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Prevalence of multidrug-resistant *Listeria monocytogenes* in retailed goat meat and offal

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A B S T R A C T

Goat meat is a major source of animal-derived protein worldwide. Listeria spp., particularly Listeria monocytogenes (L. monocytogenes) is one of the foodborne pathogens that has serious health effects. This study investigated the prevalence of Listeria spp., particularly L. monocytogenes in the meat and offal of the goat retailed in Mansoura city, Egypt. Besides, antibiogram of the recovered L. monocytogenes isolates was further screened. In addition, the recovered L. monocytogenes isolates were screened for harboring drug resistance related genes using PCR. The obtained results of the present study revealed an overall isolation rates of Listeria spp. and L. monocytogenes from goat edible tissues at 26%, and 8%, respectively. The prevalence rates of Listeria spp., in goat's muscle, liver, kidney, lungs, and rumen were 4%, 6%, 6%, 0%, and 10%, respectively. Serological identification of the isolated Listeria spp. revealed recovery of six Listeria spp. namely, L. ivanovii, L. welshimeri, L. innocua, L. seeligeri, L. grayi, and L. monocytogenes. L. monocytogenes was isolated at 4%, 6%, 6%, 0%, and 10% from goat's muscle, liver, kidney, lungs, and rumen, respectively. The recovered L. monocytogenes showed multidrug resistance profiling, particularly against tetracycline and erythromycin with 100% resistance rates. Interestingly, all isolated L. monocytogenes had tet L, and mef A coding genes for resistance against tetracycline and erythromycin. In conclusion, goat meat and offal should be considered as potential sources of Listeria spp., particularly, L. monocytogenes. Therefore, strict hygienic measures should be adopted during handling of goat meat and offal.

Keywords: Goat meat; offal; Listeria monocytogenes; multidrug resistance

1. Introduction

Goat meat production has increased worldwide, particularly in Egypt. Goat meat is regarded as a rich source of animal-derived protein, vitamins such as vitamin B group, and minerals such as zinc, and iron. Goat meat is also rich in polyunsaturated fatty acids, and therefore regarded as a healthy meat source compared with meat of other meat-producing animals (Kadim et al., 2004; Mahgoub et al., 2002). However, microbiological studies related to goat meat and offal and their role in the transmission of foodborne pathogens has received less attention.

Listeria species are ubiquitous organisms that able to grow over a wide range of temperatures and food substrates (Weller et al., 2015). Listeria monocytogenes (L. monocytogenes) has regarded as a foodborne pathogen with zoonotic importance. It causes listeriosis, a disease that arise mainly through ingestion of contaminated food and drink. The disease symptoms include gastroenteritis, fever, abortion, meningitis, and encephalitis, and might lead to death (Abdel-Malek et al., 2010; Dimic et al., 2010). Lack of hygienic measures followed during slaughtering, processing, storage, and distribution of meat and offal might lead to their contamination with foodborne pathogens such as L. monocytogenes (De Cesare et al., 2017; Liu et al., 2020). Several studies reported isolation of Listeria spp., particularly L. monocytogenes from goat products. For instances, Listeria spp. was isolated from goat meat and milk retailed in India at 17.64%. In the same study, L. monocytogenes was isolated at 6.66%, and 1.56% from goat's meat and milk samples, respectively (Barbuddhe et al., 2000). Listeria spp., were isolated from cattle and goat flesh retailed in Port Harcourt, Nigeria at 52.78%, and 30.56% (Eruteya et al., 2014). However, there is limited information available about the prevalence of multidrug resistant L. monocytogenes in retailed goat's meat and offal in Egypt.

The uncontrolled use of drugs during livestock production had led to development of multidrug resistance among the foodborne pathogens (Alsayeqh et al., 2021; Darwish et al., 2013). Multidrug resistant L. monocytogens has become a critical health issue worldwide (Barbuddhe et al., 2002).

The present study aimed at investigation of the prevalence rates of Listeria spp., particularly, L. monocytogenes in the retailed goat's meat and offal in Mansoura city, Egypt. Furthermore, antimicrobial resistance profiling was examined among the recovered L. monocytogenes isolates. Besides, screening of drug-resistance related genes among the identified L. monocytogenes isolates was done using PCR

2. Materials and Methods

1. Collection of samples:

A total number of hundred samples including 20 samples from each of fresh raw goat meat (round), lungs, liver, kidney, and rumen (each sample is 100 g in weight) were collected from butchery shops at different sanitation levels in Mansoura city, Egypt. The collected samples were transferred cooled to the laboratory for bacterial isolation and identification of Listeria spp.

Organoleptic examination:

Sensory evaluation of the collected samples was carried out based on the color, odor, and consistency (Pearson and Tauber, 1984).

Isolation and identification of Listeria spp.:

Bacteriological examination of Listeria spp. in the examined goat samples was done according to the method of APHA (2001) including the following steps:

Enrichment procedures:

Ten grams of each sample were homogenized in peptone water 1% (90 ml) for 3 min at 3000 rpm in the room temperature. The homogenate was incubated at 37°C for 24 h. A second enrichment procedure was then taken via addition of one ml of the enriched culture to 9 ml of Full Fraser broth and incubation at 37°C for 48 h.

Isolation procedures:

A loopful from the second enriched culture was streaked onto Oxford agar (Himedia, India) containing Listeria Oxford supplement (Himedia, India), followed by incubation for 48 h at 35°C. Colonies of 1-2 mm in diameter, resembling dew drop-like, and black with brown hallow colonies were regarded as Listeria colonies. Such presumptive colonies were inoculated into Tryptone Soya broth (TSB) with 0.6% yeast extract as a supplement and stored at 4oC for further identification.

Identification of Listeria isolates:

Listeria isolates were identified according to their morphological, and biochemical characteristics (FAO/WHO, 2010), and serologically using the Oxoid Listeria Test Kit (Oxoid, Basingstoke, Hampshire, England) following the manufacturer's instructions.

Antibiogram of the identified L. monocytogenes:

Evaluation of the antimicrobial resistance profiles of the recovered isolates of L. monocytogenes was carried out using the disk diffusion method. The guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2001) were followed for the choice of antimicrobials and the interpretation of the results. The tested antimicrobial discs were

purchased from Oxoid Limited, Hampshire, UK. Nutrient agar plates acted as a culture medium for L. monocytogenes. Calculation of the multiple antibiotic resistance (MAR) index for each strain was determined according to the formula stipulated by Singh et al. (2010) as follow:

MAR index= No. of resistance (Isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antibiotics.

Molecular detection of drug-resistance relates genes among L. monocytogenes isolates:

PCR was used for molecular detection of drug resistance-related genes among the recovered L. monocytogenes isolates. The screened genes were Tet L, a coding gene for tetracycline resistance, and mef A, a coding gene for macrolides resistance. The used primers (Pharmacia Biotech) in the PCR reaction were presented in Table 1

The technique recommended by Morvan et al. (2010) was applied with some modifications. L. monocytogenes isolates were refreshed on brain heart infusion broth at 37ºC. Then, the suspension was heated at 100ºC for 20 min. Five µl of each obtained lysate was used as a DNA template in the PCR reaction mixture.

Amplification reaction of L. monocytogenes:

The PCR amplification reaction was performed on a Thermal Cycler (Master cycler, Eppendorf, Germany). PCR was performed for two drug resistance related genes (tet L, and mef A). The PCR cycles started with an initial denaturation at 95°C for 2 min, followed by 40 cycles each is consisting of denaturation for 15 sec at 95°C, annealing for 30 sec at 60°C, and extension for 1 min at 72°C. A final extension step for 7 min at 72°C was employed, followed by a holding at 4°C. Amplified DNA fragments were run on 1.5% agarose gel electrophoresis (AppliChem, GmbH, Germany) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on a UV transilluminator.

3. Results and Discussion

L. monocytogenes is a critical health issue worldwide. This study investigated the isolation and identification of Listeria spp., from goat edible tissues. All examined samples had normal sensory characteristics, in terms of fresh odor, firm in consistency, and brick red color for meat, rosy red for lungs, bluish red for liver and kidney, and grayish color for rumen (data are not shown). The obtained results of the current research revealed overall isolation rates of Listeria spp., and L. monocytogenes from all examined goat samples at 26%, and 8%, respectively. The prevalence rates of Listeria spp, in goat's muscle, liver, kidney, lungs, and rumen were 4%, 6%, 6%, 0%, and 10%, respectively. While, L. monocytogenes was isolated at 4%, 6%, 6%, 0%, and 10% from these samples, respectively (Fig. 1). Serological identification of the isolated Listeria spp., revealed recovery of six Listeria spp., namely, L. ivanovii, L. welshimeri, L. innocua, L. seeligeri, L. grayi, and L. monocytogenes. The distribution of different Listeria spp., serotypes among the positive goat samples was as following: L. ivanovii was detected in one liver and kidney sample at 3.85% each, and from 2 rumen samples at 7.69%. L. welshimeri was isolated at 3.85% from each of liver, kidney, and rumen samples. L. innocua was isolated at 3.85% from each of muscle, liver, kidney, and rumen samples. L. seeligeri were isolated at 3.85% from each of muscle, liver, and kidney, and at 7.69% from rumen samples, respectively. L. grayi was isolated only from one rumen sample at 3.85%. L. monocytogenes was the most identified Listeria spp. at 30.77%. The distribution of L. monocytogenes isolates was 2 from muscle and liver samples at 7.69%, one kidney sample at 30.77%, and three rumen samples at 11.54%, respectively (Table 2). In agreement with the obtained results in the current study, L. monocytogenes was isolated at 6.66%, from the meat of the goat retailed in India (Barbuddhe et al., 2000). In addition, Listeria spp., was isolated at 30.56%, 27.78%, 17.86%, and 33.33% from goat muscle, intestine, kidney, and liver, respectively in Port Harcourt, Nigeria (Eruteya et al., 2014). In the same study, L. monocytogenes was isolated at only 1.29% while L. welshimeri was the most predominant species. Listeria spp., was isolated at higher rates (73.9%) from imported frozen beef, and at 43.5% from local beef in Malaysia. In the same study, L. monocytogenes was recovered at 75%of the frozen beef samples, and at 30.4% of local meat, but not isolated from buffalo meat (Hassan et al., 2001). In Egypt, Listeria spp., and L. monocytogenes were isolated from buffalo meat and mince at 34%, and 10%, respectively (Al-Humam et al., 2021). Contamination of goat meat and edible offal in the present study with different Listeria spp., and particularly L. monocytogenes indicates improper hygienic practices adopted during slaughtering, evisceration, or distribution (Shamloo et al., 2019). Foods contaminated with L. monocytogenes might lead to human listeriosis if ingested. This disease is characterized by the occurrence of symptoms like meningitis, encephalitis, abortion, and even death (Castellazzi et al., 2018).

The uncontrolled usage of antimicrobials during animal farming is a major cause for the development of drug resistance among several foodborne pathogens (Darwish et al., 2013). In the present study, L. monocytogenes isolates showed a 100% resistance to both of oxytetracycline, and erythromycin. The drug resistance rates for the other tested antimicrobials were as following: ampicillin (37.5%), cephalothin (37.5%), chloramphenicol (37.5%), ciprofloxacin (75%), enrofloxacin (50%), gentamicin (75%), kanamycin (62.5%), nalidixic acid (50%), neomycin (87.5%), oxacillin (25%), streptomycin (25%), and sulfamethoxazole (50%) (Fig. 2). All isolated L. monocytogenes had multidrug resistance profiles with an average MAR index of 0.471 (Table 3). Interestingly, all of the recovered L. monocytogenes harbored both of tet L, and mef A coding genes for resistance against tetracyclines, and macrolides (Fig. 3). In agreement with the obtained results in the present study, L. monocytogenes isolated from raw meat products retailed in Turkey showed multidrug resistance with 100% resistance rates to cephalothin and nalidixic acid, and 66% of isolates were resistant to sulfamethoxazole, ampicillin, and trimethoprim (Yucel et al., 2005). Besides, L. monocytogenes isolated from retailed food products in China were resistant to tetracycline and ciprofloxacin at 8.4%, and 1.8%, respectively (Zhang et al., 2007). Moreover, Maćkiw et al. (2021) in a recent study reported that 83% of L. monocytogenes isolates were resistant to ampicillin. Likely, Morvan et al. (2010) reported that drug resistance of L. monocytogenes isolates is linked to the transfer of drug resistance-coding genes among the bacterial generations, and particularly, resistance to tetracyclines and fluoroquinolones is more common and has recently emerged.

4. Conclusion

The present study demonstrates that goat meat and edible offal should be considered as potential sources of multidrug-resistant L. monocytogenes. Therefore, adoption of strict hygienic measures is highly recommended during the preparation and processing of meat and meat products before serving to humans.

Conflict of Interest: The authors declare no conflict of interest.

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Table 1: The used primers in the present study

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
Tet L(F)	5′ CCACCTGCGAGTACAAACTGG ′3	739	Morvan et al. (2010)
Tet L (R)	5′ TCGGCAGTACTTAGCTGGTGA ′3		
Mef A (F)	5′ AGTATCATTAATCACTAGTGC ′3	345	
Mef A (R)	5′ TTCTTCTGGTACTAAAAGTGG ′3		

Table 2: Distribution of different Listeria spp., among positive goat meat and offal samples

Sample	*L. ivanovii*		*L. welshimeri*		*L. monocytogenes*		*L. innocua*		*L. seeligeri*		*L. grayi*	
	No	%	No	%	No	%	No	%	No	%	No	%
Muscle	0	0	0	0	2	7.69	1	3.85	1	3.85	0	0
Liver	1	3.85	1	3.85	2	7.69	1	3.85	1	3.85	0	0
Kidney	1	3.85	1	3.85	1	3.85	1	3.85	1	3.85	1	3.85
Lungs	0	0	0	0	0	0	0	0	0	0	0	0
Rumen	2	7.69	1	3.85	3	11.54	1	3.85	2	7.69	1	3.85
Total	4	15.38	3	11.54	8	30.77	4	15.38	5	19.23	2	7.69

Table 3: Antimicrobial resistance profiling of the recovered L. monocytogenes from goat meat and offal

Isolate ID	Resistance profile	MAR index
L. monocytogenes 1	AMP, CN, CP, E, G, K, NA, N, OX, T, S, SXT	0.857
L. monocytogenes 2	AMP, CN, CP, E, G, NA, N, T, S, SXT	0.714
L. monocytogenes 3	AMP, CP, E, G, K, NA, N, T, SXT	0.642
L. monocytogenes 4	CN, CH, CP, E, G, NA, N, T	0.571
L. monocytogenes 5	CH, EN, G, K, E, N, T, SXT	0.571
L. monocytogenes 6	CH, EN, G, K, E, N, T	0.5
L. monocytogenes 7	CP, EN, K, E, N, T	0.428
L. monocytogenes 8	CP, EN, OX, E, T	0.357
Average		0.471

Fig. 1: The overall isolation rates of Listeria spp., and L. monocytogenes from goat meat and edible offal

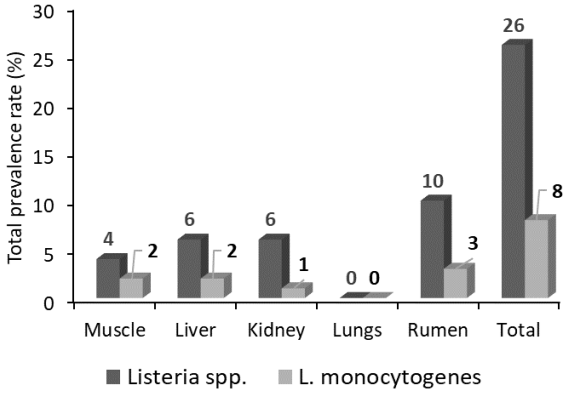

Fig. 2. Antibiogram of the recovered L. monocytogenes

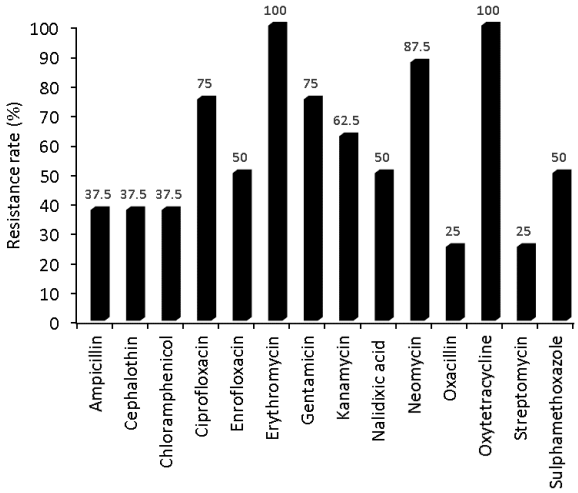

Fig. 3. Agarose gel electrophoresis of drug resistance related genes in the recovered L. monocytogenes from goat meat and offal a) Tet L, b) mef A. M refers to a 100 base pairs DNA marker, 1-8 refers to the recovered L. monocytogenes isolates.