

RESEARCH ARTICLE

Occurrence and Characterization of *Listeria* Species Isolated from Processed Meat in Qena, Egypt

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Abstract

The aims of this work were isolation, elucidation the antimicrobial susceptibility and molecular characterization of *Listeria* spp from meat products distributed in Qena Governorate, Egypt during years 2017 -2018. A total of 120 samples of raw meat products were collected from different retail outlets in Qena Governorate, Egypt and examined for the contamination with *Listeria* spp. The examined meat products were minced meat, kofta, sausage, burger, luncheon and pasterma and the prevalence of *listeria monocytogenes* was 15%, 20%, 10%, 15%, 10%, 5%, respectively. Moreover, other *Listeria* species were isolated and identified in a total percentage from the above mentioned meat products; *Listeria ivanovii* (10.8%), *L. welshimeri* (6.6%), *L. innocua* (10.8%), *L. seeligeri* (4.1%) and *L. grayi* (1.6%). Antibiogram assay detected multi-dug resistances among *L. monocytogenes*. All the isolates were resistant to neomycin and streptomycin, meanwhile, most of the isolates showed sensitivity against ampicillin. Furthermore, *L. monocytogenes* was molecularly characterized by multiplex PCR for detection of *iap*, *hylA* and *actA* virulence genes. The *iap* gene was detected in all *L. monocytogenes*. It could be concluded that processed meat products purchased in Qena Governorate harbored *L. monocytogenes* and other *Listeria* species. This in turn constitute a risk of transmission of infection to human consumer with the antibiotic resistant *listeria* spp. That gives rise to failure of treatment programs. High contamination level of meat substantiates enforcing Hazard Analysis Critical Control Points (HACCP) program during processing, handling and storage of meat products.

Keywords: *Listeria monocytogenes*, Meat Products, Antimicrobial Resistances, Virulence.

Introduction

Processed meat products are meat subjected to comminution, mincing or slicing with the addition of various amounts of fat. Salt and spices are added as flavoring agents, and other non-meat ingredients are supplemented to extend the volume and reduce the cost of the products [1]. *Listeria* is a Gram-positive bacterium, it includes many species as *L. grayi*, *L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. seeligeri*, *L. fleischmannii* and *L. weihenstephanensis*. *L. monocytogenes* and *L. ivanovii* are well known to be pathogenic to

humans and animals causing a food borne illness in human beings [2].

Listeria is a widely disseminated in the nature and has been found in various foods of animal origin. *Listeria* can grow in broad zones of temperature ranging from 1.5°C to 45°C, pH started from 4.3 till 9.40 and a salt level till 10%. The ubiquitous feature of *listeria* led to contamination of various processed meat and fermented meat products at the different stage of processing and storage [3, 4]. In contrast to other enteric pathogenic bacteria, *Listeria* can multiply at refrigeration temperature zones, which indicates that

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refrigeration of meat to 4°C could not hinder the growth of *listeria* [5].

Food-borne listeriosis is a serious disease with high fatality rates (20-30%) compared with other foodborne illness [6]. *Listeria monocytogenes* is a serious food microbial hazards that can lead to typical febrile gastroenteritis, abortion, stillbirth meningoencephalitis, and septicemia [7]. Various studies have reported the prevalence of *Listeria* spp. in a broad range of meat and its products [2, 8, 9, 10-11].

Listeria species are generally sensitive to numerous antibiotics; however, drug-resistant strains have been emerged in food [12]. Resistance in *Listeria* strains is caused by the horizontal transfer of antimicrobial resistance genes and genetic interchanges between different *Listeria* species [13, 14]. The existence of any species of *listeria* in meat and its products is an index for unsatisfied hygienic measures in a food plant. The contamination of processed meat with *Listeria* spp. is still trouble in many countries. Based on the best of our knowledge, there are not enough data about the prevalence and characteristics of *Listeria* spp in processed meat. Therefore, the goal of this research was to trace the degree of contamination with *Listeria* spp in meat products in Qena Governorate. Furthermore, the antibiotic susceptibility profiles and virulence genes were investigated.

Materials and methods

Samples' collection and preparation

One hundred and twenty random samples of meat products, (n=20) per each of minced meat, kofta, sausage, burger, luncheon, and pastirma. They were purchased from various retail groceries and supermarkets in Qena Governorate during 2017-2018 and were collected in an icebox and directly transferred to the laboratory.

The samples were prepared by thawing of frozen samples through overnight keeping in refrigerator. Aseptically, the casing of each

sample was removed and the content was blended thoroughly in sterile blender separately with sterilization after each use.

Isolation of Listeria spp.

The techniques described by the FAO [15] and the International Organization for Standardization [16] were applied for isolation of *Listeria* from food.

Primary Enrichment step

The initial step of isolation of *listeria* includes the utilization of *listeria* selective enrichment broth (CM0862, Oxoid, England) to promote the population of *Listeria*. Ten grams of the prepared samples were mixed with 90 mL *listeria* selective enrichment broth and incubated at 30°C for 7 days [17].

Secondary Enrichment

Ten mL of Fraser broth (CM08015, Oxoid, England) was inoculated with 0.1 mL of primary enrichment broth and then incubated at 35°C for 24 h.

Selective Plating

From Fraser enrichment broth, 0.1 mL was streaked into Oxford *Listeria* selective agar base (M1145, Himeida) and incubated at 35°C for up to 48 h. The typical colony was characterized by brown color with black zones around them. Suspected colonies were picked up separately into tryptone soya agar (DM277, Micro master, India) supplemented with 0.6 % yeast extract and incubated at 35 °C for 24 h for further identification. The isolates were tested for Gram's stain.

Determination of biochemical profile

The following biochemical tests: catalase, oxidase, esculin hydrolysis, sugar fermentation tests (rhamnose, xylose, mannitol) and hemolysis tests were performed based on techniques described by ISO [18].

Serological examination

Suspected *listeria* isolates were tested by Oxoid *Listeria* Test Kit (Oxoid, Basingstoke, England) which is fast latex agglutination test

used for confirmation of *Listeria* according to the manufacturer instructions [19].

Determination of antibiotics susceptibility of *Listeria monocytogenes*

The Kirby-Bauer disc diffusion assay was used to characterize the antimicrobial resistance based on the methodology described by Clinical and Laboratory Standards Institute CLSI, [20]. The following antimicrobial discs and their concentrations were used; neomycin (30 µg), streptomycin (10 µg), kanamycin (30 µg), cephalothin (30 µg), erythromycin (15 µg), sulphamethoxazole (25µg), nalidixic acid (30 µg), oxytetracycline (30 µg), chloramphenicol (30 µg), amikacin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg) and ampicillin (10 µg). According to CLSI [20], the results of antibiotics susceptibility test was expressed as resistant, intermediate and sensitive isolates.

Multiple antibiotic resistances (MARs) index for each *L. monocytogenes* isolate was calculated using the following formula [21]. MAR index = The number of antibiotics to which the isolate is resistant / the total number of antibiotics tested. MAR index higher than 0.2 indicates wide use of this antibiotic in the originating environment of this isolate. The intermediate resistant *L. monocytogenes* strains were regarded as sensitive for MAR index.

Molecular characterization of *Listeria monocytogenes*

It was performed by using multiplex PCR, the following steps were followed

DNA extraction

The methodology described by Liu [22] was followed with slight modification. The DNA was extracted from the identified *Listeria monocytogenes* by inoculation of the isolates into brain heart infusion broth (DM810, Micro master, India) at 37°C and then was heated at 100°C for 20 min. The gained lysate was utilized as a DNA template.

Multiplex polymerase chain reaction

The obtained genomic DNA of *Listeria monocytogenes* has proceeded for multiplex PCR for detection of virulence genes that exemplified by invasion-associated protein (*iap*), listeriolysin O (*hylA*) and actin associated protein (*actA*). The utilized primers for detection of *hylA* are described by Paziak-Domańska *et al.* [23], *hylAF* 5'GCA GTT GCA AGC GCT TGG AGT GAA 3' *hylAR* 5'GCA ACG TAT CCT CCA GAG TGA TCG 3'with expected product size 456bp. Mureddu *et al.* [24] described the sequences for *iap* with expected product size 131 bp *iap-F* 5' ACA AGC TGC ACC TGT TGC AG 3' *iap R5'* TGA CAG CGT GTG TAG TAG CA 3'. Rawool *et al.*[25] described the primers for *actA*, *actA* (F) 5'CGC CGC GGA AAT TAA AAA AAG A 3', *actAR* 5'ACG AAG GAA CCG GGC TGC TAG 3' with expected product size 839 bp. The amplification was carried out using a thermal cycler (Hamburg, Germany). The PCR condition was an initial denaturation at 95°C for 2 min then proceed into 35 cycles each one was 15 s, denaturation at 95°C, 30 s, annealing at 60°C and one-minute extension at 72°C and then a final extension was for 10 min at 72 °C. The PCR product was subjected to electrophoresis by using 1.5% agarose gel. DNA marker (100 bp) was utilized to estimate the size of the PCR product.

Results and Discussion

Isolation and identification of *Listeria* spp. from meat products

Meat products are popular food items in Egypt; however, these products may be contaminated during their processing and storage by microbial agents. *Listeria* is one of the major foodborne pathogens and most cases of human listeriosis are caused by *Listeria monocytogenes*. There is no sufficient information about the presence of *Listeria* spp. in meat product distributed in Qena Governorate, Egypt. In the current work, a total of 120 meat products samples were collected from various markets located in Qena Governorate and examined for the

presence of *Listeria* spp. Out of 120 samples, 56 (46.7%) were positive for *Listeria* spp. The Kofta was highly contaminated with *Listeria*

spp. (70%), followed by burger (60%), minced meat (50%), luncheon (40%), sausage (35%) and pasterma (25%) (Table 1).

Table 1: Prevalence of *listeria* spp in examined meat products samples collected from Qena Governorate markets

Sample	Number	<i>Listeria</i> spp.	
		Number	%
Minced meat	20	10	50
Kofta	20	14	70
Sausage	20	7	35
Burger	20	12	60
Luncheon	20	8	40
Pasterma	20	5	25
Total	120	56	46.7

Table 2: Prevalence of various species of *Listeria* in examined meat products samples collected from Qena Governorate markets

Samples	No.	<i>L. ivanovii</i>		<i>L. welshimeri</i>		<i>L. monocytogenes</i>		<i>L. innocua</i>		<i>L. seeligeri</i>		<i>L. grayi</i>	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Minced meat	20	2	10	1	5	3	15	4	20	0	0	0	0
Kofta	20	1	5	2	10	5	20	3	14	2	10	1	5
Sausage	20	2	10	1	5	2	10	2	10	0	0	0	0
Burger	20	3	15	2	10	3	15	2	10	1	5	1	5
Luncheon	20	2	10	2	10	2	10	1	5	1	5	0	0
Pasterma	20	2	10	0	0	1	5	1	5	1	5	0	0
Total	120	12	10	8	6.6	16	13.3	13	10.8	5	4.1	2	1.6

The species of isolated *Listeria* were identified using the biochemical reactions; *L. monocytogenes* was the most common species among the isolates (13.3%) followed by *L. innocua* (10.8%) (Table 2). The prevalence of *Listeria* spp. was higher in the burger, minced meat and kofta than other processed meat products. That might attributed to exposure of these meat products to various processing steps and manipulation throughout their processing [26, 27]. However, a lower occurrence of *Listeria* spp was recorded in pasterma that may be attributed to the inhibitory effect of salt added to pasterma [28].

The level of contamination by *L. monocytogenes* in minced meat within the current study was 15%. A higher incidence was documented by Abd El-Malek *et al.* [8].

Akpolat *et al.* [29] obtained a lower incidence (5%). *Listeria monocytogenes* was isolated from 6.81% of minced meat samples collected from Slovenia as reported by Marinsek and Grebenc [30]. Isolation and characterization of *Listeria* spp. from 300 raw meat and meat products samples collected from Nigeria were carried by Ndahi *et al.* [31], 85 *Listeria* isolates were obtained and 12 of them were identified as *L. monocytogenes*.

Concerning luncheon meat samples *listeria* spp. were isolated from eight out of 20 examined samples with a percentage of 40%. *L. monocytogenes*, *L. ivanovii* and *L. welshimeri* were isolated with a percentage of 10, each, followed by *L. innocua* and *L. seeligeri* (5 %, each), whereas *L. grayi* couldn't be identified. The low contamination level demonstrated in luncheon samples may

be explained by exposure of luncheon to a high temperature during the manufacture, which in turn led to thermal inactivation effect on *Listeria* [32].

The capability of *L. monocytogenes* to reproduce at the refrigeration zone of temperature is an additional risk of processed meat that consumed without additional heat treatment like pasturma and luncheon [33].

L. monocytogenes was isolated with a low percentage of 0.89 from ready to eat food as recorded by Gombas *et al.* [34] (0.89%), whereas Saad *et al.* [35] could not isolate *Listeria monocytogenes* from the examined luncheon samples. Machines and tools used in the processing of meat products are often contaminated with *listeria* resulting in contamination of meat products. The current study showed that *L. monocytogenes* and *innocua* were the predominant isolates among *Listeria* spp. Several researchers have indicated that the isolation rate of *L. innocua* from food is common [36].

There was a difference between the isolation rate of *L. monocytogenes* by comparing the obtained data and others from other localities. This diversity may be attributed to the difference in geographic distribution, processing of meat products and the methods used in isolation [29].

Susceptibility of *Listeria monocytogenes* to antibiotics

Fourteen antibiotics were used to examine the sensitivity of the isolated *L. monocytogenes* (n=16) to antibiotics (Table 3). All *Listeria monocytogenes* were resistance to neomycin and streptomycin, whereas 87.5% and 81.3% of the isolates were sensitive to ampicillin and gentamycin. Moreover, 87.5%, 68.8%, 62.5%, 43.8% of the isolated *Listeria monocytogenes* were resistant to kanamycin, cephalothin, erythromycin, and oxytetracycline, respectively. Resistance to sulfamethoxazole and nalidixic acid were 56.3%, each.

Table 3: The antibiotic sensitivity of the isolated *Listeria monocytogenes* (n=16) from meat products of Qena Governorate markets

Antibiotic	Susceptible		Intermediate		Resistant	
	NO.	%	NO.	%	NO.	%
Neomycin (N)	-	-	-	-	16	100
Streptomycin (S)	-	-	-	-	16	100
Kanamycin (K)	1	6.3	1	6.3	14	87.5
Cephalothin (CN)	2	12.5	3	18.8	11	68.8
Erythromycin (E)	4	25.0	2	12.5	10	62.5
Sulfamethoxazole (SXT)	5	31.3	2	12.5	9	56.3
Nalidixic acid (NA)	7	43.8	-	-	9	56.3
Oxytetracycline (T)	8	50	1	6.3	7	43.8
Chloramphenicol (C)	8	50	2	12.5	6	37.5
Amikacin (AK)	10	62.5	-	-	6	37.5
Cefotaxime (CF)	9	56.3	3	18.8	4	25.0
Ciprofloxacin (CP)	11	68.8	2	12.5	3	18.8
Gentamicin (G)	13	81.3	1	6.3	2	12.5
Ampicillin (AM)	14	87.5	1	6.3	1	6.3

Principally, the majority of *L. monocytogenes* is sensitive to antimicrobial agents acting on Gram-positive bacteria. However, within the last decades the drug resistance of *L. monocytogenes* has emerged [13]. In general, penicillin, gentamicin, and ampicillin are used for control of listeriosis. The result of antimicrobial assay of this study

showed that 87.5% and 81.3% of isolated *L. monocytogenes* were sensitive to ampicillin and gentamycin, respectively. Al-Nabulsi *et al.* [2] reported that the isolated *L. monocytogenes* from examined raw and meat products collected from Jordan were susceptible to vancomycin, ampicillin, and gentamycin and around of 56% of the isolates

were resistant to neomycin. All the isolated *L. monocytogenes* by Ndahi *et al.* [31] were resistant to penicillin, trimethoprim, sulphamethoxazole, and ampicillin.

The obtained data indicated the possibility for transfer of drug-resistant *L. monocytogenes* to human through consumption of processed meat products through the transfer of mobile genetic elements as conjugative transposon and plasmids of *L. monocytogenes*.

MAR Value below 0.20 referred to that the organism came from a lower hazard source in which the antibiotics are rarely or never utilized. MAR index value greater than 0.20 referred to that they are originated from a higher risk source, which is extremely subjected to antibiotics [32].

Concerning the data of MAR, all the examined samples were risky as their MRA value greater than 0.20 (Table 4). Strains of *L. monocytogenes* isolated from meat collected from Nigeria were sensitive to chloramphenicol, gentamycin, and erythromycin, however, they were unaffected by tetracycline and amoxicillin [37]. The isolated *Listeria* spp. from beef and sausage sample collected from Korea were sensitive to ampicillin, cephalothin, kanamycin, and streptomycin, however, resistance to nalidixic acid were detected [38]. The attention of drug-resistant pathogens should be increased notably in developing country, as there is an intensive and uncontrolled utilization of antimicrobial agents.

Table 4: Antibiotic resistance profile and multiple antibiotic resistance index for *L. monocytogenes* isolate (n=16) from meat products of Qena Governorate markes during (year)

Isolate	Antimicrobial resistance profile	MAR index
1	N, S, K, CN, E, SXT, NA, T, C, AK, CF, CP, G, AM	1
2	N, S, K, CN, E, SXT, NA, T, C, AK, CF, CP, G	0.928
3	N, S, K, CN, E, SXT, NA, T, C, AK, CF, CP	0.857
4	N, S, K, CN, E, SXT, NA, T, C, AK, CF	0.786
5	N, S, K, CN, E, SXT, NA, T, C, AK	0.714
6	N, S, K, CN, E, SXT, NA, T, C, AK	0.714
7	N, S, K, CN, E, SXT, NA, T	0.571
8	N, S, K, CN, E, SXT, NA	0.500
9	N, S, K, CN, E, SXT, NA	0.500
10	N, S, K, CN, E	0.357
11	N, S, K, CN	0.286
12	N, S, K	0.214
13	N, S, K	0.214
14	N, S, K	0.214
15	N, S	0.143
16	N, S	0.143
Average		0.509

MAR Value below 0.20 referred to that the organism came from a lower hazard source. MAR index value greater than 0.20 referred to that they are originated from a higher risk source.

Prevalence of virulence genes in isolated *Listeria monocytogenes*

Three virulence genes were screened in the isolated *L. monocytogenes* by using multiplex PCR. The occurrence of *iap* gene was demonstrated in all isolates. The actin-associated protein and listeriolysin O were

detected in 13 /16 (81.3%) and 12 /16 (75%), respectively.

The examined virulence genes in the current study were the most remarkable in determining the virulence of *L. monocytogenes* [39]. The *hlyA* gene is the characteristic gene in *L. monocytogenes*, in

this study *hlyA* was detected in 75% of the isolated *L. monocytogenes*, which is in agreement with the data achieved by Jallewor *et al.* [40]. Al-Nabulsi *et al.* [2] screened some virulence genes (*actA*, *hlyA*, *iap*, *inlB*, *inlA*, *inlC* and *inlJ*) in *L. monocytogenes* isolated from processed meat and reported that the internal gene and *inlA* were the uppermost while invasion-associated protein, *iap* had a low incidence. The occurrence of *hlyA* gene in drug-resistant *L. monocytogenes* can increase the difficulty in treating the patients suffering from *L. monocytogenes* infection.

Conclusion

This research had elucidated the occurrence of *Listeria* spp. in various meat products in Qena Governorate. Processed meat products are a potential source for transmission of drug-resistant *L. monocytogenes* to human. Thus, with an expansion in the consumption of processed meat, the regular surveillance of drug-resistant pathogens in meat products is critical to ensure the safety of meat product. HACCP and ISO 22000:2005 programs should be implemented in Qena Governorate during manufacturing of meat products. The genetic characterization of the isolated *L. monocytogenes* plays an essential aspect in exploration the outbreaks caused by foodborne pathogens as well as the epidemiology studies.

Conflict of Interest

The authors stated that there are no conflicts of interest.

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الملخص العربي

مدى تواجد وتوصيف الليستيريا المعزولة من اللحوم المصنعة بمحافظة قنا- مصر

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أجريت هذه الدراسة لعزل وتوصيف ميكروب الليستيريا من مختلف منتجات اللحوم المتداولة بمحافظة قنا وكذلك أيضا دراسة حساسية الليستيريا للمضادات الحيوية المختلفة. تم تجميع عدد ١٢٠ عينة من مختلف منتجات اللحوم من محافظة قنا خلال اعوام ٢٠١٧-٢٠١٨. كانت نسبة تواجد الليستيريا مونوسيتوجينيس في العينات والتي تكونت من اللحم المفروم، الكفتة، السجق، برجر، لنشون، بسطرمة هي ١٥%، ٢٠%، ١٠%، ١٥%، ١٠%، ٥% على التوالي. تم عزل الليستيريا إفانوفى (٨١٠%)، الليستيريا ولشميريا (٦.٦%) الليستيريا إنوكيا (١٠.٨%)، الليستيريا سيلجاري (٤.١%)، والليستيريا جرايا (١.٦%). كما أظهرت النتائج أن جميع عزلات ميكروب الليستيريا مونوسيتوجينيس مقاومة لنيومايسين والستربتومايسين، بينما معظم العزلات أظهرت حساسية للأمبيسلين، وهذا مما قد يتسبب في إصابة الإنسان بميكروب الليستيريا مونوسيتوجينيس المقاومة للمضادات الحيوية مما يؤدي الى صعوبة علاجها بالمضادات الحيوية. كما كشف اختبار تفاعل البلمرة المتسلسل عن وجود بعض جينات الضراوة (أيب، هيل، أكتا) ضمن عزلات الليستيريا مونوسيتوجينيس. وقد خلصت هذه الدراسة إلى تواجد ميكروب الليستيريا مونوسيتوجينيس وأنواع أخرى من الليستيريا في بعض منتجات اللحوم المتداولة في محافظة قنا مما يتطلب أهمية تطبيق نظام الهاسب أثناء تجهيز وتداول منتجات اللحوم في محافظه قنا. وعلى حد علمنا تعتبر هذه أول دراسة عن مدى تواجد ميكروب الليستيريا في اللحوم المصنعة بمحافظة قنا- مصر.