Survival of Clinical and Food Isolates of *Listeria monocytogenes* Through Simulated Gastrointestinal Tract Conditions

Rosário Ramalheira,¹ Marta Almeida,² Joana Azeredo,² Teresa R.S. Brandão,¹
Gonçalo Almeida,¹ Joana Silva,¹ and Paula Teixeira¹

Abstract

Twenty-seven strains of *Listeria monocytogenes* previously isolated from food ($n = 16$) and human patients of listeriosis ($n = 11$) were characterized and compared based on their ability to survive through the simulated gastrointestinal tract conditions. Cells were exposed (60 or 120 min) to low pH in the presence of pepsin, to simulate the digestion in the stomach, and subsequently to bile salts to simulate the digestion in the small intestine (60 or 120 min). Their survival was shown to be origin- (food and clinical) and strain dependent ($p < 0.001$) and also significantly dependent on the imposed simulated gastric conditions (long vs. quick exposure) ($p < 0.001$). In comparison to the food isolates, the clinical strains were in general more resistant and survived better to the two challenges imposed. Some of the tested strains, after the exposure to low pH in the presence of pepsin, became injured and subsequently more susceptible to the bile salts challenge. It was demonstrated that one of the most important natural barriers against foodborne pathogens might not be effective since it was shown that *L. monocytogenes* isolates that survived through the pH challenge were also able to survive the subsequent challenge to bile salts.

Introduction

FIRST RECOGNIZED BY MURRAY *ET AL.* (1926) *Listeria monocytogenes* is a pathogen often present in a variety of ready-to-eat foods, such as smoked fish, soft cheeses, fermented sausages, and lightly heat-treated meat products. There are also indications of their growth on food production lines and in the food processing environment, especially in zones of difficult access during normal cleaning and disinfection procedures (Nørrung, 2000). This pathogen has the ability to survive and grow under a wide range of environmental conditions, such as the presence or absence of air, refrigeration temperatures (0–4°C), high salt concentrations, water activities above 0.92, and at pH values between 4.3 and 9.6 (Ribeiro *et al.*, 2006).

L. monocytogenes have been responsible for outbreaks and sporadic cases of listeriosis, a severe infection particularly among certain groups within the general population, namely immunocompromised persons (e.g., organ transplant or cancer patients), HIV-infected individuals, pregnant women, newborn babies, and the elderly (Bell and Kyriakides, 2005;

Almeida *et al.*, 2006). Although the infection occurs infrequently, the fatality rate is high (Almeida *et al.*, 2006).

Ingestion of contaminated foods is the main route of infection (McLauchlin, 1996; Brett *et al.*, 1998; Soutos *et al.*, 2007). Virulent strains of *L. monocytogenes* that survive the passage through the stomach, where the buffering capacity of some foods may protect the organisms further along the gastrointestinal (GI) tract (McLauchlin *et al.*, 2004; Sleator *et al.*, 2007), invade the GI epithelium and enter phagocytic host cells, where the bacteria are able to survive, multiply, and be carried in the blood to various organs (particularly the brain and/or uterus) (Nørrung, 2000). The infection via the GI tract presents a series of challenges by host defense mechanisms (Miller and Czuprynski, 2002). Understanding the complex interactions between pathogenic microorganism and the host organism's GI tract is still necessary.

The aim of the present work was to characterize and compare 27 *L. monocytogenes* strains, from food and from clinical cases of listeriosis that have occurred in Portugal, representing several serotypes, based on their capacity to survive through

¹CBQF/Biotechnology School, Portuguese Catholic University, Oporto, Portugal.

²Biological Engineering Department, University of Minho, Braga, Portugal.

simulated GI tract conditions and to evaluate the possibility of cellular damage through this challenge.

Materials and Methods

Microbial strains

The isolates of *L. monocytogenes* that were used during this study are listed in Table 1. All the isolates were obtained from the Escola Superior de Biotecnologia culture collection (Porto, Portugal) and were selected based on their origin (clinical and food), serogroup (1/2b, 1/2a, 1/2c, and 4b), and resistance profile to heavy metals (arsenic, cadmium, and tetracycline). Clinical isolates collected from human patients of listeriosis that occurred in Portugal were kindly supplied by several Portuguese health institutions.

Preparation of the inoculum

Stock cultures were stored in cryotubes at -80°C in tryptic soy broth with 6 g/L yeast extract (TSBYE; Pronadisa, Madrid, Spain), with 30% (v/v) of glycerol. From the cryotube, an aliquot of *L. monocytogenes* was streaked on tryptic soy agar with 6 g/L yeast extract (TSAYE) and incubated at 37°C during 24 h. To prepare each preinoculum, a pure colony of each isolate was transferred to 10 mL of TSBYE followed by incubation at 37°C for 24 h. This culture was then subsequently diluted 1:100 in TSBYE and incubated at 37°C for 24 h to yield

cells in the stationary phase of growth. After centrifugation (7000 g, 10 min, 4°C ; Rotina 35; Hettich Zentrifugen, Oxford, CT), the pellet was resuspended to the original volume with sterile Ringer's solution (LAB M, Bury, United Kingdom).

GI conditions

The simulation of the gastric juice conditions was performed according to Madureira *et al.* (2005) in buffered peptone water (BPW; LabM, Bury, UK) adjusted to pH 2.5–3.0 (HCl, 1M) with 1000 U/mL of a filter sterilized solution of pepsin (Sigma, Madrid, Spain). After 60 min (to simulate a quick gastric transit) or 120 min (to simulate a slow gastric transit), the simulation of the effect of bile salts in the small intestine was performed by the addition of bile salts until a final concentration of 0.3% (w/v) (Pronadisa) followed by pH readjustment to 7 with sodium hydroxide. Cells were exposed to bile salts for 60 or 120 min (quick and slow digestion, respectively) (Madureira *et al.*, 2005).

Three independent controls were used: BPW at pH 7, BPW containing 0.3% (w/v) bile salts at pH 7, and BPW containing pepsin (1000 U/mL) at pH 7 (Madureira *et al.*, 2005). The solutions simulating each condition or controls were inoculated with 1% (v/v) of bacterial inoculum prepared as described above. Samples were taken at 30 min intervals, and enumeration was performed as described below. For each bacterial strain, all assays were performed in triplicate.

TABLE 1. MICROBIAL STRAINS SELECTED FOR THE STUDY AND THEIR CHARACTERIZATION ACCORDING TO THE SEROGROUP AND RESISTANCE TO ARSENIC, CADMIUM, AND TETRACYCLINE

Listeria monocytogenes strain	Source	Serogroup	Resistance to ^a		
			Arsenic	Cadmium	Tetracycline
<i>L. monocytogenes</i> _747	Food	1/2b	S	R	S
<i>L. monocytogenes</i> _925	Food	1/2b	S	R	S
<i>L. monocytogenes</i> _930	Food	1/2a	S	R	S
<i>L. monocytogenes</i> _903	Food	4b	R	S	S
<i>L. monocytogenes</i> _1044	Food	4b	R	S	S
<i>L. monocytogenes</i> _842	Food	1/2c	S	R	S
<i>L. monocytogenes</i> _854	Food	4b	S	R	S
<i>L. monocytogenes</i> _1216	Food	1/2b	S	R	S
<i>L. monocytogenes</i> _1305	Food	1/2c	R	S	S
<i>L. monocytogenes</i> _994	Food	1/2a	S	R	S
FSL-F7-001	Food	1/2c	R	R	R
FSL-F7-010	Food	1/2a	S	R	S
FSL-F7-020	Food	1/2a	S	S	S
FSL-F7-088	Food	1/2c	S	R	S
FSL-F7-099	Food	4b	R	S	S
FSL-F7-128	Food	1/2b	S	R	S
<i>L. monocytogenes</i> _1761	Clinic	1/2a	S	R	S
<i>L. monocytogenes</i> _2065	Clinic	1/2b	S	R	S
<i>L. monocytogenes</i> _2074	Clinic	4b	S	S	S
<i>L. monocytogenes</i> _2092	Clinic	4b	R	S	S
<i>L. monocytogenes</i> _2086	Clinic	1/2a	S	S	S
<i>L. monocytogenes</i> _1000	Clinic	4b	S	S	S
<i>L. monocytogenes</i> _1001	Clinic	4b	S	R	S
<i>L. monocytogenes</i> _1547	Clinic	1/2b	S	S	S
<i>L. monocytogenes</i> _1891	Clinic	1/2a	S	S	S
<i>L. monocytogenes</i> _1562	Clinic	1/2b	S	R	S
<i>L. monocytogenes</i> _2103	Clinic	1/2a	S	R	S

^aGrowth (R, resistant) or no growth (S, susceptible) onto three isosensitest agar plates (Oxoid, Basingstoke, United Kingdom) containing 500 µg/mL sodium arsenite, 75 µg/mL cadmium chloride monohydrate, and 8 µg/mL of tetracycline-hydrochloride.

Enumeration

Enumeration of the survivors was performed by plating appropriate dilutions prepared in Ringer's solution (in duplicate) on TSAYE and on Palcam agar (Merck, Darmstadt, Germany) plus selective supplement (Merck), by the drop count technique (Miles and Misra, 1938). TSAYE medium is nonselective and was used to enumerate both injured and noninjured cells. Palcam agar is selective for *L. monocytogenes* and was used for enumeration of noninjured cells. Colonies were enumerated after incubation at 37°C for 48 h.

Statistical analysis

As mentioned above, all the experiments were repeated at least three times. A one-way analysis of variance (ANOVA) was performed to test significant effects of the following: (i) slow and quick digestion, (ii) enumeration media (Palcam and TSAYE), and (iii) origin and strain on the survival of the *L. monocytogenes* through the tested conditions. Two-way ANOVA was also performed for concluding about strain/media and type of digestion/strain effects.

All calculations were carried out using the software KaleidaGraph (version 4.04; Synergy Software, Reading, PA).

Results

The survival of cells to the simulated GI conditions is illustrated in Tables 2 and 3. The resistance to both quick and slow digestions was shown to be dependent on the origin, strain, and simulated GI conditions ($p < 0.001$). Clinical isolates were significantly ($p < 0.001$) more resistant than food isolates (Tables 2 and 3); 2.7 and 3.6 log reductions (mean values) in viable counts were observed during the simulated slow GI transit of clinical and food strains, respectively. A similar tendency was observed during the simulated quick transit; 1.8 and 3.0 log reductions for clinical and food isolates, respectively.

None of the strains demonstrated sensitivity to pepsin (at neutral pH) or to bile salts challenge alone (control cells, data not shown).

Survival of *L. monocytogenes* after the exposure to the drop of pH in the presence of pepsin and subsequent challenge to

TABLE 2. SURVIVAL OF CLINICAL AND FOOD ISOLATES OF *LISTERIA MONOCYTOGENES* THROUGH SLOW DIGESTION WHEN ENUMERATION WAS PERFORMED IN SELECTIVE (PALCAM) AND NONSELECTIVE (TRYPTIC SOY AGAR WITH 6 G/L YEAST EXTRACT) MEDIA

Plating media	Source	log cfu/mL ^a				
		0 min ^b	120 min ^{c,d}		240 min ^{c,e}	
		TSAYE	Palcam	TSAYE	Palcam	TSAYE
<i>L. monocytogenes</i> _1547	Clinical	7.1 ± 0.0	4.8 ± 0.1	5.0 ± 0.1	5.3 ± 0.0	5.7 ± 0.0
<i>L. monocytogenes</i> _1761	Clinical	7.2 ± 0.1	6.0 ± 0.0	6.5 ± 0.1	5.0 ± 0.0	5.1 ± 0.1
<i>L. monocytogenes</i> _1891	Clinical	7.3 ± 0.1	4.9 ± 0.0	5.1 ± 0.1	4.1 ± 0.0	4.1 ± 0.0
<i>L. monocytogenes</i> _2065	Clinical	7.0 ± 0.0	4.8 ± 0.0	5.1 ± 0.1	2.6 ± 0.7	2.8 ± 0.5
<i>L. monocytogenes</i> _2086	Clinical	7.3 ± 0.0	4.0 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.0
<i>L. monocytogenes</i> _2092	Clinical	7.1 ± 0.0	4.0 ± 0.0	4.1 ± 0.1	3.2 ± 0.1	3.5 ± 0.6
<i>L. monocytogenes</i> _2103	Clinical	7.5 ± 0.0	5.9 ± 0.1	6.4 ± 0.2	6.0 ± 0.2	6.1 ± 0.1
<i>L. monocytogenes</i> _2074	Clinical	7.3 ± 0.0	6.2 ± 0.0	6.3 ± 0.1	6.1 ± 0.0	6.1 ± 0.0
<i>L. monocytogenes</i> _1000	Clinical	6.9 ± 0.1	4.4 ± 0.1	4.5 ± 0.2	4.1 ± 0.1	4.7 ± 0.1
<i>L. monocytogenes</i> _1001	Clinical	6.7 ± 0.1	3.5 ± 0.1	3.8 ± 0.2	<1.8 ± 0.0	<1.8 ± 0.0
<i>L. monocytogenes</i> _1562	Clinical	6.7 ± 0.0	4.5 ± 0.2	4.4 ± 0.8	4.6 ± 0.9	4.8 ± 0.1
<i>L. monocytogenes</i> _903	Food	7.1 ± 0.0	3.1 ± 0.1	4.3 ± 0.3	2.6 ± 0.5	2.8 ± 0.3
<i>L. monocytogenes</i> _1216	Food	6.9 ± 0.0	4.0 ± 0.4	4.8 ± 0.1	2.5 ± 0.1	2.7 ± 0.0
<i>L. monocytogenes</i> _1305	Food	7.3 ± 0.1	5.0 ± 0.1	5.4 ± 0.2	4.0 ± 0.1	4.4 ± 0.5
<i>L. monocytogenes</i> _747	Food	6.5 ± 0.0	<1.8 ± 0.0	<1.8 ± 0.0	<1.8 ± 0.0	<1.8 ± 0.0
<i>L. monocytogenes</i> _925	Food	6.7 ± 0.2	2.3 ± 0.1	2.7 ± 0.4	<1.8 ± 0.0	<1.8 ± 0.0
<i>L. monocytogenes</i> _930	Food	6.8 ± 0.3	<1.8 ± 0.0	<1.8 ± 0.0	<1.8 ± 0.0	<1.8 ± 0.0
<i>L. monocytogenes</i> _994	Food	6.7 ± 0.3	<1.8 ± 0.0	2.2 ± 0.1	<1.8 ± 0.0	<1.8 ± 0.0
<i>L. monocytogenes</i> _842	Food	7.1 ± 0.0	5.2 ± 0.1	5.5 ± 0.6	4.0 ± 0.0	4.1 ± 0.1
<i>L. monocytogenes</i> _854	Food	7.1 ± 0.0	3.7 ± 0.2	4.2 ± 0.1	2.3 ± 0.3	2.6 ± 0.2
<i>L. monocytogenes</i> _1044	Food	6.9 ± 0.1	3.7 ± 0.5	3.9 ± 0.1	2.2 ± 0.6	2.5 ± 0.4
FSL-F7-001	Food	6.9 ± 0.1	4.9 ± 0.0	4.9 ± 0.1	3.2 ± 0.0	3.7 ± 0.0
FSL-F7-010	Food	6.8 ± 0.2	5.9 ± 0.3	6.1 ± 0.2	5.2 ± 0.0	6.0 ± 0.0
FSL-F7-020	Food	6.8 ± 0.1	4.9 ± 0.0	5.0 ± 0.1	4.0 ± 0.1	4.2 ± 0.9
FSL-F7-088	Food	6.3 ± 0.1	5.8 ± 0.2	5.9 ± 0.2	4.7 ± 0.4	4.9 ± 0.1
FSL-F7-099	Food	6.4 ± 0.1	3.8 ± 0.3	4.0 ± 0.0	3.1 ± 0.1	3.9 ± 0.0
FSL-F7-128	Food	7.0 ± 0.0	1.9 ± 0.2	2.0 ± 0.4	2.1 ± 0.0	2.1 ± 0.0

^aSurvival represented as the media of the log cfu/mL ± the standard error of the mean.

^bInitial counts obtained in Palcam (time 0; data not shown) and TSAYE were not significantly different ($p > 0.05$).

^cCounts obtained in Palcam and TSAYE were significantly different ($p < 0.001$).

^dSurvival after exposure at pH 2.5 in the presence of pepsin.

^eSurvival after exposure to pH 2.5 in the presence of pepsin and subsequent exposure to bile salts at pH 7. TSAYE, tryptic soy agar with 6 g/L yeast extract.

TABLE 3. SURVIVAL OF CLINICAL AND FOOD ISOLATES OF *LISTERIA MONOCYTOGENES* THROUGH QUICK DIGESTION WHEN ENUMERATION WAS PERFORMED IN NONSELECTIVE (TSAYE) MEDIUM

	Source	<i>log cfu/mL</i> ^a		
		0 min	60 min ^b	120 min ^c
<i>L. monocytogenes</i> _1547	Clinical	7.1 ± 0.0	6.2 ± 0.1	6.4 ± 0.0
<i>L. monocytogenes</i> _1761	Clinical	7.2 ± 0.1	6.1 ± 0.0	5.4 ± 0.5
<i>L. monocytogenes</i> _1891	Clinical	7.3 ± 0.1	6.1 ± 0.1	5.2 ± 0.0
<i>L. monocytogenes</i> _2065	Clinical	7.0 ± 0.0	5.5 ± 0.1	4.7 ± 0.4
<i>L. monocytogenes</i> _2086	Clinical	7.3 ± 0.0	5.2 ± 0.0	5.0 ± 0.0
<i>L. monocytogenes</i> _2092	Clinical	7.1 ± 0.0	7.5 ± 0.0	4.9 ± 0.0
<i>L. monocytogenes</i> _2103	Clinical	7.5 ± 0.0	6.3 ± 0.0	6.3 ± 0.0
<i>L. monocytogenes</i> _2074	Clinical	7.3 ± 0.0	6.6 ± 0.0	6.5 ± 0.0
<i>L. monocytogenes</i> _1000	Clinical	6.9 ± 0.1	4.7 ± 0.1	4.9 ± 0.1
<i>L. monocytogenes</i> _1001	Clinical	6.7 ± 0.1	3.9 ± 0.1	4.2 ± 0.1
<i>L. monocytogenes</i> _1562	Clinical	6.7 ± 0.0	4.7 ± 0.0	4.4 ± 0.1
<i>L. monocytogenes</i> _903	Food	7.1 ± 0.0	5.1 ± 0.1	2.7 ± 0.3
<i>L. monocytogenes</i> _1216	Food	6.9 ± 0.0	5.2 ± 0.1	3.9 ± 0.2
<i>L. monocytogenes</i> _1305	Food	7.3 ± 0.1	6.5 ± 0.1	5.6 ± 0.1
<i>L. monocytogenes</i> _747	Food	6.5 ± 0.0	1.4 ± 1.9	3.6 ± 0.1
<i>L. monocytogenes</i> _925	Food	6.7 ± 0.2	3.2 ± 0.2	<1.8 ± 0.0
<i>L. monocytogenes</i> _930	Food	6.8 ± 0.3	2.5 ± 0.2	2.7 ± 0.0
<i>L. monocytogenes</i> _994	Food	6.7 ± 0.3	4.3 ± 0.3	4.0 ± 0.1
<i>L. monocytogenes</i> _842	Food	7.1 ± 0.0	6.2 ± 0.0	2.3 ± 0.6
<i>L. monocytogenes</i> _854	Food	7.1 ± 0.0	4.9 ± 0.1	3.1 ± 0.1
<i>L. monocytogenes</i> _1044	Food	6.9 ± 0.1	5.3 ± 0.0	3.1 ± 0.1
FSL-F7-001	Food	6.9 ± 0.1	6.0 ± 0.0	4.9 ± 0.2
FSL-F7-010	Food	6.8 ± 0.2	6.1 ± 0.0	6.0 ± 0.0
FSL-F7-020	Food	6.8 ± 0.1	5.5 ± 0.0	5.7 ± 0.1
FSL-F7-088	Food	6.3 ± 0.1	4.1 ± 0.0	4.8 ± 0.1
FSL-F7-099	Food	6.4 ± 0.1	4.9 ± 0.1	3.9 ± 0.1
FSL-F7-128	Food	7.0 ± 0.0	3.0 ± 0.0	3.1 ± 0.0

^aSurvival represented as the media of the *log cfu/mL* ± the standard error of the mean.

^bSurvival after exposure at pH 2.5 in the presence of pepsin.

^cSurvival after exposure to pH 2.5 in the presence of pepsin and subsequent exposure to bile salts at pH 7.

bile salts is presented in Fig. 1 (simulated quick digestion) and Fig. 2 (simulated slow digestion).

With reference to the results obtained for the slow digestion, food strain FSL-F7-088 was the most resistant to the drop in pH in the presence of pepsin with approximately 40% survivors (Fig. 2A). However, all the other tested food and clinical strains were demonstrated to be very susceptible showing a survival of 17% (strain 2074) or lower (Fig. 2). This high susceptibility was also observed for the quick digestion (Fig. 1). In general, clinical isolates were significantly ($p < 0.001$) more resistant to an exposure to low pH plus pepsin than food isolates for both quick (Fig. 1) and long exposures (Fig. 2). Cell survived better ($p < 0.001$) exposure to bile salts than to acid in the presence of pepsin (Figs. 1 and 2). For both quick and long exposures to bile salts, after-exposure to low pH and pepsin survival was dependent on the strain being investigated ($p < 0.001$) but not on its origin ($p > 0.05$).

Concerning the simulated stomach conditions, for clinical and food strains, a higher survival was observed in the quick transit than in the long exposure ($p < 0.001$). The magnitude of this behavior varied significantly ($p < 0.001$) according to the tested strain (Figs. 1 and 2). With regard to survival of the double challenge (acid and pepsin and then bile salts), clinical strains survived better than food strains ($p < 0.001$) for both simulations (slow and quick transit). In relation to the enu-

meration of *L. monocytogenes*, significant differences between the two plating media after the long digestion period were verified ($p < 0.001$). In general, and based on ANOVA results, the survivor counts were higher in the nonselective medium (TSAYE) than in the selective medium (Palcam; Table 2; $p < 0.001$). However, during the quick digestion, for food and clinical isolates, no significant differences ($p > 0.05$) were observed between the cellular counts on TSAYE and on Palcam agar media (data not shown).

Discussion

The survival of *L. monocytogenes* to the main stresses encountered during passage through the GI tract, that is, low pH in the presence of pepsin and subsequent exposure to bile salts, was investigated. No cellular reduction was observed in the presence of pepsin or bile salts, alone (controls). Begley *et al.* (2002) and Olier *et al.* (2004) have previously reported a similar resistance to bile salts. However, King *et al.* (2003) reported that bile salts might have a deleterious effect on *L. monocytogenes*. Resistance to bile salts stresses has been demonstrated to be strain dependent (Begley *et al.*, 2002; King *et al.*, 2003; Olier *et al.*, 2004). It is also important to point out that in the present study and in the studies by Begley *et al.* (2002) and Olier *et al.* (2004), survival of *L. monocytogenes* to

FIG. 1. Survival (%) of food (A) and clinical (B) isolates of *Listeria monocytogenes* through the simulated quick digestion. ■, After the exposure to the drop of pH in the presence of pepsin (expressed as % of the initial counts) and □, subsequent challenge to bile salts (expressed as % of the survivors to the previous condition).

bile salts was evaluated in nutritious media, BPW, and brain-heart infusion, respectively, whereas King *et al.* (2003) evaluated resistance to bile salts in phosphate buffer. It is well known that the bacterial resistance to a particular stress varies according to the media where it is applied being higher, for most of the situations, in more nutritious media.

For the strains investigated, a lower survival was observed in the simulated stomach conditions than in the presence of bile salts. Using a dynamic model of the stomach and small intestine, Barmpalia-Davis *et al.* (2008a, 2009) also reported more drastic reductions in listerial populations during the gastric challenge than those observed in the simulated intestinal conditions.

Differences between counts on Palcam and TSAYE have already been described by Miller *et al.* (2006); these authors reported that on the selective medium, heat-injured *Listeria innocua* cells suffer additional stresses and might fail to repair,

FIG. 2. Survival (%) of food (A) and clinical (B) isolates of *Listeria monocytogenes* through the simulated slow digestion. ■, After the exposure to the drop of pH in the presence of pepsin (expressed as % of the initial counts) and □, subsequent challenge to bile salts (expressed as % of the survivors to the previous condition).

becoming functionally inactive. Therefore, the differences in the viable cell counts between the two enumeration media suggest cellular damage, probably in the cytoplasmic membrane (Nyachuba and Donnelly, 2005; Miller *et al.*, 2006; Uyttendaele *et al.*, 2008). This was concurrent with the results obtained in the present study for the survival through the slow digestion and with those presented by Barmpalia-Davis *et al.* (2009) related to the survival of *L. monocytogenes* to 120 min gastric exposure. Damage to the lipopolysaccharide of the outer membrane and denaturation of cytoplasmic proteins has been attributed to acid exposure (Brown and Booth, 1991). This may explain the lower counts in Palcam than in TSAYE and the increased susceptibility to bile salts following exposure to low pH in the presence of pepsin observed for most of the strains. Barmpalia-Davis *et al.* (2008a)

obtained similar counts of *L. monocytogenes* on TSA and TSAYE throughout a gastric challenge. The reasons for this observation were not clear, but traits of individual strains and potential acid adaptation were proposed as possible explanations for these findings.

The capacity of *L. monocytogenes* to colonize the GI tract (Mahoney and Henriksson, 2003; Gahan and Hill, 2005; Sleator *et al.*, 2007), to resist acidic conditions (Dykes and Moorhead, 2000; King *et al.*, 2003; Vialette *et al.*, 2003; Lianou *et al.*, 2006; Ribeiro *et al.*, 2006; Barmpalia-Davis *et al.*, 2008a), to survive in the presence of bile salts (Begley *et al.*, 2002; King *et al.*, 2003; Olier *et al.*, 2004; Barmpalia-Davis *et al.*, 2008a), and to persist in the presence of pepsin (Branen and Davidson, 2000) has already been described by several authors.

Comparison of survival patterns to stress conditions of clinical versus food isolates has been addressed in several publications (Avery and Buncic, 1997; Dykes and Moorhead, 2000; Vialette *et al.*, 2003; Olier *et al.*, 2004; Lianou *et al.*, 2006; Barmpalia-Davis *et al.*, 2008a; Werbrouck *et al.*, 2008). Avery and Buncic (1997) evaluated the effect of long-term storage at 4°C on the pathogenicity and growth characteristics of 30 strains of *L. monocytogenes* (15 clinical and 15 meat isolates). Clinical isolates demonstrated a higher resistance to these unfavorable conditions than food strains, concerning to pathogenicity and lag-phase duration at body temperature. Dykes and Moorhead (2000) showed that of the 30 strains of *L. monocytogenes* investigated, 28 decreased less than 1 log cycle after exposure to pH 2.5 for 2 h. No significant differences in this reduction were detected between clinical or food (meat) strains. However, the remaining two strains, both of meat origin, were significantly reduced in numbers under similar stress conditions. Vialette *et al.* (2003) evaluated the response of *L. monocytogenes* (four clinical isolates associated to fish products and four isolates from seafood) to stress conditions, which can occur during food processing. Clinical strains displayed better adaptation to pH and osmotic stresses than seafood strains. Olier *et al.* (2004) reported that independently of the origin (human, food, or food processing environment) all the 50 strains tested were able to grow in the presence of bile salts concentrations as high as those presented in the small intestine. Bile salt degradation ability was also found to be independent of the origin of the strains. Lianou *et al.* (2006) reported variation in the response to heating at 55°C or to pH 3 (lactic acid) among *L. monocytogenes* strains of various serotypes and sources, including clinical and food isolates. These authors reported that although no relation between food and clinical isolates was observed, outbreak-related isolates of serotype 4b showed the highest acid resistance. In the current study, and probably due to the low number of strains of each serotype investigated, no relationship between the serotype and survival through the simulated gastric and intestinal conditions was observed. Barmpalia-Davis *et al.* (2008a) compared the survival of 13 *L. monocytogenes* strains in a dynamic GI model. Survival to gastric acidity was demonstrated to be growth phase and strain dependent. The inactivation rates of exponential cultures were not different between pairs (outbreak origin/food associated) of food and human isolates. The highest acid susceptibility was observed for two strains of clinical origin. However, these two strains also displayed the longest lag periods in death. Werbrouck *et al.* (2008) did not find correlation between origin (clinical

and meat) and survival of *L. monocytogenes* serotype 4b in synthetic gastric fluid.

To our knowledge, the differences in the survival through simulated GI stress conditions between food and clinical isolates observed in the present study—clinical isolates were more resistant than food isolates—have not been previously described. These results corroborate previous suggestion (Dykes and Moorhead, 2000) of the importance of acid tolerance in the infection process. However, for the strains investigated, Werbrouck *et al.* (2008) found differences in the survival capacity in gastric fluid and *in vivo* virulence potential demonstrating the difficulty of determining the virulence capacity of *L. monocytogenes* strains. Moreover, Garner *et al.* (2006) suggested that virulence-associated characteristics of *L. monocytogenes* might be affected by food-specific properties. This highlights the importance of molecular and phenotypic characterization and comparison of strains of different origins to provide a greater insight into the virulence potential of *L. monocytogenes*.

It is important to point out that the results of the present study were obtained under static conditions, not considering the gradual acidification that occurs in the stomach during digestion, and in the absence of a food matrix. Peterson *et al.* (2007) demonstrated that for *L. monocytogenes* F2365 resistance to synthetic gastric fluid was higher when growth was performed on deli turkey meat than in brain-heart infusion. Barmpalia-Davis *et al.* (2008b) reported that during the gradual pH decrease in a dynamic simulation of the stomach conditions, *L. monocytogenes* on bologna and salami slices survived during the initial stages and a high cell number attained the simulated small intestine. The same authors reported protective effects of fat against gastric destruction of the pathogen (Barmpalia-Davis *et al.*, 2009). Samara *et al.* (2009) reported that when exposed to organic acids during the decontamination of lettuce, the resistance of *L. monocytogenes* to a subsequent exposure to the simulated gastric fluid did not increase. In fact decontamination with lactic acid increased the sensibility of the cells to the simulated conditions.

Despite the limitations attributed to the model of the GI conditions used in this work, the generated information would be a valuable contribution for further studies in the context of microbiological risk assessments. Further, selection of strains for further studies concerning virulence of food isolates of *L. monocytogenes* will take advantage of these results.

Conclusions

Following the ingestion of contaminated food by a mammalian host, *L. monocytogenes* have the ability to survive through the acid environment of the stomach, the secretion of proteolytic enzymes, and the inhibitory activity of bile salts. During this study, the conditions of the GI tract were simulated, and the survival of different isolates of *L. monocytogenes* was evaluated. This simulation did not take into account the gradual acidification that normally occurs in the stomach upon ingestion of a meal, nor the protective effect of food against the lethal action of acid or bile salts. However, the ability of *L. monocytogenes* to survive through highly adverse conditions was demonstrated and was shown to be strain dependent; clinical isolates were more resistant to the tested conditions.

Acknowledgments

This work was supported by CT project—2006-016264—*Truefood. Traditional United Europe Food*, and by FCT project—PTDC/AGR-ALI/64662/2006—*Listeria monocytogenes in foods: contributing data for risk assessment*.

Disclosure Statement

No competing financial interests exist.

References

- Almeida G, Gibbs P, Hogg T, and Teixeira P. Listeriosis in Portugal: an existing but under reported infection. *BMC Infect Dis* 2006;6:152–156.
- Avery SM and Buncic S. Differences in pathogenicity for chick embryos and growth kinetics at 37°C between clinical and meat isolates of *Listeria monocytogenes* previously stored at 4°C. *Int J Food Microbiol* 1997;34:319–327.
- Barmpalia-Davis IM, Geornaras I, Kendall PA, and Sofos JN. Differences in survival among 13 *Listeria monocytogenes* strains in a dynamic model of the stomach and small intestine. *Appl Environ Microbiol* 2008a;74:5563–5567.
- Barmpalia-Davis IM, Geornaras I, Kendall PA, and Sofos JN. Survival of *Listeria monocytogenes* in a simulated dynamic gastrointestinal model during storage of inoculated bologna and salami slices in vacuum packages. *J Food Prot* 2008b;71:2014–2023.
- Barmpalia-Davis IM, Geornaras I, Kendall PA, and Sofos JN. Effect of fat content on survival of *Listeria monocytogenes* during simulated digestion of inoculated beef frankfurters stored at 7°C. *Food Microbiol* 2009;26:483–490.
- Begley M, Gahan CGM, and Hill C. Bile stress response in *Listeria monocytogenes* LO28: adaptation, cross-protection, and identification of genetic loci involved in bile resistance. *Appl Environ Microbiol* 2002;68:6005–6012.
- Bell C and Kyriakides A. *Listeria: A Practical Approach to the Organism and Its Control in Foods*. London: Blackwell Publishing, 2005.
- Branen J and Davidson PM. Activity of hydrolysed lactoferrin against foodborne pathogenic bacteria in growth media: the effect of EDTA. *Lett Appl Microbiol* 2000;30:233–237.
- Brett M, Short P, and McLauchlin J. A small outbreak of listeriosis associated with smoked mussels. *Int J Food Microbiol* 1998;43:223–229.
- Brown M and Booth I. Acidulants and low pH. In: *Food Preservatives*. Russel NJ and Gould GW (eds.). Glasgow: Blackie and Son, 1991, pp. 22–43.
- Dykes GA and Moorhead SM. Survival of osmotic and acid stress by *Listeria monocytogenes* strains of clinical or meat origin. *Int J Food Microbiol* 2000;56:161–166.
- Gahan CGM and Hill C. Gastrointestinal phase of *Listeria monocytogenes* infection. *J Appl Microbiol* 2005;98:1345–1353.
- Garner MR, James KE, Callahan MC, Wiedmann M, and Boor KJ. Exposure to salt and organic acids increases the ability of *Listeria monocytogenes* to invade Caco-2 cells but decreases its ability to survive gastric stress. *Appl Environ Microbiol* 2006;72:5384–5395.
- King T, Ferenci T, and Szabo EA. The effect of growth atmosphere on the ability of *Listeria monocytogenes* to survive exposure to acid, proteolytic enzymes and bile salts. *Int J Food Microbiol* 2003;84:133–143.
- Lianou A, Stopforth JD, Yoon Y, et al. Growth and stress resistance variation in culture broth among *Listeria monocytogenes* strains of various serotypes and origins. *J Food Prot* 2006;69:2640–2647.
- Madureira AR, Pereira CI, Truszkowska K, Gomes AM, Pintado ME, and Malcata FX. Survival of probiotic bacteria in whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract. *Int Dairy J* 2005;15:921–927.
- Mahoney M and Henriksson A. The effect of processed meat and meat starter cultures on gastrointestinal colonization and virulence of *Listeria monocytogenes* in mice. *Int J Food Microbiol* 2003;84:255–261.
- McLauchlin J. The relationship between *Listeria* and listeriosis. *Food Control* 1996;7:187–193.
- McLauchlin J, Mitchell RT, Smerdon WJ, and Jewell K. *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. *Int J Food Microbiol* 2004;92:15–33.
- Miles AA and Misra SS. The estimation of the bactericidal power of blood. *J Hyg* 1938;38:732–749.
- Miller F, Brandão T, Teixeira P, and Silva CML. Recovery of heat-injured *Listeria innocua*. *Int J Food Microbiol* 2006;12:261–265.
- Miller DC and Czuprynski CJ. Passive immunization with convalescent serum, or oral immunization with formalin-killed organisms, does not protect mice against gastrointestinal challenge with *Listeria monocytogenes*. *Comp Immunol Microbiol* 2002;25:69–75.
- Murray EGD, Webb RA, and Swann MBR. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). *J Path Bacteriol* 1926;29:407–439.
- Nørrung B. Microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. *Int J Food Microbiol* 2000;62:217–221.
- Nyachuba D and Donnelly C. Sublethal injury, pathogen virulence and adaptation. In: *Understanding Pathogen Behavior*, chapter 7. Griffiths M (ed.). Cambridge, UK: Woodhead Publishing Ltd., 2005, pp. 152–198.
- Olier M, Rousseaux S, Piveteau P, Rousset A, and Guzzo J. Screening of glutamate decarboxylase activity and bile salt resistance of human asymptomatic carriage, clinical, food, and environmental isolates of *Listeria monocytogenes*. *Int J Food Microbiol* 2004;93:87–99.
- Peterson LD, Faith NG, and Czuprynski CJ. Resistance of *Listeria monocytogenes* F2365 cells to synthetic gastric fluid is greater following growth on ready-to-eat deli turkey meat than in brain heart infusion broth. *J Food Prot* 2007;70:2589–2595.
- Ribeiro MH, Manha S, and Brito L. The effects of salt and pH stress on the growth rates of persistent strains of *Listeria monocytogenes* collected from specific ecological niches. *Food Res Int* 2006;39:816–822.
- Samara A and Koutsoumanis KP. Effect of treating lettuce surfaces with acidulants on the behaviour of *Listeria monocytogenes* during storage at 5 and 20°C and subsequent exposure to simulated gastric fluid. *Int J Food Microbiol* 2009;129:1–7.
- Sleator RD, Clifford T, and Hill C. Gut osmolarity: a key environmental cue initiating the gastrointestinal phase of *Listeria monocytogenes* infection? *Med Hypotheses* 2007;69:1090–1092.
- Soultos N, Abraham A, Papageorgiou K, and Steris V. Incidence of *Listeria* spp. in fish and environment of fish markets in northern Greece. *Food Control* 2007;18:554–557.
- Uyttendaele M, Rajkovic A, Van Houteghem N, Nico B, Olivier T, Johan D, and Frank D. Multi-method approach indicates no presence of sub-lethally injured *Listeria monocytogenes* cells after mild heat treatment. *Int J Food Microbiol* 2008;123:262–268.

- Vialette M, Pinon A, Chasseignaux E, and Lange M. Growths kinetics comparison of clinical and seafood *Listeria monocytogenes* isolates in acid and osmotic environment. *Int J Food Microbiol* 2003;82:121–131.
- Werbrouck H, Botteldoorn N, Ceelen L, Decostere A, Uyttendaele M, Herman L, and Van Coillie E. Characterization of virulence properties of *Listeria monocytogenes* serotype 4b strains of different origins. *Zoonoses Public Health* 2008;55:242–248.

Address correspondence to:

Paula Teixeira, Ph.D.

CBQF/Escola Superior de Biotecnologia

Universidade Católica Portuguesa

Rua Dr. António Bernardino de Almeida

4200-072 Porto

Portugal

E-mail: pteixeira@esb.ucp.pt