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ORIGINAL ARTICLE

Prevalence, virulence genes, and antimicrobial resistance profile of *Listeria monocytogenes* isolated from retail poultry shops in Beni-Suef city, Egypt

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ABSTRACT

Objective: This study investigated the prevalence of *Listeria monocytogenes* in retail poultry shops, characterized the antibiotic resistance profile, and detected the genotypic pattern of virulence genes.

Materials and Methods: Broiler meat (*n* = 90), intestinal content (*n* = 40), and environmental samples (*n* = 95) were collected for this study. Besides, hand swabs (*n* = 20) were obtained from the poultry shop workers and stool samples (*n* = 40) were collected from the outpatient clinics of Beni-Suef University Hospital, Egypt. The samples were subjected to isolation and identification of *L. monocytogenes* by conventional bacteriological examinations and biochemical tests, followed by confirmatory identification by the polymerase chain reaction.

Results: Among the collected samples (*n* = 285), *L. monocytogenes* could be detected in 14.4% (*n* = 41/285) of the samples, where 30.0% (*n* = 12/40) of the intestinal content was positive. Similarly, 10.0% (*n* = 9/90), 15.0% (*n* = 3/20), and 12.5% (*n* = 5/40) of the samples of meat, hand swabs, and stools were found positive for *L. monocytogenes*, respectively. A total of 12 (12.6%) out of 95 environmental samples were positive for *L. monocytogenes*. Based on the antimicrobial sensitivity profile, most of the recovered isolates were multidrug-resistant against most commonly used antibiotics.

Conclusion: The findings conclude that poultry shops play a vital role in transmitting *L. monocytogenes* to the consumers. Asymptomatic poultry shop workers should draw attention to their potentials for spreading the infection to the consumers through the contaminated carcasses. Low hygienic standards are present in commercial poultry shops that increase the risk of contamination in the sold products.

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Introduction

Over recent decades, with the terrible increase in red meat prices, poultry production witnessed a marked increase worldwide. This led to intensive poultry production with an increase in both the number of farms and flock size. In Egypt, broilers are usually raised on deep litter, resulting in poultry contamination with spoilage microorganisms and infection with zoonotic pathogens, including *Listeria monocytogenes* [1]. As a sapro-zoonosis, *Listeria* is a potentially dangerous organism widely dispersed in the environment in various non-animal reservoirs (e.g., water, soil, plants, and forages). Moreover, these are found in the intestinal tract of both diseased and healthy animals and humans. Young animals show *Listeria* infection symptoms occasionally, but most of them are healthy carriers and, therefore, are not excluded from the farm or slaughtered during *ante mortem* inspection [2]. Epidemiological data suggest that the contaminated products of animal origin, especially poultry, contribute significantly to foodborne diseases and the surrounding environment's contamination. The reduction in raw poultry contamination levels would have a considerable impact on reducing the illness incidence [3].

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In poultry, listeriosis can be seen sporadically in septicemia or localized encephalitis. The disease is occasionally observed in young chicks [4]. Intestinal colonization of poultry by *L. monocytogenes* with subsequent occurrence in feces represents a potential source for listeriosis in ruminants [5,6]. Listeriosis in humans is a severe disease manifested by septicemia, meningitis/meningoencephalitis, abortion, and baby illness at birth. The elderly, immunocompromised individuals, pregnant women, and fetuses or neonates are considered the most vulnerable population to the disease [7,8]. Although human listeriosis is rare, it is among the most important causes of death from foodborne infections in industrialized countries [9,10]. In Egypt, consumers depend on specific cultural legacies in buying retailed meat from poultry shops where they choose live birds to be slaughtered with minimal hygienic standards. Thus, it increases the risk of microbial contamination with various pathogens, including *L. monocytogenes* [11–13].

Antimicrobial drugs are routinely used for the treatment of listeriosis in humans and animals. The primary choices of antibiotics include ampicillin, gentamycin, streptomycin, or their combination [14]. Antimicrobial resistance in pathogenic bacteria possesses a tremendous public health concern. It is well documented that some *L. monocytogenes* strains have acquired an additional public health impact because of their multiple antimicrobial resistances. Accordingly, it is necessary to implement rigorous monitoring of the antimicrobial susceptibility of *L. monocytogenes* strains [15,16].

Data about antimicrobial drugs used in treating listeriosis in humans and animals and their antimicrobial resistance pattern are scarce in Egypt. Therefore, this study aimed to (i) focus on the prevalence of *L. monocytogenes* in retail poultry shops in Beni-Suef city, (ii) characterize their antibiotic resistance profile, and (iii) detect their virulence genes as an essential step toward the control of future incidence of *L. monocytogenes* at both farm animal and human infection levels.

Materials and Methods

Ethical approval

This study's methodology was ethically approved by the Institutional Animal Care and Use Committee of Beni-Suef University (BSUIACUC).

Study location and period

This study was carried out in Beni-Suef city (coordinates 29° 04′ N-31° 05′E), Beni-Suef locality, Egypt, from March to August 2019.

Sample collection

A total of 285 samples comprising broiler meat (n = 90), intestines (n = 40), environmental samples (n = 95), hand swabs (n = 20), and stool samples (n = 40) were collected. The samples were received in sterile plastic containers and kept on ice. The samples were directly transported to the Animal Hygiene and Zoonoses Laboratory of the Beni-Suef University to isolate the associated *L. monocytogenes*. In this study, retail poultry shops were selected randomly in Beni-Suef city, Beni-Suef locality, Egypt. Sanitary measures prevailing in the shops were somewhat similar, ranging from very low to low. None of the examined workers was suffering from an apparent illness. The stool samples were taken from patients attending the outpatient clinic in Beni-Suef University Hospital (20 were diarrheic, whereas the remaining were healthy). Stool samples were obtained individually from each outpatient in a sterile plastic cup and labeled carefully. The investigators filled out a standardized questionnaire for each shop worker, as well as hospital outpatients. The collected data through these questionnaires included age, gender, occupation, location of individual's residence, and the recorded signs and symptoms. The level of sanitary measures adopted was registered for those working in the shops.

Isolation and identification of L. monocytogenes

The collected samples were directly immersed in *Listeria* enrichment broth (LEB; Oxoid, Cat. No. CM0862), supplemented with acriflavine-HCL (15 mg/l), nalidixic acid (40 mg/l), and cycloheximide (50 mg/l) [17,18]. All the primary enrichment broths were incubated at 30°C for 48 h. For poultry meat, 25 g of the sample was aseptically transferred into sterile Stomacher closure bags containing 500 ml of half-strength Fraser enrichment broth with CCFA supplement (pre-enrichment broth) and was homogenized for 1 min, followed by incubation of the samples in pre-treatment broth at 30°C for 48 h. The pre-enriched cultures were diluted 1:100 into 10 ml of full-strength Fraser enrichment broth with CCFA supplement (enrichment broth), then incubated at 37°C for 48 h [17].

Following enrichment, a loopful from each broth culture was streaked onto Oxford *Listeria* selective agar (Oxoid, Cat. No. CM0856), supplemented with *Listeria* selective supplement (Oxoid, Cat. No. SR0140), containing cycloheximide (400 mg/l), colistin sulfate (20.0 mg/l), acriflavine (5.0 mg/l), cefotetan (2.0 mg/l), and phosphomycin (10.0 mg/l). The plates were incubated at 37°C for 24–48 h [18]. As a result of aesculin hydrolysis, grayish green colonies surrounded by black zones were supposed to be *Listeria* spp. The suspected colonies grown on

the Oxford medium's surface were selected and streaked onto tryptone soya agar (Oxoid, Cat. No. PO0163) plates supplemented with 0.6% Tryptic Soya Agar-Yeast Extract (TSA-YE) for purification, and were incubated at 37°C for 24 h for further identification.

Biochemical tests verified the suspected colonies, and further confirmation was carried out by the polymerase chain reaction. The methods of conventional identification of pure isolates included Gram stain, catalase, oxidase, motility (20°C–25°C), acid formation in Kliger iron agar, methyl red and Voges-Proskauer reaction, nitrate reduction, sugar fermentation test, blood hemolysis onto 5.0% sheep blood agar plates, and Christie–Atkins–Munch-Peterson (CAMP) test [19–21].

Species-specific identification of *L. monocytogenes* using the *16SrRNA* gene [22] was carried out. DNA extraction from the suspected samples was conducted using the QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's recommendations. Molecular characterization was achieved at the Biotechnology Center in the Animal Health Research Institute, Egypt.

Detection of L. monocytogenes virulence genes

The detection of hemolysin A (*hlyA*), phosphatidylinositol phospholipase C (*pIcA*), invasion-associated protein p60 (encoded by the *iap* gene), a pleiotropic virulence regulator, *prfA* (a 27-kDa protein encoded by *prfA*), and internalin (*inlA* and *inlB*) genes were carried out using the specific primer sequences listed in Table 1.

Antimicrobial susceptibility pattern

The antimicrobial sensitivity pattern was conducted on 20 randomly selected isolates from the recovered strains proven to be *L. monocytogenes* by biochemical and molecular techniques, using the disk diffusion method [27], and tested with most of the commonly used antibiotics in the treatment of listeriosis in humans and animals. The antibiotics included penicillin (10 µg), ampicillin (30 µg), amoxicillin/clavulanic acid (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), streptomycin (10 mg), sulfamethoxazole/trimethoprim (25 µg), tetracycline (30 µg), and vancomycin (30 µg).

Results

The results in Table 2 and Figure 1 show that *L. monocytogenes* was detected in 41 (14.4%) samples out of 285. A total of 12 (30.0%) poultry intestinal samples were positive for *Listeria*. In the case of poultry meat samples, nine (10.0%) samples were positive. Similarly, three (15.0%) and five (12.5%) of hand swabs and stool samples were positive for *L. monocytogenes*, respectively. Besides, *L. monocytogenes* was recovered from 12 (12.6%) environmental swabs.

Table 1. Sequences, target genes, and amplicon sizes for the identification and virulence characterization of *L. monocytogenes* isolated in the study.

Target gene	Primers sequences	Amplified segment (bp)	References
16SrRNA	GGA CCG GGGCTA ATA CCG AAT GAT AA TTC ATG TAG GCG AGT TGC AGC CTA	1,200	[22]
hlyA	GCA TCT GCA TTC AAT AAA GA TGT CAC TGC ATC TCC GTG GT	174	[23]
plcA	ACA AGC TGC ACC TGT TGC AG TGA CAG CGT GTG TAG TAG CA	1,484	[24]
iap	CTG CTT GAG CGT TCA TGT CTC ATC CCC C CAT GGG TTT CAC TCT CCT TCT AC	131	
prfA	TCT CCG AGC AAC CTC GGA ACC TGG ATT GAC AAA ATG GAA CA	1,052	[25]
inlA	ACG AGT AAC GGG ACA AAT GC CCC GAC AGT GGT GCT AGA TT	800	[26]
inlB	CTG GAA AGT TGT ATT GGG AAA TTT CAT AAT CGC CAT CAC	343	

Table 2. Prevalence of *L. monocytogenes* in the examined samples.

Samples/Swabs	No. of samples examined	No. of positive samples	Percentage (%)
Meat	90	9	10.0
Intestine	40	12	30.0
Hand swab	20	3	15.0
Stool	40	5	12.5
Environmental	95	12	12.6
Total	285	41	14.4

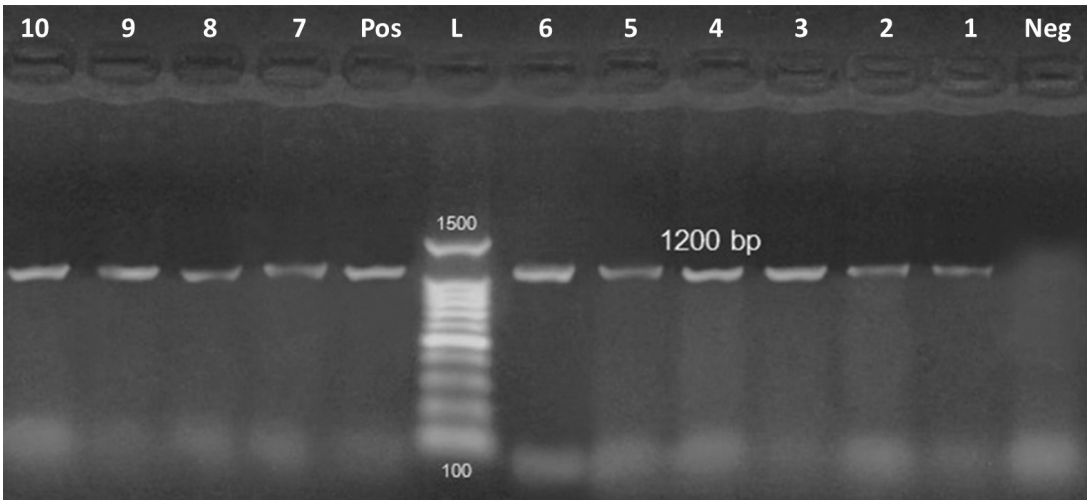

Figure 1. Gel electrophoresis of the PCR product using *16S rRNA* (amplified 1200 bp) of *L. monocytogenes* gene-specific primer. L = Ladder 100–1,500 bp, Neg = Control negative; Pos = Control positive. Lane 1–10: Number of examined samples.

Table 3. Distribution of virulence genes of *L. monocytogenes* in the examined samples.

Samples/ Swabs	Positive No. (%)	Genes screened (%)					
		hlyA	*plcA*	*iap*	*prfA*	*inlA*	*inlB*
Meat	9 (10.0)	9 (100.0)	– (0.0)	– (0.0)	– (0.0)	– (0.0)	– (0.0)
Intestine	12 (30.0)	12 (100.0)	– (0.0)	– (0.0)	– (0.0)	3 (25.0)	– (0.0)
Hand swab	3 (15.0)	3 (100.0)	0 (0.0)	– (0.0)	1 (33.3)	1 (33.3)	1 (33.3)
Stool	5 (12.5)	5 (100.0)	– (0.0)	– (0.0)	– (0.0)	1 (20.0)	– (0.0)
environmental	12 (12.6)	12 (100.0)	– (0.0)	– (0.0)	– (0.0)	– (0.0)	– (0.0)

The frequent distribution of virulence genes in the recovered traits (Table 3) was detected only in the intestinal samples, hand swabs, and stool samples. There were no detectable target genes in the environmental or meat samples. The findings showed that the internalin genes (*inlA*) were detected in three (25.0%) intestinal samples, one (33.3%) hand swab sample, and one (33.3%) stool sample (Fig. 2A). Furthermore, *inlB* and *prfA* genes were found only in one hand swab sample (33.3% each) (Fig. 2B and C). *hlyA* gene was detected in all recovered isolates of *L. monocytogenes* (100.0% each) (Fig. 2D). On the contrary, *pIcA* and *iap* genes were not recorded in any examined samples (Fig. 2E and F).

Table 4 demonstrates the antimicrobial sensitivity pattern of the isolated *L. monocytogenes* (n = 41) from different examined samples, revealing that most of these

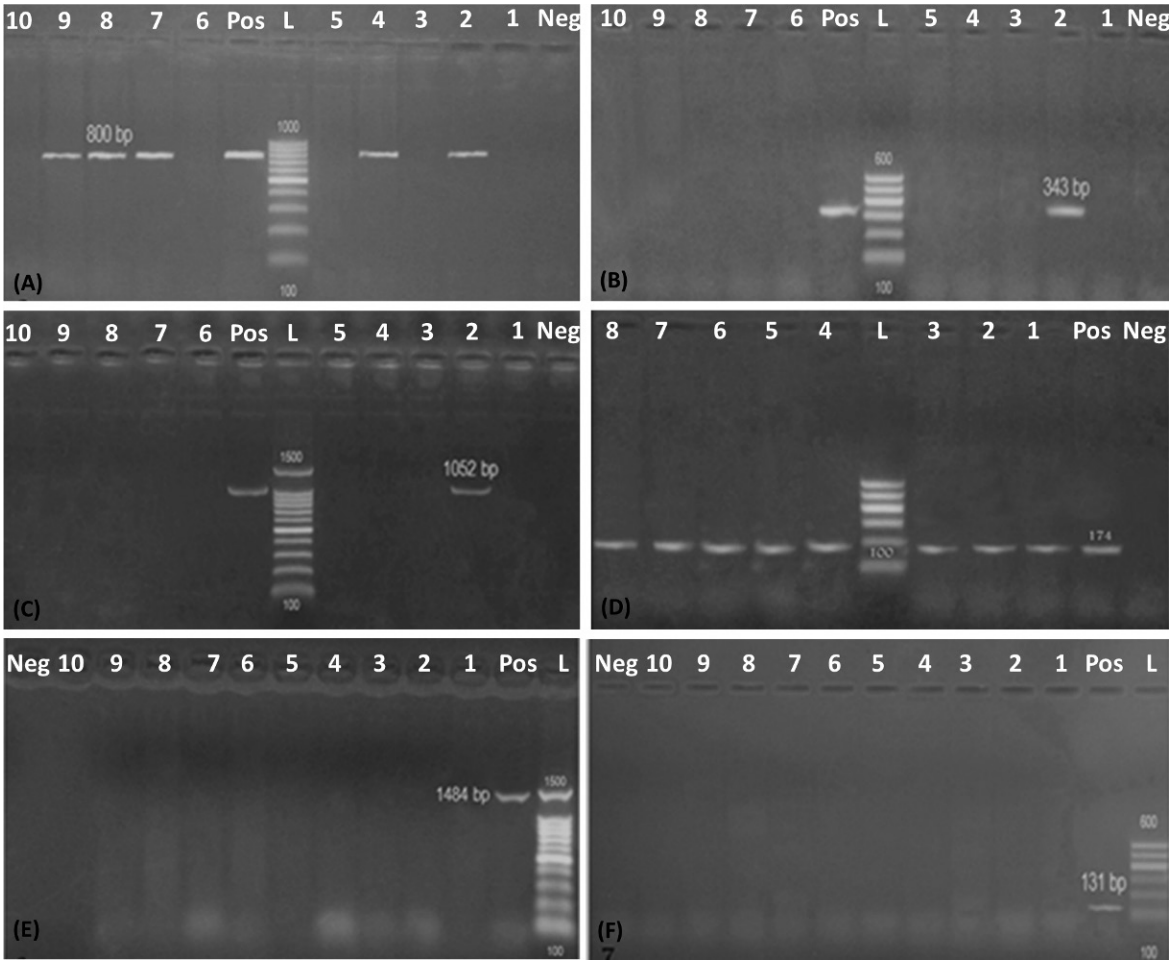

Figure 2. PCR amplification of different virulent genes. (A) PCR product of *inlA* gene (800 bp). (B) PCR product of *inlB* gene (343 bp). (C) PCR product of *prfA* gene (1,052 bp). (D) PCR product of *hlyA* gene (174 bp). (E) PCR product of *pclA* gene (1,484 bp). (F) PCR product of *iap* (131 bp). L = Ladder 100–1,500 bp, Neg = Control negative; Pos = Control positive. Lane 1–10: Test samples.

isolates were multidrug-resistant, especially to penicillin, ampicillin, erythromycin, and tetracycline. However, the isolates recovered from human stool samples showed high sensitivity to most tested drugs. Environmental samples considered the most resistant isolates showing complete resistance (100.0%). However, the isolates were still sensitive to a variable degree to erythromycin, streptomycin, and vancomycin. Isolates obtained from the intestine showed nearly equal sensitivity to amoxicillin/clavulanic acid and vancomycin, followed by sulfamethoxazole/trimethoprim to a lesser degree. *L. monocytogenes* isolates recovered from meat samples exhibited high sensitivity to sulfamethoxazole/trimethoprim (88.8%) followed by vancomycin and streptomycin (77.7% each), then chloramphenicol (66.6%). *Listeria* isolates obtained from human samples (both hand swabs and stool samples) were sensitive to amoxicillin/clavulanic acid, chloramphenicol, and sulfamethoxazole/trimethoprim (66.7 and 100.0%, 66.7 and 100.0%, and 66.7 and 80.0%, respectively). Furthermore, *Listeria* recovered from human stool samples was sensitive to ampicillin, erythromycin, streptomycin, and vancomycin (100.0, 100.0, 80.0, and 60.0%, respectively) (Table 4).

Discussion

The prevalence of *L. monocytogenes* in the present study was slightly higher than the reports of 8.0% by Weber et al. [28], 4.36% by Kalender [15], 8.0% by Abd El-Malek et al. [16], 7.14% by Al-Ashmawy et al. [29], 4.0% by Awadallah and Suelam [30], 4.2% by Shaker and Hassanien [31], and

Table 4. *In-vitro* susceptibility testing of antimicrobial profile of *L. monocytogenes* isolates recovered from the examined samples.

Samples/ swabs (*n*)	Antibiotics /dose (µg/disc)%																							
	Penicillin G (10 mg)			Amoxicillin + Clavulanic (30 µg)			Ampicillin (30 µg)			Chloramphenicol (30 µg)			Erythromycin (15 µg)			Tetracycline (30 µg)			Vancomycin (30 µg)			Streptomycin (10 mg)			Sulfamethoxazole/ Trimethoprim (25 µg)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Meat (9)	0.0	0.0	100.0	44.4	0.0	55.5	0.0	0.0	100.0	66.6	0.0	33.3	22.2	11.1	66.6	11.1	0.0	88.8	77.7	0.0	22.2	77.7	11.1	11.1	88.8	0.0	11.1
Intestine (12)	0.0	0.0	100.0	58.3	16.7	25.0	0.0	0.0	100.0	16.7	8.3	75.0	0.0	0.0	100.0	0.0	0.0	100.0	50.0	16.7	33.3	8.3	8.3	83.0	25.0	8.3	66.7
Hand (3)	0.0	0.0	100.0	66.7	0.0	33.3	0.0	0.0	100.0	66.7	0.0	33.3	0.0	0.0	100.0	0.0	0.0	100.0	33.3	33.3	33.3	0.0	33.3	66.7	66.7	0.0	33.3
Stool (5)	0.0	100.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	60.0	20.0	20.0	80.0	0.0	20.0	80.0	0.0	20.0
environmental (12)	0.0	0.0	100.0	16.6	16.6	66.7	0.0	0.0	100.0	0.0	0.0	100.0	50.0	0.0	50.0	0.0	0.0	100.0	25.0	25.0	50.0	41.6	8.3	50.0	16.6	0.0	83.3

N = Number of samples; S = Susceptible; I = Intermediate; R = Resistant.

7.5% by Osman et al. [32]. On the contrary, Rahmat et al. [33] detected the bacterial pathogen in 62.5% of poultry meat samples, and Carvalho et al. [34] detected *L. monocytogenes* in 94.6% of poultry meat and meat processing environments. The prevalence of *L. monocytogenes* in different examined samples in this study indicated relatively higher isolation rates of the pathogenic bacteria from other types of samples than previous studies. This might be attributed to the complete dispersal of the pathogen that could be found in untreated water, soil, and silage, which can contaminate food products of animal and plant origin easily [35]. The fact that a wide variety of saprophytic and pathogenic organisms is found in poultry meat such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Listeria* spp. support the results obtained in this study [36]. On the other hand, there is a considerable relationship between hygienic standards in retail poultry shops under examination and bacterial contamination. The lower the sanitary measures practiced in these shops, the more bacterial contamination, and subsequent isolation.

Several virulence genes proved to play an essential role in the pathogenicity of *L. monocytogenes*, including *hlyA*, *actA*, *inlA*, *inlB*, *inlC*, *inlJ*, *prfA*, *plcA*, and *iap* [37]. In the present study, *hlyA*, *inlA*, *inlB*, and *prfA* virulence genes were detected in intestinal and human (hand swabs and stool samples) isolates, which suggested a significant threat to public health through consumption of contaminated carcasses together with a low level of hygienic standards in retail poultry shops and food processing areas [26]. Nearly similar results were obtained by Moreno et al. [38] in Brazil, Wang et al. [39] in China, Jamali et al. [40] in Iran, and Gelbicova and Karpiskova [41] in the Czech Republic. This study's findings suggest that the environment is the reservoir of this bacterial pathogen and considers the link between human and animal infections. It might play a role in the cross-transfer of virulence genes between virulent and non-virulent strains, increasing their public health significance.

Listeria spp. are usually sensitive to many antimicrobial drugs, including penicillin, chloramphenicol, aminoglycosides, tetracyclines, and macrolides. Increasing resistance rate has been detected lately in variable serovars [37]. Similar results were obtained by Jamali et al. [40] and Rahimi et al. [42], who detected that *Listeria* spp. was sensitive to gentamycin and vancomycin and resistant to penicillin. Reciprocally, Wellinghausen [43] and Arslan and Baytur [10] proved *Listeria* spp. as sensitive to penicillin (100.0%). Much lower results were obtained by Wellinghausen [43], who mentioned that only 10.0% of *Listeria* spp. were resistant to sulfamethoxazole/trimethoprim. Multidrug-resistant *L. monocytogenes* strains isolated from food and human samples have been reported by Safdar and Amstrong [44] and

Marian et al. [45]. The marked increase in the rate of antimicrobial resistance among *Listeria* spp. against the most used antibiotics in animal and human listeriosis treatment might be attributed to misuse and/or abuse of antibiotics [46].

Conclusion

The current study concludes that retail poultry shops play a vital role in the transmission of *L. monocytogenes* to the consumers. The poultry shop workers may act as asymptomatic carriers of this pathogen, which focuses on their potentiality for spreading the infection to the human population through the contaminated carcasses. The cleaning and sanitation level in retail poultry shops also has a leading role in determining contamination in the sold product. It is realized that the lower the hygienic standards in poultry shops, the more chances of contamination of the surrounding environment. As a result, it increases the subsequent reduction of the quality of the sold products. Further studies should be applied to find an effective disinfectant to break the link between animal and human infections in the environment.

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Conflict of interest

No conflict of interest was found between the authors.

Authors' contributions

Both authors contributed equally in designing, experimentation, analysis, and manuscript preparation and finalization. All the authors finally approved the publication of the article.

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