1990 when the first antibiotic-resistant strains of *L. monocytogenes* had been reported (Poyart-Salmeron et al., 1990), there has been continuing observation of the emergence of strains of *Listeria* spp. isolated from food or from clinical cases of listeriosis, which are resistant to one or multiple antibiotics (Zhang et al., 2007). Our results agree with those of Rota et al. (1996) and (Walsh et al., 2001) indicating that higher percentage of *L. innocua* was resistant to antibiotics than *L. monocytogenes*.

DISCUSSION

This research confirms the observation of *Listeria* being a common contaminant in food products, and Taiwan is no exception. In lack of knowledge of documented listeriosis outbreaks, it is important to identify this possibility. More studies on the occurrence of *Listeria* spp. are needed to establish microbiological criteria of foods in other parts of the country. In addition, a recommendation for *L. monocytogenes* in food needs to be made by a governmental authority. The information obtained from the

present study demonstrated that a wide and rapidly expanding range of undesirable and, in some cases, multiresistant determinants, is currently present in members of the *Listeria* genus with significant potential for transfer to the currently pathogenic species, that is, *L. monocytogenes.* These data may well provide a background of antibiotic resistance of strains isolated from food in public health studies of *L. monocytogenes.*

Our finding would provide useful information to evaluate health risks for consumers, and to determine the susceptibility of *Listeria* spp. isolated from retail food products in Taiwan in the use of antimicrobials.

REFERENCES

Arslan S, Ozdemir F (2008). Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese. Food Control, 19: 360–363.

Baek SK, Lim SY, Lee DH, Min KH, Kim CM (2000). Incidence and characterization of *Listeria monocytogenes* from domestic and imported foods in Korea. J. Food Protect., 63: 186–189.

Bell C, Kyriakides A (2005). *Listeria*, A Practical Approach to the Organism and its Control in Foods, 2nd Ed. Blackwell Pub., London, pp 62–69.

Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, Ianieri A (2009). Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. Int. J. Food Microbiol., 128: 497-500.

De Simon M, Tarrago C, Ferrer MD, (1992). Incidence of *Listeria monocytogenes* in fresh foods in Barcelona (Spain). Int. J. Food Microbiol., 16: 153–156.

Dhanashree B, Otta SK, Karunasagar I, Goebel W, Karunasagar I (2003). Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India, Food Microbiol., 20: 447–453.

EFSA (European Food Safety Authority), (2007). Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness — Scientific Opinion of the Panel on Biological Hazards. The EFSA J., 599: 1–42.

Gianfranceschi MV, Gattuso A, D'Ottavio MC, Fokas S, Aureli P (2007). Results of a 12-month long enhanced surveillance of listeriosis in Italy. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=746.

Gibbons IS, Adesiyun A, Seepersadsingh N, Rahaman S (2006). Investigation for possible source(s) of contamination of ready-to-eat meat products with *Listeria* spp. and other pathogens in a meat processing plant in Trinidad. Food Microbiol., 23: 359–366.

Griffiths MW (1989). *Listeria monocytogenes*: itsimportance in the dairy industry. J. Sci. Food Agric., 47: 133–158.

Hsieh YH, Liu JG, Hwang JC (2010). Survival of Listeria monocytogenes inoculated in retail yogurt products. Proceedings of the World Academy of Science, Engineering and Technology, May 26, Tokyo, Japan.

Hsieh YH, Yan M, Liu JG, Hwang JC (2011). The synergistic effect of nisin and garlic shoot juice against *Listeria spp.* in soymilk. J. Taiwan Inst. Chem. Eng., 42(4): 576-579.

Hudson JA, Mott SJ, Delacy KM, Edridge AL (1992). Incidence and coincidence of *Listeria* spp. motile aeromonads and Yersinia enterocolitica on ready-to-eat fleshfoods. Int. J. Food Microbiol., 16: 99–108.

Jalali M, Abedi D (2008). Prevalence of *Listeria* species in food products in Isfahan, Iran. Int. J. Food Microbiol., 122: 336–340.

Li Q, Sherwood JS, Logue CM (2007). Antimicrobial resistance of *Listeria* spp. recovered from processed bison. Lett. Appl. Microbiol., 44: 86–91.

Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P (1990). Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. Lancet, 335: 1422–1426.

Rota C, Yanguela J, Blanco D, Carraminana JJ, Arino A, Herrera A (1996). High prevalence of multiple resistance to antibiotics in 144 *Listeria* isolates from Spanish dairy and meat products. J. Food Protect., 59: 938–943.

Schlech WF (2000). Foodborne listeriosis. Clin. Infect. Dis., 31: 770–775.

Stonsaovapak S, Boonyaratanakornkit M (2010). Prevalence and antimicrobial resistance of Listeria species in food products in Bangkok, Thailand, J. food safety 30(1):145-161.

Uyttendaele M, De Troy P, Debevere J (1999). Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. Int. J. Food Microbiol., 53: 75–80.

Walsh D, Duffy G, Sheridan JJ, Blair IS, Mcdowell DA (2001). Antibiotic resistance among Listeria, including *Listeria monocytogenes*, in retail foods. J. Appl. Microbiol., 90: 517–522.

Yan M, Li CW, Hsieh YH (2011). Synergistic inhibition of *Listeria monocytogenes* by nisin and green onion extract. J. Food Saf., 31: 505-510.

Zhang Y, Yeh E, Hall G, Cripe J, Bhagwat AA Meng J (2007). Characterization of *Listeria monocytogenes* isolated from retail foods. Int. J. Food Microbiol., 113: 47-53.