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Original article

Prevalence, identification and antimicrobial resistance of *Listeria monocytogenes* and *Listeria* spp. isolated from poultry and pork meat

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Summary The aim of this work was to evaluate the prevalence of *Listeria monocytogenes* and other *Listeria* spp. in chicken, duck, quail, turkey and pork meat, including the antibiotic resistance of isolated strains. A total of 184 meat samples were collected from different retailers in La Rioja (Spain). The presence of *Listeria* spp. and *L. monocytogenes* were detected in 24.46% and 10.32% of the meat samples respectively. *L. monocytogenes* was the predominant *Listeria* spp. found in chicken, quail and pork meat, while *L. innocua* and *L. welshimeri* were the predominant species in duck and turkey meat respectively. A total of thirty-three strains (55.93%) of *Listeria* spp. were found to be multi-resistant (resistant to ≥3 families of antibiotics). The highest multi-resistant rates were observed in *L. monocytogenes* (73.68%) and *L. innocua* (70.59%), followed by *L. ivanovii* (50%). Resistance to ampicillin and trimethoprim–sulfamethoxazole were found in *L. monocytogenes* strains isolated from chicken, being of special concern, since these antibiotics are used in the treatment of listeriosis. Special measures should be taken to reduce meat contamination such as adequate handling, correct preparation (cooking) and cleaning and disinfection in order to avoid cross-contamination.

Keywords chicken, duck, food pathogens, food safety, quail, turkey.

Introduction

Listeria is Gram-positive, rod-shaped, non-spore-forming bacteria that are ubiquitous in nature (Doijad *et al.*, 2018). These bacteria can grow in adverse conditions such as environments with high osmolarity, low pH and low temperatures (Chin *et al.*, 2018, González-Fandos *et al.*, 2021a, González-Fandos *et al.*, 2021b). Taxonomically, twenty species have been described within the genus *Listeria* (Doijad *et al.*, 2018; Leclercq *et al.*, 2019). These are divided into two groups based on their genetic and phenotypic relationship: (i) *Listeria sensu stricto*, which includes the species *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. marthii*; and (ii) *Listeria sensu lato*, which includes the species *L. grayi*, *L. rocourtiae*, *L. fleischmannii*, *L. weihenstephanensis*, *L. floridensis*, *L. aquatica*, *L. cornellensis*, *L. riparia*, *L. grandensis*, *L. booriae*, *L. newyorkensis*, *L. costaricensis*, *L. goaensis* and *L. thailandensis* (Doijad *et al.*, 2018; Leclercq *et al.*, 2019). Recently, new species of *Listeria* spp. have been isolated, such as *L. valentina* (Quereda *et al.*, 2020), *L. ilorinensis* (Raufu et al., 2022) and *L. cossartiae*, *L. farberi*, *L. immobilis*, *L. portnoyi* and *L. rustica* (Carlin *et al.*, 2021).

Among the different species of *Listeria*, *L. monocytogenes* and *L. ivanovii* are the only species pathogenic to both animals and humans (Matle *et al.*, 2020). Listeriosis in humans attributed to *L. ivanovii* remains infrequent, with only two documented cases in the scientific literature (Rossi *et al.*, 2022). Additionally, *L. monocytogenes* is considered one of the most significant foodborne pathogens worldwide (Leclercq *et al.*, 2019). On the other hand, the presence of *Listeria* spp. in food may indicate inadequate hygienic conditions (Cufaoglu *et al.*, 2021).

Listeria monocytogenes is widely distributed in the environment and foods (Hoelzer *et al.*, 2015; González-Fandos *et al.*, 2021a). This pathogen can survive for long periods in food processing environment due to its ability to survive under adverse conditions (Ph and low temperatures) and its ability to form biofilms (Knudsen *et al.*, 2017; González-Fandos *et al.*, 2021b).

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Serotype 1/2a strains of *L. monocytogenes* are frequently isolated from the food, while serotype 4b strains are often associated with human listeriosis (Hoelzer *et al.*, 2015). In 2022, 2738 listeriosis cases were reported in the European Union, with 1330 hospitalisations and a high fatality rate (18.1%) (EFSA, 2023). Clinical listeriosis mainly affects high-risk groups: pregnant women, newborns, elderly and immunocompromised people (Hoelzer *et al.*, 2015). In fact, in the European Union in 2022, 70.9% of listeriosis cases were reported in people over 64 years, being 59.8% of the fatal cases in the age group 65–84 years and 24.3% in the age group >84 years. (EFSA, 2023).

On the other hand, emergence of antibiotic resistance strains poses challenges to the clinical treatment in high-risk groups. The data on the evolution of multiple antibiotic resistance should be taken into account in order to mitigate the risk (Wai *et al.*, 2020). Antibiotic treatments such as aminopenicillin (ampicillin or amoxicillin) and benzylpenicillin (penicillin G and gentamicin) often used with aminoglycosides, trimethoprim alone (or combined with sulfamethoxazole), erythromycin and tetracyclines are considered the most commonly used therapeutic treatment for infections caused by *L. monocytogenes* (Kayode & Okoh, 2022). In situations where patients have allergies or certain disease conditions, second-level agents are used, which include vancomycin, trimethoprim–sulfamethoxazole, erythromycin (applicable during pregnancy) and fluoroquinolones. In cases of immunocompromised patients with a high risk of listeriosis, empirical therapy with broad-spectrum antibiotics such as carbapenems or piperacillin–tazobactam may be administered (Kayode & Okoh, 2022).

In the last years, a growth in the prevalence of antibiotic resistance in *L. monocytogenes* isolated from food products has been observed (Olaimat *et al.*, 2018; Carvalho *et al.*, 2019; Kayode & Okoh, 2022). The resistance to antimicrobials in *L. monocytogenes* is a growing concern, not only in human listeriosis due to clinical antibiotic use but also in food and the environment (Li *et al.*, 2022). Cephalosporins, fosfomycin and macrolides have been identified as less effective against this bacterium.

The first report of multiple antibiotic-resistant clinical *L. monocytogenes* isolates was described in France in 1988, and since then, an increasing number of cases have been recorded (Kayode & Okoh, 2022). Several reports describe the resistance profiles of *L. monocytogenes* recovered from different sources, including chicken and pork (Pesavento *et al.*, 2010; Chin *et al.*, 2018; Shen *et al.*, 2022). However, the information available on the antimicrobial resistance of this pathogen isolated from quail, duck and turkey meat is limited (Rahimi *et al.*, 2012; Jamali *et al.*, 2014).

Therefore, the objective of this study was to determine the prevalence in addition to identifying and characterising the antimicrobial resistance of *Listeria* spp. and *L. monocytogenes* isolated from pork and poultry meat.

Material and methods

Sampling and microbiological analysis

A total of 184 meat samples (thirty-five chicken, thirty-seven quail, thirty-one duck, thirty-seven turkey and thirty-nine pork) were collected from different retailers in La Rioja (Spain). The samples were transported to the university laboratory under refrigeration and kept at 4 °C for no longer than 1 h before analysis.

For the enumeration of *L. monocytogenes* and *Listeria* spp., 10 g of meat were aseptically weighed in a laminar flow cabinet and homogenised with 90 mL of 0.1% sterile peptone water (Oxoid, Basingstoke, Hampshire, UK) in a Masticator blender (IUL Instruments, Barcelona, Spain) for 2 min. Serial dilutions were prepared with the same diluent and 1 mL was plated on 140 mm ALOA® plates (BioMérieux, Marcy l'Etoile, France), which were incubated in a Memmert Incubator (Memmert, Schwabach, Germany) for 48 h at 37 °C. The detection limit was 1 log CFU g^{-1}.

To determine the presence or absence of *L. monocytogenes* and *Listeria* spp., 10 g was weighed under aseptic conditions, homogenised with 90 mL of half Fraser broth (Oxoid, Basingstoke, Hampshire, UK) and incubated for 24 h at 30 °C. After this time, 1 mL was taken and transferred to a tube with 9 mL of Fraser broth (Oxoid). incubated for 24 h at 37 °C, then cultures were plated onto ALOA® plates and incubated at 37 °C for 24–48 h.

Isolation and identification of *L. monocytogenes* and others *Listeria* spp.

Between three and five colonies suspected of being *L. monocytogenes* (blue colonies with a halo) or *Listeria* spp. (blue colonies without halo) were isolated and identified from each meat sample analysed. Isolates were purified in Tryptone Soy Agar (Scharlau) and Brain Heart Infusion broth (Scharlau). The purified isolates were kept at −80 °C. Bacterial identification was performed by matrix-assisted laser desorption/ionisation–time-of-flight mass spectrometry (MALDI-TOF MS) Biotyper (Bruker, Billerica, MA, USA).

Antimicrobial susceptibility of *L. monocytogenes* and others *Listeria* spp.

Of the total number of isolated strains, one was selected per sample and identification to carry out the susceptibility to twenty-nine antimicrobials using the disk diffusion method on Mueller–Hinton agar.

The following antibiotic disks (Oxoid, Basingstoke, Hampshire, UK) were used: amikacin (AK, 30 μg), gentamicin (CN, 10 μg), streptomycin (S, 10 μg), tobramycin (TMN, 10 μg), amoxycillin/clavulanic acid (AUG, 30 μg), ampicillin (AMP, 2 μg), penicillin G (PNG, 10 μg), oxacillin (OX, 1 μg), cefotaxime (CTX, 30 μg), ceftaroline (CPT, 30 μg), imipenem (IPM, 10 μg), meropenem (MEM, 10 μg), ciprofloxacin (CIP, 5 μg), enrofloxacin (ENR, 5 μg), levofloxacin (LEV, 5 μg), norfloxacin (NOR, 10 μg), doxycycline (DO, 30 μg), minocycline (MH, 30 μg), tetracycline (TE, 30 μg), tigecycline (TGC, 15 μg), teicoplanin (TEC, 30 μg), vancomycin (VA, 30 μg), chloramphenicol (C, 30 μg), erythromycin (ERY, 15 μg), linezolid (LZD, 30 μg), nitrofurantoin (F, 300 μg), quinupristin/dalfopristin (QD, 15 μg), rifampicin (RD, 5 μg) and trimethoprim/sulphamethoxazole 1:19 (SXT, 25 μg). After incubation at 37 °C for 18–24 h, inhibition zones were measured and scored as susceptible, intermediate (reduced susceptibility) or resistant according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020).

Statistical analysis

Analysis of variance was carried out using SPSS version 26 software (IBM SPSS Statistics). Tukey's test for comparison of means was performed using the same program. The level of significance was determined at $P < 0.05$.

Results

Enumeration and prevalence of *L. monocytogenes* and other *Listeria* spp. isolated from pork and poultry meat

The presence of *Listeria* spp. was detected in 45 samples of the 184 samples tested (24.46%). According to the type of meat, presence was determined in nine of thirty-five chicken samples (25.71%), thirteen of thirty-one duck samples (41.94%), seven of thirty-seven quail samples (18.92%), seven of fifty-one turkey samples (13.73%) and nine of thirty pork samples (30%) (Table 1).

Listeria monocytogenes was detected in 19 samples of the 184 tested (10.32%). According to the type of meat, the presence of this pathogen was found in six of thirty-five chicken samples (17.14%), seven of thirty-seven quail samples (18.92%), two of fifty-one turkey samples (3.92%) and four of thirty pork samples (13.33%). *L. monocytogenes* was not detected in any duck meat sample (Table 1).

Of the forty-five samples with the presence of *Listeria* spp., a total of four samples, two of pork and two of quail, showed *L. monocytogenes* counts higher than 1 log CFU^{-1}. The pork samples showed counts of 1.30 and 1.60 log CFU g^{-1}, while the quail samples showed counts of 2.15 and 2.94 log CFU g^{-1}. In the two quail samples, only *L. monocytogenes* was found. However, in the pork samples after enrichment, *L. innocua* was found in one sample and *L. ivanovii* in the other pork sample. The four samples with *L. monocytogenes* counts were obtained from only two of the retailers evaluated.

Counts of *Listeria* spp., other than *L. monocytogenes*, were only obtained in five duck samples (1.30 log CFU g^{-1} in two samples, 2 log CFU g^{-1} in two samples and 2.73 log CFU/g in one sample). In four samples, only one *Listeria* spp. was identified, while in one sample, two *Listeria* spp. were identified. After enrichment, *L. innocua* was found in the five samples; in four of them, it was only isolated after enrichment. These samples were obtained

Table 1 Number of samples with presence of *Listeria* spp. and *L. monocytogenes* and species identified

Type of meat (*n**)	Number of *Listeria* spp.-positive samples	Number of *L. monocytogenes*-positive samples	Species identified (*n*†)
Chicken (35)	9	6	*L. monocytogenes (3)* *L. monocytogenes, L. innocua (1)* *L. monocytogenes, L. welshimeri (2)* *L. innocua (1)* *L. welshimeri (2)*
Duck (31)	13	0	*L. innocua (7)* *L. welshimeri (1)* *L. welshimeri, L. innocua (4)* *L. welshimeri, L. grayi, L. innocua (1)*
Quail (37)	7	7	*L. monocytogenes (7)*
Turkey (51)	7	2	*L. monocytogenes (1)* *L. monocytogenes, L. welshimeri (1)* *L. innocua (2)* *L. welshimeri (2)* *L. welshimeri, L. ivanovii (1)*
Pork (30)	9	4	*L. monocytogenes (2)* *L. monocytogenes, L. innocua (1)* *L. monocytogenes, L. ivanovii (1)* *L. welshimeri (4)* *L. welshimeri + L. grayi (1)*
Total (184)	45	19	

*Number of samples.
†Number of samples.

from the same retailers where the positive *L. monocytogenes* samples were purchased. In the other thirty-six samples, counts were below 1 log CFU/g, and only after enrichment, *Listeria* spp. were detected. In twenty-nine samples, only one *Listeria* spp. was identified (*L. monocytogenes* in ten samples), and in the other seven samples, two *Listeria* spp. were identified (*L. monocytogenes* in four samples).

Table 1 shows the *Listeria* spp.-positive samples by type of meat. A total of 164 strains were isolated from the forty-five positive samples. The largest number of isolates was identified as *L. monocytogenes* (35.37%), followed by *L. innocua* (32.93%) and *L. welshimeri* (28.05%). Only 1.83% were identified as *L. ivanovii* and *L. grayi*. *L. monocytogenes* was isolated from all types of meat except duck. *L. innocua* and *L. welshimeri* were found in all types of meat except quail. *L. ivanovii* was only isolated from turkey and pork meat, while *L. grayi* was only isolated from duck and pork meat (Table 1).

In 28.9% of the samples (thirty of forty-five samples), more than one species of *Listeria* was identified as can be seen in Table 1. The prevalence of *Listeria* spp. in chicken meat was as follows: *L. monocytogenes* (14.29%), *L. welshimeri* (2.57%) and *L. innocua* (2.57%). In duck meat, the prevalence was as follows: *L. innocua* (38.71%) followed by *L. welshimeri* (19.35%), and to a lesser extent, *L. grayi* (3.23%). In quail meat, only *L. monocytogenes* was isolated (18.92%). In turkey meat, *L. welshimeri* was the predominant species (7.84%) followed by *L. monocytogenes* (3.92%), *L. ivanovii* (1.96%) and *L. innocua* (1.96%). In pork meat, the predominant one was *L. monocytogenes* (10%), followed by *L. welshimeri* (6.67%), with a 3.33% *L. ivanovii*, *L. innocua* and *L. grayi*.

Of the total number of isolated strains, one was selected per sample and identified to carry out the analysis of susceptibility to antibiotics. In total, 59 isolates were selected: 19 from *L. monocytogenes*, 2 from *L. grayi*, 17 from *L. innocua*, 2 from *L. ivanovii* and 19 from *L. welshimeri*.

Antimicrobial resistance of *L. monocytogenes* and other *Listeria* spp. isolated from pork and poultry meat

Table 2 shows the resistance patterns of the fifty-nine strains studied. Of the fifty-nine strains, a total of thirty-three (55.93%) were found to be multi-resistant (resistant to ≥3 families of antibiotics). The highest multi-resistant rates were observed in *L. monocytogenes* (fourteen of nineteen strains, 73.68%) and *L. innocua* (twelve of seventeen strains, 70.59%), followed by *L. ivanovii* (one of two strains, 50%) and *L. welshimeri* (two of nineteen strains, 10.53%). However, none of *L. grayi* strains was multi-resistant. By animal species, the highest multi-resistance rates were found in quail (85.71%, six of seven strains were multi-resistant), followed by chicken (50%, six of twelve), duck (47.37%, nine of nineteen), turkey (44.44%, four of nine) and pork (33.33%, four of twelve).

A strain isolated from turkey identified as *L. innocua* was resistant to a total of nine antibiotics. Two turkey

Table 2 Antimicrobial resistance phenotype of *Listeria* spp. isolated from chicken, duck, quail, turkey and pork meat

Type of meat	Species (number of isolates)	Antibiotic resistance phenotype (number of isolates)
Chicken	*Listeria monocytogenes* (6)	AMP-OX-CTX-MEM (1)
		OX-MEM-SXT (1)
		OX-CTX (1)
		OX-CTX-CPT-MEM (1)
		OX-CTX-CPT (1)
		AMP-OX-CTX-CPT-MEM-F (1)
	Listeria welshimeri (4)	OX-CTX (4)
	Listeria innocua (2)	OX-CTX-CPT-TE (1)
		OX-CTX-F (1)
Quail	*Listeria monocytogenes* (7)	OX-CTX-CPT-MEM (3)
		OX-CTX-CPT-ENR (1)
		OX-CTX-CPT-MEM-F (1)
		OX-CTX-CPT (1)
		OX-CTX-MEM-F (1)
Duck	*Listeria welshimeri* (6)	OX-CTX (3)
		OX (3)
	Listeria innocua (12)	OX-CTX-CPT (1)
		OX-CTX-CPT-F (5)
		OX-CTX-F (2)
		OX-MEM-CIP-DO-MH-TE-ERY-QD-RD (1)
		OX-CTX-TE-F (1)
		OX-CTX (2)
	Listeria grayi (1)	OX-CTX (1)
Turkey	*Listeria monocytogenes* (2)	OX-CTX-CPT-ENR-F (1)
		OX-CTX-CPT-MEM-F (1)
	Listeria welshimeri (4)	OX-MEM-MH-TE-ERY-QD (1)
		OX (2)
		OX-CTX-CPT (1)
	Listeria innocua (2)	OX-CTX-CPT (1)
		OX-CTX-CPT-TE (1)
	Listeria ivanovii (1)	OX-TE (1)
Pork	*Listeria monocytogenes* (4)	OX-CTX-CPT (1)
		OX-CTX-F (2)
		OX-CTX (1)
	Listeria welshimeri (5)	OX-CTX (2)
		OX (3)
	Listeria innocua (1)	OX-CPT-CIP-F (1)
	Listeria ivanovii (1)	OX-CTX-CPT-ENR-F (1)
	Listeria grayi (1)	OX-CTX (1)

AK, Amikacin; AMP, ampicillin; AUG, amoxycillin/clavulanic acid; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; CPT, ceftaroline; CTX, cefotaxime; DO, doxycycline; ENR, enrofloxacin; ERY, erythromycin; F, nitrofurantoin; IPM, imipenem; LEV, levofloxacin; LZD, linezolid; MEM, meropenem; MH, minocycline; NOR, norfloxacin; OX, oxacillin; PNG, penicillin; QD, quinupristin/dalfopristin; RD, rifampicin; S, streptomycin; SXT, trimethoprim/sulphamethoxazole 1:19; TE, tetracycline; TEC, teicoplanin; TGC, tigecycline; TMN, tobramycin; VA, vancomycin.

strains, one *L. monocytogenes* and one *L. welshimeri*, were resistant to five and six antibiotics respectively. A chicken strain identified as *L. monocytogenes* was resistant to six antibiotics.

No strain was resistant to any antibiotic tested from the aminoglycoside or glycopeptide family. Furthermore, no strain was resistant to amoxicillin/clavulanic acid, penicillin G, imipenem, levofloxacin, norfloxacin, tigecycline, chloramphenicol and linezolid.

Of the group of beta-lactams, in penicillin, resistance against two antibiotics was found: ampicillin (3.39%) and oxacillin (100%). Continuing with beta-lactams, of the two cephalosporins evaluated, high percentages of resistance were found against cefotaxime, 100% of the strains were resistant in quail, 91.67% in chicken, 78.95% in duck, 55.56% in turkey and 66.67% in pork. Against ceftaroline, the following resistance rates were found in strains isolated from chicken, quail, duck, turkey and pork – 33.33%, 85.71%, 31.58%, 55.56% and 25.00% respectively. Of the other beta-lactams, resistance rates against meropenem of 33.33%, 71.43%, 5.26% and 22.22% were found in strains isolated from chicken, quail, duck and turkey respectively. However, no resistance against meropenem was detected in strains isolated from pork.

Of the quinolones evaluated, only ciprofloxacin in the strains isolated from duck (5.26%) and pork (8.33%) and enrofloxacin in those isolated from quail, turkey and pork with values of 14.29%, 11.11% and 8.33%, respectively, presented resistance.

In the tetracycline group, only one strain isolated from duck (5.26%) was resistant to doxycycline. One strain isolated from duck (5.26%) and one from turkey (11.11%) were resistant to minocycline. In the case of tetracycline, one strain isolated from chicken (8.33%), two from duck (10.53%) and three from turkey (33.33%) were resistant.

For nitrofurantoin, at least one strain of each type of meat was resistant, two in chicken (16.67%), quail (28.5%) and turkey (22.22%), eight in duck (42.11%) and four in pork (33.33%). Only one chicken strain was resistant to trimethoprim/sulfamethoxazole (8.33%). Only one strain from duck and another from turkey meat were resistant to erythromycin and quinupristin–dalfopristin. Moreover, only one strain from duck was resistant to rifampicin and one strain from chicken to trimethoprim–sulfamethoxazole.

Discussion

Enumeration and prevalence of *L. monocytogenes* and other *Listeria* spp. isolated from pork and poultry meat

In the present work, the presence of *Listeria* spp., was detected in 24.6% of the meat samples. The percentage of positive samples depended on the type of meat: 25.71% in chicken, 41.94% in duck, 18.92% in quail, 13.73% in turkey samples and 30% in pork meat. Other authors have reported a higher prevalence of *Listeria* spp. in chicken meat (41.5%–42.6%) (25.71% in our study) (Yücel *et al.*, 2005; Chin *et al.*, 2018) and turkey meat (40.9%) (13.73% in the present work) (Aras & Ard, 2015). Also, higher prevalence of *Listeria* spp. has been reported by Rahimi *et al.* (2012) in quail meat (22.4%) than in the present study (18.92%). In contrast, lower prevalence has been reported by Rahimi *et al.* (2012) in chicken meat (16.9%) and turkey meat (11%). Jamali *et al.* (2014) reported a lower prevalence of *Listeria* spp. in duck meat (14.1%) than in the present work (41.94%). The differences between studies maybe due to the initial contamination of birds, and also to differences in the hygienic conditions of the processing plants. *Listeria* spp. is widely distributed in birds, and its presence in poultry meat can be due to faecal contamination during evisceration in the slaughterhouse since birds are usually asymptomatic carriers of this bacterium and they can ingest this bacterium through contaminated food, water or soil (Dhama *et al.*, 2015; Luque-Sastre *et al.*, 2018). On the other hand, *Listeria* spp. may be present on the equipment and installations in poultry processing plants, and they can contaminate the surface of poultry meat (Gonçalves-Tenório *et al.*, 2018). Similar prevalence has been reported by Pesavento *et al.* (2010) in pork meat, with a percentage of 28.5% compared to the 30% obtained in our samples. The prevalence of *Listeria* spp. is higher in pork meat than in pigs; pork meat is mainly contaminated with *Listeria* spp. during processing (Kanuganti *et al.*, 2002). In consequence, special care should be given to hygienic measures during meat processing/handling and packaging of pork meat.

Similar prevalence of *L. monocytogenes* has been reported by Cufaoglu *et al.* (2021) in chicken meat (19.1% and 17.14% in the present work) and Liu *et al.* (2020) in pork meat (11.3% and 10.26% in the present work). However, higher prevalence has been reported by Ochiai *et al.* (2010) in chicken (28.7%, 17.14% in the present work) and pork meat (35.7%, 13.33% in the present work). Also, higher prevalence of *L. monocytogenes* was found by Aras & Ard (2015) in turkey meat (10.43%). We did not find *L. monocytogenes* in duck meat. However, other authors have reported a prevalence of 4.8% in this type of meat (Jamali *et al.*, 2014). Lower prevalence of *L. monocytogenes* has been found by Rahimi *et al.* (2012) in chicken, quail and turkey meat (1.9%, 5.2% and 0.9%, respectively) (17.14%, 18.92% and 3.92% in the present work). *L. monocytogenes* can be present in the environment of pig and poultry farms, being common asymptomatic carriage animals (Schoder *et al.*, 2022; Lagarde *et al.*, 2024). Both pigs and poultry can harbour the bacterium in their digestive tract and excrete it into the

environment, being considered the primary source of *L. monocytogenes* in carcass contamination at the slaughterhouse (Luque-Sastre *et al.*, 2018; Lagarde *et al.*, 2024). Differences in the prevalence of this pathogen can be explained by the contamination in animals and the hygienic conditions during processing (Luque-Sastre *et al.*, 2018; Oswaldi *et al.*, 2022; Lagarde *et al.*, 2024).

We observed that the dominant *Listeria* spp. depended on the meat type. It should be highlighted that in quail meat, we only isolated *L. monocytogenes* (18.92%), while Rahimi *et al.* (2012) reported that *L. innocua* was the predominant species (12% of the samples) followed by *L. monocytogenes* (5.2%), *L. welshimeri* (2.2%) and *L. seeligeri* (1.7%).

We observed a prevalence of *L. monocytogenes* of 14.29%, *L. welshimeri* of 2.57% and *L. innocua* of 2.57% in chicken meat, while Rahimi *et al.* (2012) reported that *L. innocua* was the predominant species (13.8%), followed by *L. monocytogenes* (19%) and *L. welshimeri* (1.3%).

In duck meat, we observed that the highest prevalence of *Listeria* spp. was *L. innocua* (38.71%), followed by *L. welshimeri* (19.35%), and to a lesser extent, *L. grayi* (3.23%). In contrast, Jamali *et al.* (2014) reported lower prevalence of *L. innocua* (1.4%) and the presence of others: *Listeria* spp. *L. ivanovii* (5.5%), *L. innocua* 2.57% (4.8%) and *L. seeligeri* (2.4%) (Jamali *et al.*, 2014).

In turkey meat, we found that *L. welshimeri* was the predominant species (7.84%), followed by *L. monocytogenes* (3.92%), *L. ivanovii* (1.96%) and *L. innocua* (1.96%), while Aras et al. pointed out that *L. innocua* was the predominant species (14.8%), followed by *L. grayi* (13%), *L. monocytogenes* (10.43%) and *L. welshimeri* (Aras & Ard, 2015).

We found that in pork meat, the predominant species was *L. monocytogenes* (10%), followed by *L. welshimeri* (6.67%), *L. ivanovii*, *L. innocua* and *L. grayi* (3.33%). However, Pesavento *et al.* (2010) reported that the predominant species was *L. innocua* (50%). *L. monocytogenes*, *L. innocua* and *L. welshimeri* have often been isolated at slaughterhouses from pigs and processing environments (Oswaldi *et al.*, 2022).

Poultry and pork meat can be contaminated with *L. monocytogenes* and *Listeria* spp. during (i) animal production, (ii) processing at the slaughterhouse from healthy carrier animals, environment or equipment and (iii) meat processing from environment, hands or equipment (Nesbakken *et al.*, 1996; Upmann *et al.*, 2000; Perez-Arnedo *et al.*, 2021; González-Fandos *et al.*, 2021a, 2021b). Even more *L. monocytogenes* can adapt to determined niches in processing plants, and persist for long periods, causing cross-contamination (Colagiorgi *et al.*, 2017). Consequently, equipment, walls and floors can be a source of contamination with *L. monocytogenes* during processing. As sources of *Listeria* spp. in meat, the following have been pointed out: carcass gut content, contaminated equipment, contaminated surfaces and food handlers. *L. monocytogenes* can find its way into food processing facilities through various means, including cross-contamination by workers and transportation of animals (Quereda *et al.*, 2021). Although poultry meat can be contaminated with *L. monocytogenes* after evisceration, washing and chilling, portioning operation has been pointed out as the most critical stage (Upmann *et al.*, 2000, Perez-Arnedo *et al.*, 2021). In fact, in the present work, all the samples that presented *L. monocytogenes* count and 73.68% of those with the presence of *Listeria* spp. were obtained from two of the retailers evaluated. These findings suggest the relevance of meat handling and cross-contamination and highlight the relevance of keeping good hygienic practices (Perez-Arnedo *et al.*, 2021; Siluma et al., 2023).

Antimicrobial resistance of *L. monocytogenes* and other *Listeria* spp. isolated from pork and poultry meat

Since the first case reported in the early 1990s, concern about antibiotic resistance in *Listeria* spp. and *L. monocytogenes* has been increasing. Over time, several studies have found resistant strains of *Listeria* spp. and *L. monocytogenes* isolated from various sources (Escolar *et al.*, 2017). The diversity of antimicrobial resistance patterns observed in the last 25 years can be explained by exposure to different types of antimicrobials in different geographic locations and time periods (Escolar *et al.*, 2017).

The first-line drugs in the treatment of listeriosis are ampicillin, penicillin and gentamicin, either alone or in combination. If the patient is allergic to penicillin, trimethoprim–sulfamethoxazole (STX) can be successfully used (Cufaoglu *et al.*, 2021). Cufaoglu *et al.* (2021) reported higher resistance rates to these antibiotics than us. We only found two *L. monocytogenes* strains resistant to ampicillin and one to SXT, all of them isolated from chicken (resistant rates of 3.38% and 1.69% considering the 59 strains evaluated). Also, Yücel *et al.* (2005) only found resistance against trimethoprim–sulfamethoxazole in *L. monocytogenes*. Other authors have reported high resistance rates to trimethoprim–sulfamethoxazole in *L. monocytogenes* strains isolated from turkey (50%) (Aras & Ard, 2015). However, Cufaoglu *et al.* (2021) pointed out resistance rates of 27.2% for ampicillin and 97.3% for SXT in strains isolated from different foods including chicken meat. Yücel *et al.* (2005) have also reported high resistance rates against ampicillin in *L. monocytogenes*, *L. innocua* and *L. welshimeri* strains isolated from meat and meat products (66%, 96% and 100%, respectively), while Rahimi *et al.* (2012) observed the following resistance rates for these species: 11.1%, 9.2% and 22.2% in raw meat.

As in the present work, other authors have not found resistance to gentamicin, penicillin and vancomycin in *Listeria* spp. isolated from chicken or pork meat (Chin *et al*., 2018; Shen *et al*., 2022). However, Aras & Ard (2015) found resistance to gentamicin and penicillin in *Listeria* spp. isolated from turkey meat.

While Chin *et al*. (2018) reported resistance against chloramphenicol and erythromycin, of 6.25% and 12.5%, respectively, in strains isolated from chicken meat, we observed that all the strains from chicken were susceptible to these antibiotics. However, we observed resistance to erythromycin in two strains: one isolated from turkey and another from duck meat, none of them was *L. monocytogenes*. Although Chin *et al*. (2018) observed resistance to tetracycline in strains isolated from chicken meat (25%), we only observed resistance to this antibiotic in two strains isolated from turkey and two strains isolated from duck meat.

According to Aras & Ard (2015), the percentages of resistance to oxacillin in strains isolated from duck meat ranged from 75% to 93.3%. In contrast, we observed that 100% of the strains were resistant to this antibiotic. We also observed 83.3% *L. monocytogenes* isolated from chicken meat and 100% of those isolated from quail, turkey and pork were resistant to ceftaroline. Also, Panera-Martínez *et al*. (2022) found high resistance to oxacillin among *Listeria* spp. isolated from chicken (above 90%).

As in the present work, other authors have only found resistance against ciprofloxacin in *L. innocua* (Yücel *et al*., 2005; Aras & Ard, 2015). Resistance to enrofloxacin has also been reported by other authors (Panera-Martínez *et al*., 2022).

We only found one *L. innocua* strain isolated from duck resistant to rifampicin, but not from turkey. Neither Chin et al found resistance to rifampicin in *Listeria* spp. isolated from chicken (Chin *et al*., 2018). However, Aras & Ard (2015) found resistance against this antibiotic in *L. innocua* (18.8%) and *L. monocytogenes* (8.3%) isolated from turkey.

Listeria monocytogenes strains resistant to nitrofurantoin have also been reported in chicken meat by other authors (Panera-Martínez *et al*., 2022). We also found *L. monocytogenes* strains resistant to nitrofurantoin in quail, turkey and pork meat.

We found the highest multi-resistance rates in quail meat (85.71%), followed by chicken (50%), duck (47.37%), turkey (44.44%) and pork (33.33%). We observed that 73.68% of *L. monocytogenes* strains were multi-resistant, higher percentage than that reported by other authors (33.3%–49.1%) (Jamali *et al*., 2014; Panera-Martínez *et al*., 2022). Similar to Aras & Ard (2015), we observed that the highest multi-resistant rates corresponded to *L. monocytogenes*. Other authors have also reported a similar percentage of *Listeria* spp. multi-resistant (55.3%, 55.93% in the present work) (Aras & Ard, 2015). Multi-resistant *L. innocua* has been reported in meat at high levels (62%) as in the present study (Aras & Ard, 2015).

As in the present work, Pesavento *et al*. (2010) isolated *L. innocua* and *L. monocytogenes* from poultry resistant to a high number of antibiotics. We isolated one *L. innocua* strain from turkey resistant to nine antibiotics, and one *L. monocytogenes* and one *L. welshimeri* strain from turkey resistant to five and six antibiotics respectively. We also found one *L. monocytogenes* strain from chicken resistant to six antibiotics.

Different measures have been recommended to control *L. monocytogenes* in foods (FAO and WHO, 2007, 2022); additionally, surveillance of the prevalence of *L. monocytogenes* in foods has been carried out (EFSA, 2023). In fact, the prevalence of this pathogen in foods has not shown a significant increase in the European Union in the period 2018–2022 (EFSA, 2023); even some authors have reported a decrease in the last years (Hanes & Huang, 2022; Maung *et al*., 2023). Since antimicrobial resistance is an increasing health issue, control programmes have been adopted at the national and international levels (FAO, 2021; WHO, 2023). Some authors have indicated that the percentage of *L. monocytogenes* multi-resistant strains in poultry and pork meat has not increased or even it has reduced in the last years due to the measures taken at farm level (Hanes & Huang, 2022; Maung *et al*., 2023). However, some studies indicate that most of the isolates harboured virulence genes (Maung *et al*., 2023). Moreover, as in the present work, resistance to antibiotics that are used in the treatment of human listeriosis has been observed (Panera-Martínez *et al*., 2022), and in consequence, control measures are needed (Maung *et al*., 2023).

Conclusions

Listeria monocytogenes was the predominant *Listeria* spp. found in chicken, quail and pork meat, while *L. innocua* and *L. welshimeri* were the predominant species in duck and turkey meat respectively. Our results show that chicken, quail and turkey can be a source of multi-resistance *L. monocytogenes* strains. Resistance to ampicillin and trimethoprim–sulfamethoxazole was found in *L. monocytogenes* isolated from chicken, which is of special concern for consumers' health since these antibiotics are used in the treatment of listeriosis. Special measures should be taken in order to reduce meat contamination such as adequate handling, correct preparation (enough cooking) and cleaning and disinfection in order to avoid cross-contamination.

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Author contributions

Alba Martinez-Laorden: Investigation; formal analysis; data curation; writing – original draft. **Celia Arraiz-Fernandez:** Data curation; investigation. **M. Jesús Cantalejo:** Data curation. **Elena Gonzalez-Fandos:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; writing – review and editing; formal analysis; project administration; data curation; supervision; resources; visualization.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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