

ORIGINAL ARTICLE

The characterization of *Listeria* spp. isolated from food products and the food-processing environment

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Keywords

antimicrobial resistance, *Listeria monocytogenes*: ready-to-eat foods, PFGE: serotyping.

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2009/1998: received 18 November 2009, revised 10 July 2010 and accepted 27 July 2010

doi:10.1111/j.1472-765X.2010.02928.x

Abstract

Aim: To enhance the information pertaining to the epidemiology of a collection of 378 *Listeria* spp. isolates obtained from several food-processing plants in Ireland over a 3- year period (2004–2007).

Methods and results: The collection was characterized by pulsed-field gel electrophoresis (PFGE). The most prevalent pulse-type was PFGE profile I ($n = 14.5\%$) that consisted mainly of environmental *Listeria* spp. samples. Serotyping of 145 *Listeria monocytogenes* isolates was performed. The most common serovar was 1/2a and comprised 57.4% ($n = 77$) of the *L. monocytogenes* collection. The other serovars were as follows: 4b (14.1%, $n = 19$), 1/2b (9.7%, $n = 13$), 4c (4.4%, $n = 6$) and 1/2c (6.7%, $n = 9$), respectively. Eleven isolates were identified as non-*Listeria* spp., the remaining ten *L. monocytogenes* isolates were nontypeable. The antimicrobial susceptibility testing revealed the antibiotic that isolates displayed the most resistance to was gentamicin (5%) followed by sulfamethoxazole-trimethoprim (2%), tetracycline and ciprofloxacin (1.5%).

Conclusions: The subtyping has indicated the diversity of the *Listeria* spp. The presence of serotype 1/2a, 1/2b and 4b in both raw and cooked ready-to-eat food products is a public health concern, as these serotypes are frequently associated with foodborne outbreaks and sporadic cases of human listeriosis. In addition, the emergence of antimicrobial-resistant *L. monocytogenes* isolates could have serious therapeutic consequences.

Significance and Impact of Study: The molecular subtyping and the further characterization of these isolates may be valuable particularly in the context of a suspected common source outbreak in the future.

Introduction

Listeria monocytogenes is a Gram-positive bacterium that is responsible for causing the foodborne disease listeriosis in humans (Cossart 2007). Foodborne outbreaks of human listeriosis have only been documented since the 1980s when the first human listeriosis outbreak was proven to be directly linked to the consumption of *Listeria*-contaminated food (Schlech *et al.* 1983). Within the genus, there are six *Listeria* species *L. monocytogenes*, *L. welshimeri*, *L. grayi*, *L. seeligeri*, *L. innocua* and *L. ivanovii*. *Listeria* is a genus of Gram-positive, facultative

bacteria, widely present in the environment and commonly isolated from water and soil and plant material undergoing decay such as silage (Farber and Peterkin 1991; Fenlon 1999). A variety of foods such as dairy products (milk, soft cheeses in particular), raw and cooked meats, raw vegetables, and raw and smoked seafood have been associated with disease transmission of foodborne listeriosis (Rocourt *et al.* 2003; Aguado *et al.* 2004; Gudbjornsdottir *et al.* 2004; Chou and Wang 2006).

Pulsed-field gel electrophoresis (PFGE) is a molecular subtyping method that has been successfully used to characterize *L. monocytogenes* isolates (Vela *et al.* 2001a;

Revazishvili *et al.* 2004; Corcoran *et al.* 2006; Wagner *et al.* 2007; Filiouis *et al.* 2009; Mammina *et al.* 2009; Kerouanton *et al.* 2010). PFGE has a high discriminatory power, is reproducible, can be standardized and can also reveal the genetic relatedness of the isolates. Serological classification method groups *L. monocytogenes* into 13 different serovars, but only three of the 13 serotypes (1/2a, 1/2b and 4b) are regularly implicated in clinical cases (Jacquet *et al.* 2002). Serotype 4b in particular has been implicated in c. 40% of sporadic cases and epidemic listeriosis outbreaks in Europe and North America (Schuchat *et al.* 1991).

Listeria monocytogenes has been closely monitored for antimicrobial resistance since the first emergence of multiresistant strains in France in 1988 (Poyart-Salmeron *et al.* 1990), and other strains resistant to one or more antibiotics have been recovered from environmental, food and from sporadic cases of human listeriosis (Safdar and Armstrong 2003; Zhang *et al.* 2007; Arslan and Ozdemir 2008; Davis and Jackson 2009). The importance of continuous monitoring of environmental, food and clinical *Listeria* isolates for antibiotic resistance is emphasized by the slow and gradual emergence of antimicrobial-resistant strains.

In Ireland, between 2004 and 2007, a total of 51 listeriosis cases were notified to the Health Service Executive (HSE). There were two fatalities (one adult and one neonate) and three intrauterine deaths. Since 2004, the occurrence of listeriosis cases has been reported as increasing. In 2007, there were 21 cases, a threefold increase in 2006 when seven cases were reported. Ready-to-eat (RTE) foods such as soft cheese and sliced cooked meats were reported as suspected sources, but this could not be proven (HPSC 2008). Owing to the rise in listeriosis cases and the suspected link to contaminated RTE food products, the key objective of this study was to characterize *L. monocytogenes* and other *Listeria* spp. isolated from various food categories (cooked & raw, meat & fish, dairy, fruit & vegetable) and the food-processing environment (internal walls, floors and drains) using traditional and molecular subtyping methods (serotyping and PFGE). Previous studies have shown human listeriosis cases have been associated with serotype 4b, 1/2b and 1/2a (Raybourne 2002; Borucki and Call 2003). This study aimed to examine the prevalence of such serotypes within our collection and to determine the prevalence and association of such serotypes with RTE products. Recent studies have shown that levels of antimicrobial resistance are gradually increasing (Conter *et al.* 2009; Davis and Jackson 2009); therefore, a subset of the collection was examined. Data obtained by this study will be submitted to a national database to enhance epidemiological surveillance of *Listeria* spp. in Ireland.

Materials and Methods

Bacterial isolates and identification

The collection in this study consisted of 378 *Listeria* isolates that were obtained from the Central Veterinary Research Laboratory (CVRL), during the period 2004–2007. The isolates were sourced from several food-processing plants within Ireland. The initial characterization of all isolates was performed following the United States Department of Agriculture, Food Safety and Inspection Service traditional culture protocol (USDA 2008). Within the collection, a set of 273 isolates were identified to species level by the commercial bio-typing kit api[®]-*Listeria* system (bioMerieux, Marcy-l'Etoile, France) and by the combined methods of real-time PCR and 16s rRNA sequencing (data not shown). The following species *L. monocytogenes* ($n = 145$), *L. welshimeri* ($n = 58$), *L. innocua* ($n = 42$), *L. grayi* ($n = 2$) and *L. seeligeri* ($n = 11$) were identified. A further 15 isolates were identified as non-*Listeria* spp. [*Enterococcus* spp. ($n = 12$), *Micrococcus* spp. ($n = 1$), *Entobacter* spp. ($n = 1$) and *Staphylococcus* spp. ($n = 1$)].

The *Listeria* isolates were collected from a variety of food categories, raw meat (porcine/poultry/bovine/fish or unspecified) ($n = 46$), cooked meat (fish/bovine/porcine) ($n = 52$), dairy (cheese/garlic butter) ($n = 10$), vegetables and fruit (apple, raw swede, mixed salad or unspecified) ($n = 13$), food ingredients, cover brine, stuffing, ($n = 8$), cereal (garlic baguette, pizza base) ($n = 8$), unknown raw food product ($n = 1$) and the food-processing environment ($n = 135$). Environmental isolates came from internal drains, floors, walls and door handles from several food-processing plants in Ireland. All isolates were stored in Protect[™] bacterial preservation vials (Medical Supply Ltd, Dublin, Ireland) in cryopreservation fluid at -70°C . Cultures were grown in brain heart infusion (BHI) agar or BHI broth (Oxoid, Hampshire, UK) at 37°C from beads when required.

Serotyping

The *L. monocytogenes*-positive isolates in the collection ($n = 145$) were serotyped using the ELISA as described by Palumbo *et al.* 2003. Briefly, the *L. monocytogenes* isolates were cultivated in BHI motility agar (BHI broth with 0.3% technical agar) (Oxoid) for 24 h at 30°C . The BHI motility agar culture was used to inoculate 2×10 ml BHI broth in 30-ml plastic universal tubes and incubated at 30°C for 24 h. The two broth cultures of each isolate were centrifuged (at 1920 g), one pellet was used for the O and the other for H antigen determination. For the somatic (O) antigen, a pellet was resuspended in 1 ml of

0.2% NaCl, and the cells were autoclaved at 121°C for 30 min to kill the cells. For the H antigen, the second pellet was resuspended in 1 ml 4% formaldehyde – 0.2% NaCl and incubated for 1 h to also kill cells. Both samples were centrifuged at 16 060 *g* for 10 min and resuspended in 1 ml of 0.2% NaCl. The optical density of the cells was adjusted in a Jenway 6300 Spectrophotometer (Lennox, Dublin, Ireland) at 600 nm to 0.35 using 0.2% NaCl. The ELISA was recorded on the μ Quant Microtiter Plate Reader (Medical Supply Co. Ltd). The reaction was measured at A_{405} until 1.1 was reached before 30 min. The reaction was stopped and the results recorded for each antiserum.

Pulsed-field gel electrophoresis

The PFGE typing was performed according to the CDC standardized PulseNet 30-h protocol, (Graves and Swaminathan 2001) with the following amendment. The gel was prepared with 0.5 \times Tris–borate EDTA buffer (TBE) (Bio-Rad Laboratories, Hercules, CA, USA) containing 50 μ mol l⁻¹ thiourea (Sigma-Aldrich, Dublin, Ireland) and electrophoresed on a CHEF-DR II apparatus (Bio-Rad Laboratories). The gels were stained with 1 μ g ml⁻¹ ethidium bromide (Sigma Aldrich), and the gel images were captured under UV illumination by the Syngene Gene Genius Bio-imaging system (Syngene; Synoptics Ltd, Cambridge, UK).

Data analysis

PFGE patterns were saved in TIFF format and transferred to BioNumerics Version 5.0 software (Applied Maths; NV Keistraat, Sint-Martens-Latem, Belgium). Similarity between fingerprints was determined by the Dice coefficient and using a band position tolerance of 1%. The dendrograms were generated to demonstrate similarities by the unweighted pair group method; using arithmetic averages (UPGMA).

Antimicrobial susceptibility testing

A subset of the collection was selected for antimicrobial susceptibility testing based on the PFGE profile. A dendrogram comparing all *Listeria* species was examined (data not shown). Within the PFGE clusters, if isolates were >80% related, only one isolate from within this cluster was randomly selected. Five species were tested – *L. monocytogenes*, *L. welshimeri*, *L. innocua*, *L. grayi* and *L. seeligeri*; in total, 213 isolates were selected for antimicrobial susceptibility testing. Antibiotic susceptibility of all isolates was established by the disc diffusion method recommended by the Clinical & Laboratory Standards Institute

formerly known as the National Committee for Clinical Laboratory Standards (NCCLS 2000). The antimicrobial agents used and their corresponding concentrations were as follows: penicillin (PEN) (10 units), ampicillin (10 μ g), cefaclor (30 μ g), ciprofloxacin (5 μ g), chloramphenicol (30 μ g), gentamicin (10 μ g), vancomycin (30 μ g), nalidixic acid (30 μ g), amoxicillin-clavulanic acid (30 μ g), sulfamethoxazole-trimethoprim (1.25/23.75 μ g) and tetracycline (30 μ g) (Oxoid). The diameter of the zone around each disc was measured to the nearest mm and interpreted in accordance with the breakpoints recommended by CLSI for Gram-positive micro-organisms and breakpoints utilized by Vela *et al.* 2001a,b. The breakpoints are as follows: penicillin (≤ 19) ampicillin (≤ 13), cefaclor (≤ 14), ciprofloxacin (≤ 15), chloramphenicol (≤ 12), gentamicin (≤ 12), vancomycin (≤ 12), amoxicillin-clavulanic acid (≤ 13), sulfamethoxazole-trimethoprim (≤ 10) and tetracycline (≤ 14). The control strain used was *Streptococcus pneumoniae* ATCC 49619.

Results

Serotyping

A total of 145 *L. monocytogenes* isolates identified by the commercial bio-typing *apti*[®]-*Listeria* kit were serotyped. A total of five different serotypes were found. The most common serovar was 1/2a and comprised 57.4% ($n = 77$) of the *L. monocytogenes* collection. The other serovars were as follows: 4b (14.1%, $n = 19$), 1/2b (9.7%, $n = 13$), 4c (4.4%, $n = 6$) and 1/2c (6.7%, $n = 9$), respectively. Molecular identification using the combined methods of real-time PCR and 16s rRNA DNA sequencing (data not shown) revealed that 11 isolates were not *Listeria* spp. Eight isolates were identified as *Enterococcus faecalis*, one as *Staphylococcus epidermidis* and one as *Micrococcus luteus*. The remaining ten isolates were identified as *L. monocytogenes* spp. using the molecular identification methods. However, these isolates could not be typed by both traditional slide agglutination and ELISA serotyping methods.

PFGE

The total collection of isolates ($n = 378$) was subtyped by PFGE. Twenty isolates were nontypeable by *ApaI* and *AscI* restriction. The nontypeable isolates were found to be contaminated or mixed cultures when plated using conventional selective media (real-time PCR negative). *L. monocytogenes* genomic DNA was digested using *AscI*, which generated 103 pulse-field types (PFTs) for 112 isolates (Fig. 1). The pulse-field types were labelled I-CXVI. Each profile had between 8 and 12 DNA fragments with

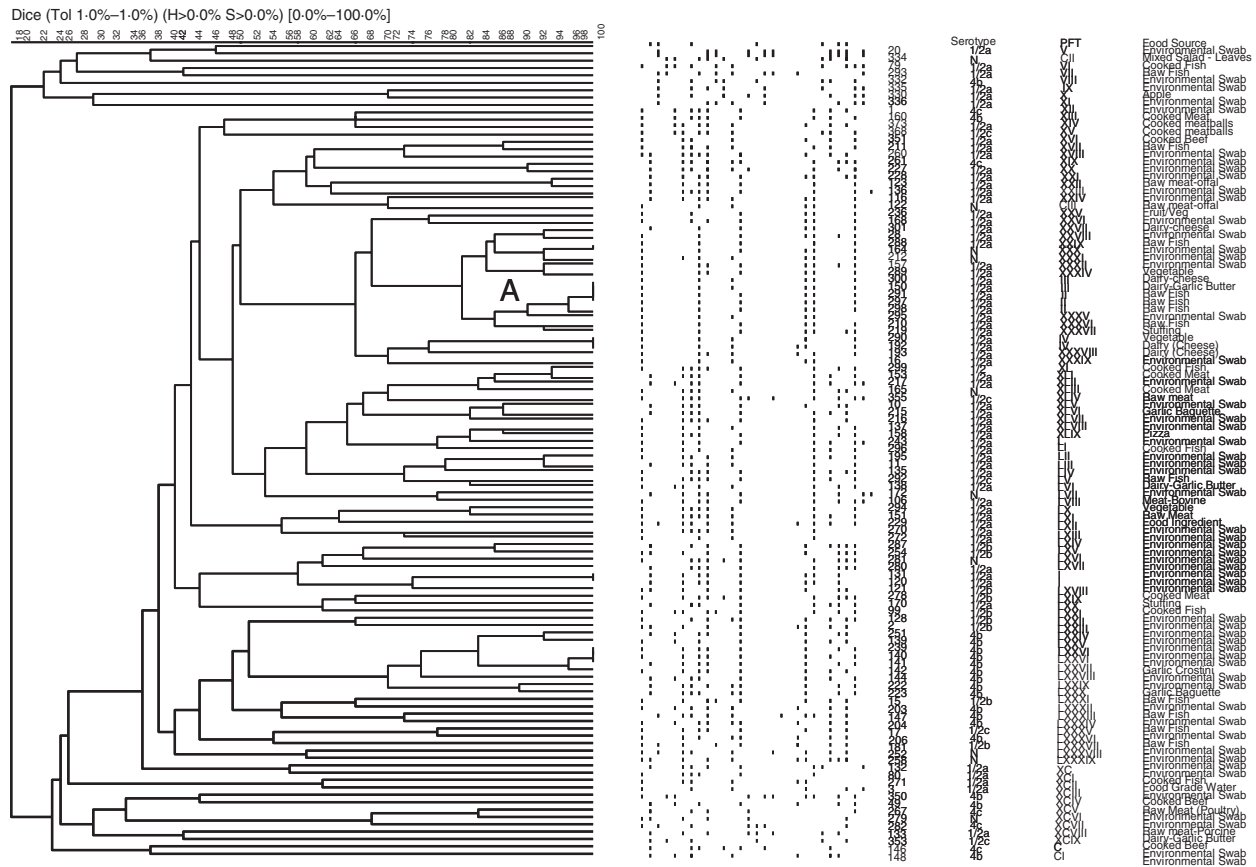


Figure 1 Pulsed-field gel electrophoresis dendrogram representing *Listeria monocytogenes* isolates obtained from a variety of food types and the food-processing environment.

sizes ranging from 50 to 770 kbp. There was no clear correlation between pulse-field type and a particular food type. The PFGE typing also revealed that there is a large genetic diversity amongst the *L. monocytogenes* isolate collection. There is one cluster above 80% similarity that there is correlation between the pulse-field types and the serotype 1/2a (Fig. 1).

Antimicrobial susceptibility of *Listeria* isolates

A subset of the isolates from the collection ($n = 213$) was selected based on the level of relatedness in a PFGE cluster (Fig. 1). Six of the 213 isolates selected were uncultivable and therefore could not be tested. A final collection of 207 *Listeria* spp. isolates were tested and the results analysed. It was found that 14.4% ($n = 30$) displayed resistance to at least one of the ten antibiotics tested. Resistance to one antibiotic was more common than multiple antibiotic resistance. Resistance to gentamicin (5%) was the most common followed by sulfamethoxazole-trimethoprim (2%), tetracycline and ciprofloxacin (1.5%)

(Table 1). Of the isolates identified to species level, 18.4% of *L. monocytogenes*, 18.1% of *L. seeligeri*, 13.6% of *L. innocua* and 8% of *L. welshimeri* isolates displayed resistance to at least one antibiotic. All *L. grayi* isolates were susceptible to all antibiotics tested. Two *L. monocytogenes* isolates were resistant to two antibiotics. None of the isolates tested were found to be resistant to more than three antibiotics.

Discussion

Serotyping and PFGE

In this study, five serotypes were identified (Table 2). The ELISA method of serotyping described by Palumbo *et al.* (2003) proved to be cost-effective and allowed the high throughput required for this study. The main advantages of this method over the traditional slide agglutination is the reduced variability of the antiserum quality by using a commercially available antisera and also the reduction of inconsistencies in judgement associated with weakly

Table 1 The profile of antibiotic-resistant *Listeria monocytogenes*, *L. welshimeri*, *L. seeligeri*, *L. innocua* isolates sampled from various food and environmental sources

I.D. No	Species	Source	Serotype*	PFT	Resistance profile
8	<i>L. monocytogenes</i>	Env (Unspecified)	4b	NT	SXT
35	<i>L. monocytogenes</i>	Mixed salad leaves	4b	NT	GM C
95	<i>L. monocytogenes</i>	Vegetable	4b	NT	CIP
136	<i>L. monocytogenes</i>	Env (Unspecified)	1/2a	XXIII	AM
137	<i>L. monocytogenes</i>	Env (Unspecified)	1/2a	XLVIII	AM
179	<i>L. monocytogenes</i>	Env (Unspecified)	1/2b	NT	PEN
181	<i>L. monocytogenes</i>	Env (Unspecified)	1/2b	NT	SXT
217	<i>L. monocytogenes</i>	Env (Unspecified)	1/2a	XLII	SXT
224	<i>L. monocytogenes</i>	Raw meat (Porcine)	1/2a	NT	TE
278	<i>L. monocytogenes</i>	Cooked meat	1/2b	LXIX	GM
280	<i>L. monocytogenes</i>	Env (Unspecified)	1/2b	LXVII	GM
306	<i>L. monocytogenes</i>	Salad	1/2†	NT	SXT
348	<i>L. monocytogenes</i>	Cooked beef	4b	NT	CIP
350	<i>L. monocytogenes</i>	Cooked beef	4b	XCIII	TE CIP
353	<i>L. monocytogenes</i>	Cooked Beef	1/2c	XCIX	GM
274	<i>L. seeligeri</i>	Env (Unspecified)		CCLV	GM
40	<i>L. welshimeri</i>	Cranberry stuffing		CLX	RA
154	<i>L. welshimeri</i>	Raw meat		CCLVI	CEC
374	<i>L. welshimeri</i>	Cooked beef		CCLXI	GM
75	<i>L. innocua</i>	Env (Unspecified)		CCXXXVII	GM TE
369	<i>L. innocua</i>	Cooked beef		CCXXX	GM

*Serotyping was only performed on *L. monocytogenes* isolates.

†The H antigen could not be determined for this *L. monocytogenes* isolate by traditional slide agglutination or by the serotyping ELISA.

agglutinating antigen–antiserum combinations (Palumbo *et al.* 2003). Ten isolates could not be typed by either the traditional slide agglutination or ELISA serotyping methods. Nadon *et al.* 2001 have suggested that horizontal gene transfer or point mutations in genomic DNA can result in phenotypic shifts that may affect antigen detection and therefore lead to serotypes that are nontypeable by traditional serotyping methods.

The majority of human listeriosis cases are caused by three serotypes (4b, 1/2b and 1/2a) (Nadon *et al.* 2001). In Ireland, the predominant serotype reported from patients was 4b, but 1/2 was found to be the predominant serotype from the food-positive samples (HPSC 2008). Food products that were frequently implicated in outbreaks of listeriosis were found to be dairy products (milk, soft cheeses in particular), raw and cooked meats, raw vegetables, and raw and smoked seafood (Fleming *et al.* 1985; Linnan *et al.* 1988; Gugnani 1999; Ryser 1999; Capita *et al.* 2001; Aguado *et al.* 2004; Gudbjornsdottir *et al.* 2004; Reissbrodt 2004; Chou and Wang 2006). In this study, it was observed that serotype 1/2a was the predominant serotype, this serotype was present in all food categories (Table 2). However, serotypes 1/2b and 4b, which are more associated with human listeriosis, were present in cooked & raw, meat & fish and vegetable food categories. This data indicates that these food products, which may not be cooked prior to consumption, may

Table 2 *Listeria monocytogenes* serotypes found in the different food categories and the environment determined by the serotyping ELISA

Source	Serotype					
	1/2a	1/2b	1/2c	4b	4c	Nontypeable
Food-processing environment	37	7	5	10	3	4
Raw meat	8	1	0	0	1	2
Cooked meat	1	1	2	3	2	2
Raw fish	13	1	0	4	0	0
Cooked fish	4	0	0	0	0	0
Dairy cheese	4	0	0	0	0	0
Garlic butter	2	0	2	0	0	0
Ingredient	2	1	0	0	0	0
Vegetable	4	2	0	2	0	1
Fruit	1	0	0	0	0	1
Food grade water	1	0	0	0	0	0
	77	13	9	19	6	10
Total	134					

serve as vehicles to transmit virulent *L. monocytogenes* to humans.

Legislation criteria for control of *L. monocytogenes* in ready-to-eat foods products vary from country to country. In the USA, the US Food and Drug Administration and the Food Safety and Inspection Service have established

a policy of zero tolerance (i.e. no detectable levels of permitted). In Ireland, the European Regulation on Microbiological Criteria for Foodstuffs (Commission Regulation (EC) No 2073/2005) states that RTE foods intended for infants and for special medical purposes must not have any *L. monocytogenes* cells present in 25 g but for RTE foods not intended for infants or special medical purposes can have a limit of 100 CFU g⁻² during the products shelf life. Quantitative microbiological risk assessment for *L. monocytogenes* is difficult as the disease process is complex with multiple routes of infection (McLauchlin *et al.*, 2004). Even with a zero tolerance policy in USA towards *L. monocytogenes*, which has led to multimillion dollar recalls because of contamination, several outbreaks of listeriosis still occurred (Revazishvili *et al.*, 2004). This has demonstrated that even with strict legislative limits, the removal of *L. monocytogenes* from the food chain is very difficult.

PFGE is considered to be the gold standard subtyping method because of the documented reproducibility in previous epidemiological studies and its high discriminatory power (Graves and Swaminathan 2001; Vela *et al.* 2001a,b; Autio *et al.* 2002). Genotypic analysis has shown that *L. monocytogenes* is grouped into four distinct genetic lineages (Rasmussen *et al.* 1995; Wiedmann *et al.* 1997; Liu *et al.* 2006; Roberts *et al.* 2006; Sauders *et al.* 2006; Ward *et al.* 2008). Lineage I consists of serotypes 1/2b, 3b, 4b, 4d and 4e and lineage II (serotypes 1/2a, 3a, 1/2c and 3c). A third lineage (lineage III) includes serotypes 4a and 4c as well as some serotype 4b strains (Brosch *et al.* 1994; Nadon *et al.* 2001; Ward *et al.* 2004 and Wiedmann *et al.* 1997).

However, other studies have observed either no correlation or not a clear-cut association of PFGE types with specific serotypes (Gianfranceschi *et al.* 2002; Revazishvili *et al.* 2004). In this study, the results of the PFGE typing and serotyping suggest that there is also not a clear association of PFGE type with serotype. The PFGE typing also revealed that there is a large genetic diversity amongst the *L. monocytogenes* isolate collection (for the 112 isolates, 103 PFGE types were identified). This result is similar to the high genetic diversity found among *L. monocytogenes* isolates from retail food in the USA (Zhang *et al.* 2007), and the PFGE analysis generated 120 PFGE types for 167 *L. monocytogenes* isolates sampled from various RTE meats, raw chicken and fresh produce.

Previous studies have found that serotype 1/2a was the predominate serotype of *L. monocytogenes* food and environmental isolates. (Boerlin *et al.* 1997; Corcoran *et al.* 2006; Gilbreth *et al.* 2005; Gudbjornsdottir *et al.*, 2004; Lukinmaa *et al.* 2003). One explanation could be the ability of this specific serotype to survive better in certain foods. When frankfurters were spiked with a mixture of

L. monocytogenes strains of different serotypes and sampled over a 90-day period of storage at 4°C, it was found that 1/2a serotype isolate was the dominant isolate recovered. (Porto *et al.* 2003) Another explanation could be that the selective compounds found in University of Vermont medium I (UVM I) promotes the bias selection of 1/2a serotype over 1/2b and 4b serotypes. All isolates grew with identical growth rates in the nonselective media but differed in their growth rate in the selective UVM I media (Bruhn *et al.* 2005). This selective broth was utilized in this study.

Antimicrobial susceptibility testing

Listeria spp. was considered relatively susceptible to a wide range of antibiotics until the discovery of an antibiotic-resistant *L. monocytogenes* in 1988; since then, the rate of antimicrobial-resistant food-related *Listeria* has slowly increased. (Charpentier and Courvalin 1999; Vela *et al.* 2001b; Walsh *et al.* 2001; Prazak *et al.* 2002; Conter *et al.* 2009). The results of this study differ to those of Davis and Jackson 2009; Rota *et al.* 1996 and Walsh *et al.* 2001. Those studies demonstrated that a higher percentage of *L. innocua* or *L. welshimeri* spp. was resistant to antibiotics than *L. monocytogenes* spp. In the study by Davis and Jackson 2009, they observed that majority of *L. monocytogenes* isolates were resistant to oxacillin (99%, 89/90) and that most of the *L. welshimeri* (67%, 4/6) were resistant to sulfamethoxazole-trimethoprim. The results of our study indicated that *L. monocytogenes* and *L. seeligeri* actually displayed higher percentage of resistance to the antibiotics tested than any other *Listeria* spp. The highest level of resistance displayed by *L. monocytogenes* was to gentamicin (33%, 4/15) and sulfamethoxazole-trimethoprim (33%, 4/15) followed by ciprofloxacin (25%, 3/15) and tetracycline (13%, 2/15) (Table 1). Our results also indicated that there did not appear to be any association between PFGE pattern or lineage and antimicrobial resistance profiles. This may be because of the fact the PFGE detects variation of the bacterial genome, whereas most known antimicrobial resistance genes are carried on plasmids (Zhang *et al.* 2007).

In this study, it was found that while the overall incidence of antibiotic resistance in food and environmental *L. monocytogenes* and other *Listeria* spp. isolates is still relatively low, there is an emerging pattern of antibiotic resistance amongst *L. monocytogenes* food and environmental isolates. This emergence of resistance has also been noted by other studies. (Zhang *et al.* 2007; Arslan and Ozdemir 2008; Conter *et al.* 2009). From an Irish perspective, Walsh *et al.* (2001) observed gentamicin resistance displayed by one *L. innocua* isolate; therefore, there is a concern that four of *L. monocytogenes* isolates

in this study were found to be resistant to gentamicin. Currently, the treatment of choice of human listeriosis utilizes β -lactam and aminoglycoside antimicrobial drugs alone or in combination (Hof 2003). However, the combination of trimethoprim-sulfamethoxazole is utilized for the treatment of listeriosis among patients who are allergic to the penicillins (Jones and Macgowan 1995). The emergence of *L. monocytogenes* strains' resistance to clinically important antibiotics may have future implications for the effective treatment of listeriosis.

In conclusion, the continual monitoring and molecular subtyping of *Listeria*-positive food isolates within Ireland is required. The genetic diversity seen in this study among the *L. monocytogenes* isolates suggests that a number of sources could be linked with food product contamination. With the increase in sporadic cases of listeriosis in Ireland, future work is needed to link *Listeria* food isolates from specific foods produced at specific plants to establish routes of food contamination. Molecular subtyping data for *L. monocytogenes* isolates from foods and food-processing plants can help detect potential outbreak sources (Fugett et al. 2007). This study also underlines the importance of continuous surveillance of any emerging antimicrobial resistance in all food and environmental *Listeria* spp. isolates.

Acknowledgements

This research was funded by the Department of Agriculture, Fisheries and Food under the Food Institutional Research Measure (FIRM). We thank Dr. Mary Murphy, Senior Executive Scientist, Veterinary Food Safety Laboratory, Cork, for kindly providing training and access to BioNumerics version 5.0 software for the PFGE typing analysis. Dr. Lee Coffey in Waterford Institute of Technology is also gratefully acknowledged for technical assistance with the serotyping assay.

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