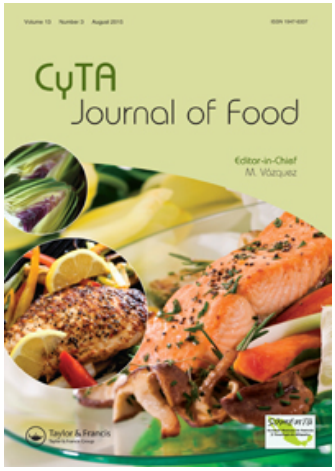

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Occurrence and antibiotic susceptibility of Listeria monocytogenes isolated from raw and processed meat products in Amman, Jordan

Anas A. Al-Nabulsi[a], Tareq M. Osaili[a], Abi A. Awad[a], Amin N. Olaimat[b], Reyad R. Shaker[a] & Richard A. Holley[b]

[a] Department of Nutrition and Food Technology, Jordan University of Science and Technology, Irbid, Jordan

[b] Department of Food Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

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Occurrence and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw and processed meat products in Amman, Jordan

Incidencia y susceptibilidad antibiótica de *Listeria monocytogenes* aislada de productos cárnicos crudos y procesados en Amán, Jordania

Anas A. Al-Nabulsi[a]*, Tareq M. Osaili[a], Abi A. Awad[a], Amin N. Olaimat[b], Reyad R. Shaker[a] and Richard A. Holley[b]

[a]*Department of Nutrition and Food Technology, Jordan University of Science and Technology, Irbid, Jordan;* [b]*Department of Food Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada*

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A total of 270 samples of raw and processed meat products were collected at retail in Amman, Jordan and tested for the presence of *Listeria* spp. by conventional bacteriological methods. *L. monocytogenes* isolates were confirmed by PCR using *L. monocytogenes* virulence gene primers *actA, hlyA, iap, inlA, inlB, inlC* and *inlJ*. The overall occurrence of *L. monocytogenes* was 24.4% using the culture method and 21.9% using the PCR technique. *L. ivanovii* was the main *Listeria* spp., with an overall occurrence of 27%, while *L. welshimeri* and *L. seeligeri* showed the lowest occurrence in both types of product. All confirmed the *L. monocytogenes* isolates were sensitive to ampicillin, gentamicin and vancomycin while 56.6, 10, 6.7 and 5% of isolates were resistant to neomycin, tetracycline, kanamycin and erythromycin, respectively. This first report on the presence of *Listeria* in raw and processed meats in Jordan indicates that these products may pose a risk of listeriosis.

Keywords: *L. monocytogenes*; raw meat; processed meat; ready-to-eat meat; antibiotic susceptibility

Se reunieron un total de 270 muestras de productos cárnicos crudos y procesados obtenidos en puntos de venta en Amán, Jordania y estos fueron analizados en busca de la presencia de *Listeria* spp. utilizando métodos bacteriológicos convencionales. El aislamiento de *L. monocytogenes* fue confirmado mediante PCR utilizando cebadores del gen de virulencia *L. monocytogenes: actA, hlyA, iap, inlA, inlB, inlC* y *inlJ*. El total de incidencia de *L. monocytogenes* fue 24,4% utilizando el método de cultivo y 21,9% utilizando la técnica PCR. *L. ivanovii* fue la principal *Listeria* spp. con un total de incidencia de 27%, mientras que *L. welshimeri* y *L. seeligeri* mostraron el menor grado de incidencia en los dos tipos de productos. Todo ello confirmó que el aislamiento de *L. monocytogenes* fue sensible a la ampicilina, gentamicina y vancomicina mientras que 56,6%, 10%, 6,7% y 5% de los aislamientos resultaron resistentes a la neomicina, tetraciclina, kanamicina y eritromicina, respectivamente. Este primer estudio sobre la presencia de *Listeria* en carnes crudas y procesadas en Jordania indica que estos productos pueden presentar riesgo de listeriosis.

Palabras claves: *L. monocytogenes*; carne cruda; carne procesada; carne lista para el consumo; susceptibilidad antibiótica

1. Introduction

Listeria monocytogenes is a Gram-positive bacterium and one of 10 species that comprise the genus *Listeria*. Other species include *L. innocua, L. seeligeri, L. welshimeri, L. ivanovii* and *L. grayi*, plus the recently added *L. rocourtiae* (Leclercq et al., 2010), *L. marthii* (Graves et al., 2010), *L. weihenstephanensis* (Halter, Neuhaus & Scherer, 2013) and *L. fleischmannii* (Bertsch et al., 2013). Only *L. monocytogenes* and *L. ivanovii* are pathogenic to man and animals, the latter species more frequently infecting animals. *L. monocytogenes* is widely distributed in nature and has been isolated from many different foods of animal and plant origin. Since it can be one of the most virulent foodborne pathogens for immunodeficient individuals, it represents a constant challenge for the food industry, health regulatory officials and consumers (Selby et al., 2006). However, the incidence of listeriosis is low compared with illness caused by other foodborne pathogens such as *Campylobacter jejuni* or *Salmonella* spp. *L. monocytogenes* has been extensively studied over the past few decades because of its high case/fatality rate (20–30%), its high burden of healthcare costs during chronic episodes of infection and its ability to survive for longer periods under adverse environmental conditions than many other non-spore-forming bacteria (Fenlon, 1999).

The prevalence of *L. monocytogenes* in raw and cooked ready-to-eat (RTE) meat products is of concern to the meat industry, particularly in RTE products that enable its growth even at refrigeration temperatures without further inhibitory treatment such as cooking. Byelashov et al. (2009) reported that 34% of raw meat used in the processing of dry-fermented sausages may be contaminated with *L. monocytogenes*, resulting in contamination of up to 72% of ground and stuffed product prior to ageing, fermentation and drying. Additionally, Pao and Ettinger (2009) reported that 29% of frozen beef patties and 5.0% of raw ground beef samples sold in Virginia, USA were contaminated with *L. monocytogenes*. Similarly, 7.9% of sliced RTE meats sampled in the UK were contaminated with *L. monocytogenes* (Little, Sagoo, Gillespie, Grant & McLauchlin, 2009). Despite the lower contamination rates of *L. monocytogenes* in RTE meat products than in raw meat, the former is considered a high risk for transmitting listeriosis to susceptible populations since these foods are preserved refrigerated and some can allow growth of *L. monocytogenes* (Mbandi & Shelef, 2002). RTE meat products are often sliced and packaged at the retail market where further transfer of the pathogen can occur, depending on the level of hygienic practice used by food handlers (Garrido, Vitas & García-Jalón, 2009). Although

*Corresponding author. Email: anas_nabulsi@just.edu.jo

L. monocytogenes is often present in raw dry-fermented sausages, levels are normally <10 CFU/g. It does not grow in dry sausages when properly processed (pH ≤5.3, water activity ≤0.86) and has not been shown to be a public health problem in this class of RTE product (Uyttendaele, De Troy & Debevere, 1999). In contrast with USDA, which has zero tolerance for *L. monocytogenes* in all RTE meats, Canada and most EU countries allow ≤100 CFU/g *L. monocytogenes* in products that do not support its growth (Lara-Lledó, Olaimat & Holley, 2012).

Successful treatment of an invasive case of listeriosis is reliant upon an extended course of antibiotic therapy. In general, antibiotic treatment of listeriosis involves the use of β-lactams such ampicillin or penicillin, alone or combined with gentamicin. However, with patients who have an allergy to β-lactams, trimethoprim and a sulphonamide have been used as an alternative with success (Conter et al., 2009). *L. monocytogenes* is usually susceptible to a wide range of antibiotics, but in 1988 a multidrug-resistant strain was found in France (Poyart-Salmeron, 1990). Since then other strains resistant to one or more antibiotics have been recovered from food, the environment and from sporadic cases of human listeriosis (Conter et al., 2009). The occurrence of antibiotic resistance complicates therapy and lengthens convalescence from illness. Antibiotic use in clinical medicine (appropriate and otherwise) has contributed to the emergence of multidrug-resistant strains, but another contributor has been the use of antibiotics in animal feed as growth promoters (Harakeh et al., 2009). The objectives of the present study were to determine the occurrence of *L. monocytogenes* and *Listeria* spp. in raw and processed meat products sold in Amman, Jordan and to assess the susceptibility of isolated *L. monocytogenes* strains to selected antibiotics used for treating listeriosis.

2. Materials and methods

2.1. *Bacterial culture*

L. monocytogenes (ATCC 7644) (MicroBioLogics Inc., St Cloud, MN, USA) was used as a reference strain for biochemical and PCR analyses while *Staphylococcus aureus* (ATCC 25923) and *Rhodococcus equi* (ATCC 6939) (MicroBioLogics) were used as reference strains for the CAMP diagnostic test for listeriolysin production. The bacterial strains were kept as frozen cultures at –18°C in Brain Heart Infusion (BHI, Oxoid Ltd, Basingstoke, UK) containing 20% glycerol. These strains were refreshed bi-weekly, cultured on Tryptone Soya Agar (TSA) supplemented with 0.6% yeast extract (TSBYE) (Oxoid Ltd., Basingstoke, UK) and stored at 4°C.

2.2. *Meat samples*

Eighty raw meat and 190 processed meat samples were collected from local markets and fresh meat butchers located in Amman, Jordan. Samples were collected aseptically and transported at 4°C in an ice box to the Food Microbiology Lab at Jordan University of Science and Technology. Samples were stored at 4ºC and analysed within 24 h of collection.

2.3. *Isolation and identification of* Listeria *spp.*

All samples were tested for the presence of *L. monocytogenes* following the procedure recommended by the International Organization for Standardization (International Organization for Standarization, 2004). Briefly, a 25 g meat sample was aseptically homogenized in 225 ml pre-enrichment half-Fraser broth (Oxoid Ltd) supplemented with half-Fraser supplement (SR0166E, Oxoid Ltd) in Stomacher bags (Seward Ltd, West Sussex, UK) for 30 s using a Stomacher circulator (Easy Mix, AES Laboratoire, Bruz, France), followed by incubation at 30ºC for 24 h. Then 0.1 ml half-Fraser broth was added to 10 ml Fraser broth containing Fraser supplement and incubated at 37ºC for 48 h.

At the end of incubation, a loopful of Fraser broth was streaked on the surface of *Listeria* selective agar (Oxford agar) supplemented with Modified *Listeria* Selective Supplement (SR0206E, Oxoid Ltd). A loopful was also streaked on chromogenic *Listeria* agar (ALOA) supplemented with Brilliance *Listeria* Differential Supplement (SR0228E, Oxoid Ltd) and incubated at 35ºC for 24 h. Presumptive *Listeria* spp. were identified on Oxford agar by their greyish colonies surrounded by black halos after 24 h, and on chromogenic *Listeria* agar by their green–blue colonies surrounded by an opaque halo. Incubation was extended to 48 h when growth was weak or when none was noted after 24 h. For biochemical identification of *L. monocytogenes*, five suspect colonies from each plate were streaked on TSA supplemented with yeast extract (0.5%) and incubated at 37ºC for 18–24 h.

2.4. *Biochemical confirmation of* Listeria *spp.*

Gram staining, oxidase, catalase, motility, haemolysis and CAMP tests in addition to carbohydrate utilization tests using the Microbact™ *Listeria* 12 L Kit System (Oxoid Ltd) were carried out on all suspect isolates in order to differentiate *L. monocytogenes* from other *Listeria* spp. as described by ISO (1996).

2.5. *Confirmation of isolated* L. monocytogenes *by PCR*

2.5.1. *DNA extraction*

DNA extraction of *L. monocytogenes* (ATCC 7644), used as reference strain, and of *Listeria* isolates that were biochemically confirmed as *L. monocytogenes* was carried out as described by Promega Corp. (Wizard® DNA Purification Kit, Madison, WI, USA). This involved five stages: cell wall lysis, nuclear disruption, RNase treatment, protein precipitation and unfolding of DNA by isopropanol.

2.5.2. *PCR protocol*

One and one-half µl of DNA, prepared as described in the DNA extraction procedure, was amplified with primers for the following virulence genes: *actA* (actin-associated protein); *iap* (invasion-associated protein); *hlyA* (listeriolysin O); and *inlB, inlA, inlC* and *inlJ* (the internalins) (Table 1). The gene amplification methods followed were after Osaili, Alaboudi and Nesiar (2011) for *actA* and *hlyA* genes, after Furrer, Candrian, Hoefelein and Luethy (1991) for the *iap* gene, and after Liu, Lawrence, Austin and Ainsworth (2007) for the *inlB, inlA, inlC* and *inlJ* genes. The amplifications were carried out using a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) and PCR products were visualized with a UV transilluminator and photographed with a gel documentation system (Gel Doc 2000, Bio-Rad, Hercules, CA, USA). A standard 100 bp DNA ladder (GeneRuler, Fermentas, Thermo Fisher Scientific, Waltham MA, USA) was used to determine the size of the amplified fragments.

Table 1. Primer sequences used in PCR assays for *L. monocytogenes*.

Tabla 1. Secuencias de los cebadores utilizados en los ensayos PCR para *L. monocytogenes*.

Target gene	Primer sequences (5′ → 3′)	Product size (bp)	Concentration (µl)	Reference
actA	01 (5′-GCTGATTTAAGAGATAGAGGAACA-3′), 02 (5′-TTTATGTGGTTATTTGCTGTC-3′)	827	1	Osaili et al. (2011)
hlyA	234 (5′- CATCGACGGCAACCTCGGAGA-3′), 319 (5′-ATCAATTACCGTTCTCCACCATT-3′)	417	1	Osaili et al. (2011)
iap	5′-ACA AGC TGC ACC TGC TGC AG-3′ and 5′- TTGA CAG CGT GTG TAG TAG CA-3′	131	1	Furrer et al. (1991)
inlA	ACGAGTAACGGGACAAATGC CCCGACAGTGGTGCTAGATT	800	1	Liu et al. (2007)
inlB	TGGGAGAGTAACCCAACCAC GTTGACCTTCGATGGTTGCT	884	1	Liu et al. (2007)
inlC	AATTCCCACAGGACACAACC CGGGAATGCAATTTTTCACTA	517	1	Liu et al. (2007)
inlJ	TGTAACCCCGCTTACACAGTT AGCGGCTTGGCAGTCTAATA	238	1	Liu et al. (2007)

2.6. *Susceptibility to antimicrobial agents using micro-broth dilution*

2.6.1. *Antibiotics*

The antibiotics used in the current study were chosen based on their mode of action and use in clinical therapy (Al-Nabulsi et al., 2015). Isolates of *L monocytogenes* confirmed by PCR were tested for their susceptibility to the following antibiotics: streptomycin sulfate 65%, gentamicin sulfate 59%, kanamycin sulfate 75%, neomycin sulfate 60%, tetracycline hydrochloride 90%, ampicillin 84.5%, vancomycin 98% (Bio Basic Inc., Markham ON, Canada), ciprofloxacin 98.7% (Biochemika Int., Hangzhou, China), doxycycline HCL 100% (Tocelo, Chemicals B.V., Hertogenbosch, Netherlands) and erythromycin-SCN 84% (Sigma-Aldrich, St Louis, MO, USA).

2.6.2. *Preparation of antibiotics and* L. monocytogenes *cultures*

Antibiotic stock solutions were prepared following the manufacturers' recommendations. Doxycycline and ciprofloxacin were dissolved in sterile distilled water with a few drops (each ≤0.05 ml) of 1 N HCL. The remaining antibiotics were dissolved directly in distilled water. All stock solutions were sterilized using 0.20 µm disposable syringe filter units (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and serially diluted to provide the desired concentrations (0.01–1000 µg/ml) in the reaction mixture.

The 59 *L. monocytogenes* isolates confirmed by PCR and *L. monocytogenes* ATCC 7644 were maintained on TSA slants at 4°C and transferred bi-weekly to maintain viability. One loopful was transferred to 10 ml Tryptic Soy Broth (TSB, Oxoid, Ltd) and incubated at 37°C for 24 h. After that, 0.1 ml of prepared cultures was transferred to 100 ml TSB and incubated as described before. The fresh cultures were diluted in Mueller Hinton Broth (MHB, Oxoid, Ltd) to give a final concentration of approximately 6 $\log_{10}$ CFU/ml in the reaction mixtures.

2.6.3. *Antimicrobial assays (broth micro-dilution method)*

The minimal inhibitory concentrations (MICs) of antibiotics against isolates were tested in MHB at 37ºC using the micro-dilution method described by Al-Nabulsi et al. (2011) and Klare et al. (2005).

2.6.4. *Interpretation of results*

The MIC break-points of selected antibiotics against *L. monocytogenes* isolates were determined using reference values proposed by the Comité de L'Antibiogramme de la Société Française de Microbiologie (CA-SFM) in France (Acar et al., 1998).

3. Results

3.1. *Occurrence of* L. monocytogenes *and* Listeria *spp. in raw and processed meats using conventional culture methods*

L. monocytogenes was isolated from 40/80 (50%) of raw meat samples (Table 2A) and from 26/190 (13.7%) of processed meat samples (Table 2B) based on growth on selective media, Gram reaction, oxidase and catalase tests, as well as on Microbact™ biochemical tests. *L. ivanovii* was isolated from 19/80 (23.7%) raw meat samples but was the most frequently found species in processed meat samples (54/190 or 28.4%), while *L. welshimeri* and *L. seeligeri* showed the lowest occurrence in both raw and processed meat product samples (Tables 2A and B).

3.2. *Incidence of* L. monocytogenes *in raw and processed meats using PCR*

Thirty-five (43.8% of samples) of the 40 *L. monocytogenes* strains isolated by cultural methods from raw meat samples were also confirmed as *L. monocytogenes* by having one or more of the seven virulence genes present and detected by PCR using the primers. Additionally, 24 (12.6% of samples) of the 26 *L. monocytogenes* strains isolated from processed meat samples by conventional methods were similarly confirmed as *L. monocytogenes* by PCR (Table 3). However, no single primer was able to identify all *L. monocytogenes* isolates confirmed by cultural methods. The occurrence of the internalin gene, *inlA*, in isolates (53/59) was the highest among all primers examined, while the gene for the invasion-associated protein, *iap*, was the lowest (Table 3).

3.3. *Susceptibility to antimicrobial agents*

When 10 antibiotics were used to evaluate the susceptibility of *L. monocytogenes* isolates, the MIC ranged from 0.06 to

Table 2. Prevalence of *L. monocytogenes* and other *Listeria* spp. in raw and processed meat products using conventional culture.

Tabla 2. Prevalencia de *L. monocytogenes* y otras *Listeria* spp. en productos cárnicos crudos y procesados utilizando cultivo convencional.

Sample type	Number of samples	*L. monocytogenes* no. (%)	*L. ivanovii* no. (%)	*L. grayi* no. (%)	*L. seeligeri* no. (%)	*L. welshimeri* no. (%)	*L. innocua* no. (%)
A- Raw meat							
Fresh meat							
Beef	35	18 (51.4)	8 (22.8)	3 (8.6)	2 (5.7)	1 (2.9)	4 (11.4)
Lamb	15	5 (33.3)	7 (46.6)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
Frozen meat							
Beef	15	9 (60.0)	1 (6.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lamb	15	8 (53.3)	3 (20.0)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
Total	80	40 (50.0)	19 (23.7)	5 (6.3)	2 (2.5)	1 (1.25)	6 (7.5)
B- Processed meat							
Fresh	10	1 (10.0)	1 (10.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)
Frozen	10	0 (0.0)	3 (30.0)	1 (10.0)	1 (10.0)	1 (10.0)	2 (20.0)
Beef hotdog							
Fresh	10	4 (40.0)	8 (80.0)	2 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)
Frozen	27	8 (29.6)	7 (25.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Beef kofta balls[1]							
Fresh	7	3 (42.8)	5 (18.5)	1(14.2)	0 (0.0)	0 (0.0)	1 (14.2)
Frozen	7	1 (14.2)	4 (57.1)	2 (28.5)	1 (14.2)	0 (0.0)	0 (0.0)
Mortadella[2]	32	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	1 (3.1)	0 (0.0)
Beef salami	65	4 (6.2)	16 (24.6)	9 (13.8)	4 (6.15)	0 (0.0)	8 (12.3)
Beef scallop	2	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	(0.0)
Frozen kebab[3]							
Beef	5	0 (0.0)	2 (40.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)
Lamb	5	2 (40.0)	2 (40.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)
Kibbeh[4]	10	3 (30.0)	3 (30.0)	1 (10.0)	1 (10.0)	1 (10.0)	0 (0.0)
Total	190	26 (13.7)	54 (28.4)	19 (10.0)	8 (4.2)	5 (2.63)	11 (5.8)
Overall occurrence	270	66 (24.4)	73 (27.0)	24 (8.9)	10 (3.7)	6 (2.2)	17 (6.3)

Notes: [1]Meatball; [2]bologna meat; [3]ground meat mixed with spices used for grilling; [4]ground meat mixed with bulghur stuffed with spiced meat.

Notas: [1]Albóndiga; [2]mortadela; [3]carne picada mezclada con especies utilizada a la parrilla; [4]carne picada mezclada con relleno de bulgur y carne especiada.

Table 3. Prevalence of confirmed *L. monocytogenes* in raw and processed meat products using PCR.

Tabla 3. Prevalencia de *L. monocytogenes* confirmada en productos cárnicos crudos y procesados utilizando PCR.

			PCR profile						
Sample type	Number of samples	*L. monocytogenes* no. (%)	*actA*	*hlyA*	*iap*	*inlB*	*inlA*	*inlC*	*inlJ*
A- Raw meat									
Fresh meat									
Beef	35	16 (45.7)	4	4	3	3	14	5	6
Lamb	15	4 (26.7)	3	2	2	3	3	2	1
Frozen meat									
Beef	15	7 (46.7)	2	1	1	4	7	3	2
Lamb	15	8 (53.3)	4	4	1	7	7	5	6
Total	80	35 (43.8)	13	11	7	17	31	15	15
B- Processed meat									
Beef burger									
Fresh	10	1 (10.0)	0	0	0	0	1	0	0
Frozen	10	0 (0.0)	0	0	0	0	0	0	0
Beef hotdog									
Fresh	10	4 (40.0)	0	2	1	0	4	3	2
Frozen	27	8 (29.6)	2	1	2	5	6	1	1
Beef kofta balls[1]									
Fresh	7	2 (42.8)	2	1	0	0	2	2	1
Frozen	7	1 (14.2)	0	0	1	0	0	0	0
Mortadella[2]	32	0 (0.0)	0	0	0	0	0	0	0
Beef salami	65	4 (6.2)	4	1	0	4	5	4	4
Beef scallop	2	0 (0.0)	0	0	0	0	0	0	0
Frozen kebab[3]									
Beef	5	0 (0.0)	0	0	0	0	0	0	0
Lamb	5	2 (40.0)	0	0	0	0	2	1	1
Kibbeh[4]	10	2 (20.0)	1	0	0	0	2	1	1
Total	190	24 (12.6)	9	5	4	11	22	11	8
Overall occurrence	270	59 (21.9)	22	16	11	28	53	26	23

Notes: [1]Meatball; [2]bologna meat; [3]ground meat mixed with spices used for grilling; [4]ground meat mixed with bulghur stuffed with spiced meat.

Notas: [1]Albóndiga; [2]mortadela; [3]carne picada mezclada con especies utilizada a la parrilla; [4]carne picada mezclada con relleno de bulgur y carne especiada.

Table 4. MIC (mg/ml) of selected antibiotics against 59 *L. monocytogenes* isolates and *L. monocytogenes* ATCC 7644, and their antibiotic resistance classification based on MIC break-points proposed by Acar et al. (1998).

Tabla 4. CMI (mg/ml) de antibióticos seleccionados contra aislamientos de 59 *L. monocytogenes* y *L. monocytogenes* ATCC 7644 y su clasificación según resistencia antibiótica basada en los puntos de corte CMI propuestos por Acar et al. (1998).

			MIC break-point (μg/ml)			*L. monocytogenes* no. (%)		
Antibiotic	MIC (μg/ml) ATCC 7644	MIC (μg/ml)	S[a]	I	R	S	I	R
Tetracycline	0.25	0.06–32	≤4	>4, ≤8	>8	54 (90.0)	0 (0.0)	6 (10.0)
Streptomycin	2	1–32	≤8	>8, ≤16	>16	58 (96.7)	0 (0.0)	2 (3.3)
Gentamicin	0.125	0.06–16	≤4	>4, ≤8	>8	60 (100.0)	0 (0.0)	0 (0.0)
Kanamycin	0.5	0.5–32	≤8	>8, ≤16	>16	56 (93.3)	0 (0.0)	4 (6.7)
Neomycin	16	4–256	≤8	>8, ≤16	>16	23 (38.3)	3 (5.0)	34 (56.7)
Ampicillin	0.5	0.125–2	≤4	>4, ≤16	>16	60 (100.0)	0 (0.0)	0 (0.0)
Vancomycin	0.5	0.125–1	≤4	>4, ≤16	>16	60 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	1	0.125–2	≤1	>1, ≤2	>2	57 (95.0)	3 (5.0)	0 (0.0)
Doxycycline	0.125	0.06–16	≤4	>4, ≤8	>8	55 (91.7)	4 (6.7)	1 (1.7)
Erythromycin	0.25	0.125–16	≤1	>1, ≤4	>4	54 (90.0)	3 (5.0)	3 (5.0)

Notes: [a]S: susceptible, I: intermediate, R: resistant.

Notas: [a]S: susceptible, I: intermedio, R: resistente.

256 μg/ml. *L. monocytogenes* ATCC 7644 was sensitive to all antibiotics used here except neomycin, while all *L. monocytogenes* meat isolates were sensitive to ampicillin, gentamicin and vancomycin (Table 4). Furthermore, ≥90% of tested isolates were sensitive to tetracycline, streptomycin, kanamycin, ciprofloxacin, doxycycline and erythromycin, whereas 56.7% of isolates examined were resistant to neomycin.

4. Discussion

The overall occurrence of *Listeria* spp. was 70 and 47.4% in raw and processed meats, respectively (results not shown). Indrawattana et al. (2011) also isolated *Listeria* spp. from 58.7% of raw meat samples in Bangkok. However, our findings showed higher values than those reported by Gibbons, Adesiyun, Seepersadsingh and Rahaman (2006) in Trinidad, Li, Sherwood and Logue (2004) in the United States, Nadhi et al. (2014) in Nigeria and Abd El-Malek, Ali, Hassanein, Moemen and Elsayh (2010) in Egypt. In the present study, the highest occurrence among *Listeria* spp. was *L. ivanovii* in both raw and processed meat products. Ndahi et al. (2014) also found that *L. ivanovii* occurred with the highest frequency (52.8%) among *Listeria* spp. isolated from Nigerian raw and processed meats. *L. grayi, L. seeligeri, L. innocua* and *L. welshimeri* were also isolated from meat products, although the latter occurred with the lowest frequency among *Listeria* spp. Awaisheh (2010) also found that *L. welshimeri* was the least frequently isolated *Listeria* strain from 56 beef and 36 poultry samples in Jordan. The occurrence of *Listeria* spp. in raw meats is difficult to prevent, but in processed meats improper sanitation during the preparation of these products contributes to its distribution (Fenlon, Wilson & Donachie, 1996).

Conventional culture methods detected seven more strains of *L. monocytogenes* than were confirmed by the PCR technique. It is evident that the primers used were unable to detect all *L. monocytogenes* isolates due to the absence of the targeted virulence determinants in some of the biochemically confirmed isolates. The panel of virulence genes used in the present work included those thought most important in determining virulence (Liu et al., 2007; Osaili et al., 2011), though some known virulence genes of *L. monocytogenes* were not part of the panel (i.e. regulatory gene, *prfA*, the phospholipase genes *plcA, plcB* and associated metalloprotease, *mpl*). It is possible that some of the biochemically confirmed but PCR-negative strains were avirulent forms of *L. monocytogenes* (Liu et al., 2007; Rawool, Malik, Shakuntala, Sahare & Barbuddhe, 2007) because of an evolutionary change that may have affected the distribution of pathogenic genes. Rantsiou, Alessandria, Urso, Dolci and Cocolin (2008) also found that the prevalence of *L. monocytogenes* in RTE meat products as determined by real-time PCR did not agree with cultural methods. The choice of target genes is important for detection of virulent *L. monocytogenes* by PCR. However, the detection of one virulence-associated gene by PCR is not always adequate to identify pathogenic *L. monocytogenes* (Rawool et al., 2007; Shakuntala, Malik, Barbuddhe & Rawool, 2006). Although *hlyA* is considered a distinctive gene for identifying *L. monocytogenes*, it was not detected in all *L. monocytogenes* isolates in the current study. To overcome this problem the presence of the six other virulence genes was targeted. The absence of *hly*A may be explained by the occurrence of a specific evolutionary event that resulted in alteration of the profile of genes responsible for pathogenesis. A similar observation was made by Ndahi et al. (2014), in which of 12 *L. monocytogenes* isolates from raw and processed meat products, only one harboured the *hlyA* gene.

The occurrence of *L. monocytogenes* in raw meat (43.8%) was higher than in processed meat (12.6%), and this discrepancy may have been the direct result of cooking during processed meat manufacture. Nonetheless, the presence of *L. monocytogenes* in processed meats is a more serious public health issue because the organism can often grow in products having extended shelf-lives during refrigerated storage and reach levels that facilitate the establishment of invasive infections. Problems in processed meat plants often arise because of inadequate sanitation of food contact surfaces and subsequent cross-contamination that occurs through raw materials, utensils, humans, rodents, insects and even birds (Jemmi & Stephan, 2006). The occurrence of *L. monocytogenes* in processed meat reported in the current study is lower than the 19.2% positive rate reported by Awaisheh (2010) in Jordanian RTE beef products, but is substantially higher than the 1.5% reported by Osaili et al. (2014) in Mediterranean cooked RTE beef products in Jordan.

L. monocytogenes is widely distributed in the environment and is present in the gastrointestinal tract of animals; therefore, low levels of *L. monocytogenes* can naturally be present in raw

meat (Selby et al., 2006). The occurrence of *L. monocytogenes* in raw meat in the present study was higher than that found in other countries, including 34.9% in Spain (Vitas & Garcia-Jalon, 2004), 27.5% in Greece (Filiousis, Johansson, Frey & Perreten, 2009) and 15.4% in Thailand (Indrawattana et al., 2011). Preventative guidelines should be developed in Jordan to reduce *L. monocytogenes* contamination of raw meat and prevent contamination of processed meat products. These guidelines include establishment of good manufacturing practices, appropriate cleaning, sanitation and hygiene programmes, adequate temperature control during processing, storage and distribution, as well as implementation of food safety education and training programmes for food handlers and food retail and service managers (Lianou & Sofos, 2007).

In general, most *L. monocytogenes* strains isolated from clinical, food and environmental sources are susceptible to the antibiotics active against Gram-positive bacteria; however, antibiotic resistance to *L. monocytogenes* has emerged over the last few years (Charpentier & Courvalin, 1999). The drug of choice for treating listeriosis is a β-lactam (ampicillin or penicillin) alone or combined with an aminoglycoside (gentamicin) (Ramaswamy et al., 2007). Fortunately, all confirmed *L. monocytogenes* isolates found in the present study were sensitive to ampicillin, gentamicin and vancomycin. However, 56.6, 10, 6.7 and 5% of these isolates were resistant to neomycin, tetracycline, kanamycin and erythromycin, respectively. Similarly, when Osaili et al. (2011) studied the antibiotic resistance of 17 *L. monocytogenes* isolates from raw or RTE chicken in Jordan it was found that most strains were sensitive to the antibiotics tested; however, three isolates were resistant to tilimicosin and two were resistant to tetracycline. The present results indicate that it is possible to detect antibiotic-resistant *L. monocytogenes* isolates in raw and processed Jordanian meat products. Listeriosis in humans is frequently transmitted via food products; consequently antibiotic-resistant *L. monocytogenes* isolates can have important public health consequences, especially in developing countries where there is widespread and often uncontrolled use of antibiotics. Since antibiotic resistance in *L. monocytogenes* is mainly due to acquisition of mobile elements such as plasmids and conjugative transposons (Charpentier & Courvalin, 1999), it is realistic to anticipate increased observations of bacterial antibiotic resistance in the future.

5. Conclusions

Listeria spp. including *L. monocytogenes, L. ivanovii, L. grayi, L. welshimeri, L. seeligeri* and *L. inncoua* were isolated from 70% of raw meat and 47.4% of processed meat products sold in Jordan. In parallel, *L. monocytogenes* was isolated from 43.8% of raw and 12.6% of processed meat products by the PCR technique using primers targeting seven virulence genes. Among the genes detected by the primers used, *inlA* (internalin A) was most frequently found in isolates. All *L. monocytogenes* isolates were sensitive to ampicillin, gentamicin and vancomycin. However, 56.6, 10, 6.7 and 5% of these isolates were resistant to neomycin, tetracycline, kanamycin and erythromycin, respectively. The data from this study should serve as motivation to develop guidelines in order to ensure the overall safety of raw and processed meat products in Jordan.

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