

Clinical and Environmental Prevalence and Antibiotic Susceptibility of *Listeria monocytogenes* in Dakahlea Governorate, Egypt

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Abstract

Listeria monocytogenes (*L. monocytogenes*) is a gram-positive bacterium with public health problem. A total of 870 samples were collected. These include of 470 clinical samples from ascites of end stage liver disease and gastric lavages of diseased infants (0.8-2 month old) as well as of 400 environmental samples taken from the market such as lettuce, carrot, ice cream, soft cheese, frozen meat, liver and hamburgers. They are collected during March 2010 and December 2014, from Dakahlea Governorate, Egypt. *Listeria* cultures were carried out, isolated, and investigated. *Listeria* colonies were recovered only in 50 (26 from patients and 24 from environmental) out of 870 samples and assessed by biochemical tests. Antibiotic susceptibility of *L. monocytogenes* to different 17 antibiotics was carried out. The *Listeria* isolates were appeared susceptible to amoxicillin/ clavulanate and cotrimoxazole and showed different resistance levels of chloramphenicol, ampicillin, streptomycin and tetracycline and were completely resistant to cefipime and ceftazidime. Using the iodometric overlay method of 50 *Listeria* isolates, 32 (64%) were β -lactamase(s) producers. Following PCR assessment of antimicrobial resistance genes, 28/50 *L. monocytogenes* isolates contained more one antimicrobial resistance gene sequence. A high frequency of *penA* (46%) was detected compared to *strA* (38%), *tetM* (20%), and *ampC* (18%). The authors finally concluded that although *L. monocytogenes* was detected in environmental and clinical samples at low rates, it exerted pathological symptoms and are susceptible to amoxicillin/ clavulanate and cotrimoxazole with a high frequency of antimicrobial resistant *penA* gene.

Keywords: *Listeria monocytogenes*; Ascites; Gastric aspirates; Environmental samples; Antibiotic resistance; Resistance genes

Introduction

Listeria currently includes a total of six species; *L. monocytogenes*, *L. ivanovi*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*. Of these species, *L. monocytogenes* and *L. ivanovi* are the only species found to be pathogenic to humans and other animals [1]. *L. monocytogenes* is food-borne pathogens contributed to the development of septicemia, meningitis, encephalitis and gastroenteritis, especially in infants, elderly and immunosuppressed individuals. It is also showed miscarriage in pregnant women [2]. It is detected in food stuffs such as vegetables, fruits, dairy products and some processed food items [3].

Some studies revealed that *L. monocytogenes* is susceptible to a wide range of antibiotics but resistant to cephalosporins and fosfomycin [4,5]. It is clearly evident that there is a difficulty in its treatment due to high resistance to antibiotics which may give risks to human health [6].

Identification of *Listeria* is difficult and need more time for investigation taking approximately 5-10 days in routine investigations [7], and these may interrupt the control of food safety. Recently, the applied molecular techniques represent promising alternatives tools for rapid identification. Polymerase chain reaction (PCR) facilitates rapid identification with of a high selective specificity and potential for automation [8].

The present study aimed to determine the incidence of Listerial infection and antimicrobial resistance profile of *L. monocytogenes* against several antimicrobials as well as the genotype profiles of the isolated *L. monocytogenes* in Egypt, using molecular biology tools.

Materials and Methods

Collection of human samples

Listerial infections were investigated in 270 of hospitalized infants (8 M old-2 Y- old) suffering from fever and vomiting during March and December 2010-2014 in Dakahlea Governorate, Egypt. The control study was conducted. Vomiting gastric fluids were collected from admitted infants. In case of patients with end liver disease, ascitic fluid was collected from 200 patients admitted at Mansoura University Hospital. Paracentesis was carried out without ultrasound guidance using a standard sterile technique. The clinical samples were delivered directly to the lab for investigations. A written informed consent had been taken beforehand from all patients or parent of infants.

Collection of environmental samples

Four hundred random samples of lettuce, carrot, ice cream, soft cheese, raw milk, frozen meat, liver and hamburgers, raw ground sausages and raw poultry were collected from the markets in Dakahlea Governorate. The samples were transferred directly in ice boxes to laboratory and analyzed for *L. monocytogenes*.

Enrichment procedures

The isolates were subjected for cold enrichment to allow increase growth of *L. monocytogenes* progeny. Subculture was performed after 24 hrs on Listeria selective agar (Oxford Formulation). Culture was further incubated at 37°C for 24 hrs and colonies were identified.

Biochemical confirmation techniques

Listeria isolates were tested for Gram stain; catalase reaction; motility test; blood haemolysis test and CAMP test according to Bergey's Manual of Systematic Bacteriology [9].

Characterization of flagella by electron microscopy

L. monocytogenes cells were collected in pellets, fixed with glutaraldehyde 2% (volume in volume, v/v) in 50 mM sodium phosphate buffer pH 7.2 for 24 hrs, followed by negatively stained in 1% phosphotungstic acid for 10 seconds according to Hayat and Miller [10]. The grids were viewed with a Joel 100 CX transmission electron microscope.

Genomic DNA extraction

DNA was extracted from each isolate by using purification kit (Bio Basic DNA Mini Kit, Canada) as recommended by the manufacturer instructions

PCR identification of *Listeria monocytogenes*

Two primers were selected based on the *prfA* (transcriptional activator of the virulence factor) gene for *L. monocytogenes* according to Germini et al. [5]. The forward and reverse sequences were mentioned in Table 1. All PCR reactions were carried out using 25 µL containing 2 µL of extracted DNA. Each reaction mixture contained 12.5 µL Taq Master Mix (GenoOn, Germany), 1 µL of 500 M forward primer (LIS-F); 1 µL of 500 M reverse primer (LIS-R) and 8 µL of Ultra-Pure DNase/RNase-Free distilled water (Fermentas, USA). The DNA amplification reactions were carried out in thermal cycler (Techne, UK) as follows: pre-incubation at 95°C for 5 min; 40 cycles consisting of ds DNA denaturation at 95°C for 30 s, primer annealing at 54°C for 30 s, primer extension at 72 °C for 30 s; final elongation at 72 °C for 5 min.

Target gene	Primer sequence (5'-3')	Amplified fragment length	Reference
<i>prfA</i> gene	LIS-F: TCA TCG ACG GCA ACC TCG G	217 bp	Germini et al. [5]
	LIS-R: TGA GCA ACG TAT CCT CCA GAG T		

Table 1: Primer used for identification of *L. monocytogenes* by PCR.

Gel electrophoresis

All amplification products were carried out in 1.5 % agarose gel, stained with ethidium bromide, under a short-wavelength UV light source, and photographed. A standard 100 bp DNA ladder (GenoOn, Germany) was used to determine the size of the amplified fragments. *Escherichia coli* ATCC 25922 as standard for Gram negative.

Phenotypic detection of antimicrobial resistance in *L. monocytogenes* isolates

Antimicrobial susceptibility test was performed of each isolate by a disc diffusion method on Mueller-Hinton agar according to Bauer et al. [11]. The tested antimicrobial discs (Oxoid, England) were ampicillin (AMP/10 µg), amoxicillin (AMX/25 µg), ampicillin/clavulanate (AMC/30 µg), cefotaxime (CTX/30 µg), ceftriaxon (CRO/30 µg), ceftazidime (CAZ/30 µg), cefepime (FEB/30 µg), amikacin (AK/30 µg), gentamicin (CN/10 µg), streptomycin (S/10 µg), erythromycin (E/15 µg), ciprofloxacin (CIP/5 µg), norfloxacin (NOR, 30 µg), chloramphenicol (C/30 µg), sulphamethazole/trimethoprim (SXT/25 µg), tetracycline (TE/30 µg), vancomycin (VA/30 µg).

Detection of β-lactamase production

Each of the positive tested isolates was applied onto the surface of nutrient agar plates. After overnight incubation at 37°C, the plates were overlaid with 1% molten agarose containing 0.2% soluble starch, 1% penicillin G and 0.2% toluene. The plates were incubated for 15 min at room temperature and thin homogenous film of iodine solution was done onto the surface of the agar plates and incubated at room temperature until discoloration zones appeared around β-lactamase producing colonies [12].

PCR reaction and condition of antimicrobial resistance genes

Nine antimicrobial resistance genes were tested such as encoding tetracycline efflux pump (*tetA* and *tetM*), streptomycin phosphotransferases (*strA* and *strB*), penicillin binding protein gene (*penA*), chloramphenicol transporter non-enzymatic chloramphenicol-resistance protein (*cmlA*), adenine methylase related to resistance of erythromycin (*ermA*), erythromycin resistance methylase (*ermB*) and beta lactamase-ampicillin resistance gene (*ampC*). Oligonucleotide sequences and amplicon sizes of antimicrobial resistance genes were illustrated in Table 2. The antimicrobial resistance genes were determined by PCR technique according to Srinivasan et al. [13]. Primers were supplied from Integrated DNA Technologies (Bilolegio, Netherlands). Amplification of target genes was performed using a DNA thermal cycler (Techne, UK). And the Taq polymerase kit (GenoOn, Germany) in 0.5 mL of 96-well PCR plates (Fisher Scientific Co., Pittsburgh, PA). The reaction mixture (50 µL total volume) consisted of 30 µL of sterile water, 5 µL of PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl), 2 L of 15 mM MgCl₂, 2 µL of deoxyribonucleoside triphosphates (2.5 mM each dATP, dTTP, dGTP and dCTP), 1.0 µL of each primer (stock concentration, 25 µM), 1–10 µL of template, and 0.5 µL (5 U/µL) of Taq DNA polymerase. After overlaying with sterile seal tape (Fermentas, USA), samples were subjected to PCR amplification. Thirty PCR cycles were run under the following conditions: denaturation at 94°C for 45 sec, primer annealing at optimum temperature for 45 sec, and DNA extension at 72°C for 45. PCR tubes were incubated for 7 min at 72°C and then at 4°C. Twenty µL of the reaction mixture were analyzed by standard agarose (1.5%) gel electrophoresis (Cambrex Bio Science, Rockland, ME) with Tris-borate- EDTA buffer system. Reaction products were visualized by staining with ethidium bromide (0.5 µg/mL in the running buffer). *Escherichia coli* ATCC 25922 as standard for Gram negative.

Results and Discussion

Occurrence of *L. monocytogenes*

Fifty samples (5.74%)/870 tested clinical and environmental samples exhibited the presence of *L. monocytogenes* (Table 3). These include 26 (52%) for clinical and 24 (48%) of the environmental ones.

Target gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
<i>tet A</i>	Fw	5'GGCCTCAATTCCTGACG	372	Guillame et al.[14]
	Rv	5'AAGCAGGATGTAGCCTGTGC		
<i>tet M</i>	Fw	5'GTGGACAAAGGTACAACGAG	405	Poyart-Salmeron et al. [15]
	Rv	5'-CGGTAAAGTTCGTACACAC		
<i>str A</i>	Fw	5'-CTTGGTGATAACGGCAATTC	548	Gebreyes and Altier [16]
	Rv	5'-CCAATCGCAGATAGAAGGC		
<i>str B</i>	Fw	5'-ATCGTCAAGGGATTGAAACC	509	Gebreyes and Altier [16]
	Rv	5'-GGATCGTAGAACATATTGGC		
<i>erm B</i>	Fw	5'-GAAAAGGTACTCAACCAAATA	639	Okamoto et al. [17]
	Rv	5'-AGTAACGGTACTTAAATGTTTAC		
<i>erm A</i>	Fw	5'-AACACCCTGAACCCAAGGACG	420	Sutcliffe et al. [18]
	Rv	5'-CTTCACATCCGGATTGCTCGA		
<i>cml A</i>	Fw	5'-CCGCCACGGTGTGTTGTTATC	698	Gebreyes and Altier [16]
	Rv	5'-CACCTTGCCTGCCATCATTAG		
<i>pen A</i>	Fw	5'-ATCGAACAGGCGACGATGTC	500	Antignac et al. [19]
	Rv	5'-GATTAAGACGGTGTTTACGG		
<i>amp c</i>	Fw	5'-TTCTATCAAMACTGGCARCC	550	Lanz et al. [20]
	Rv	5'-CCYTTTTATGTACCCAYGA		

Table 2: Primers used for detection of genes encoding resistance to different antimicrobials in *L. monocytogenes* isolates.

Biochemical & PCR techniques

The positive clinical and environmental isolates showed positive catalase test, beta-haemolysis, umbrella-shaped motility motility test and positive CAMP test. Applying forward and reverse *prfA* (transcriptional activator of the virulence factor) gene detected 217 bp. PCR amplification gives all amplicons Figure1.

Antibiotics susceptibility

Table 4 summarized the activities of 17 antibiotics tested against the 50 *L. monocytogenes* isolated from the clinical and environmental samples. Amoxicillin-clavulanate showed highest sensitivity reaching to 86% of the tested isolates. However, high rates of resistance were observed for streptomycin (90%), cefotaxime (94%), ceftriaxone (96%) and ceftazidime and cefpime (100%), while low rate of resistance was remarked to chloramphenicol (18%), vancomycin and cotrimoxazole (24%), and tetracycline (26%).

Ultrastructural characterization of *L. monocytogenes* flagella

L. monocytogenes appeared rod-shaped structures with many flagella distributed in a peritrichous manner with many points of attachment (Figure 2).

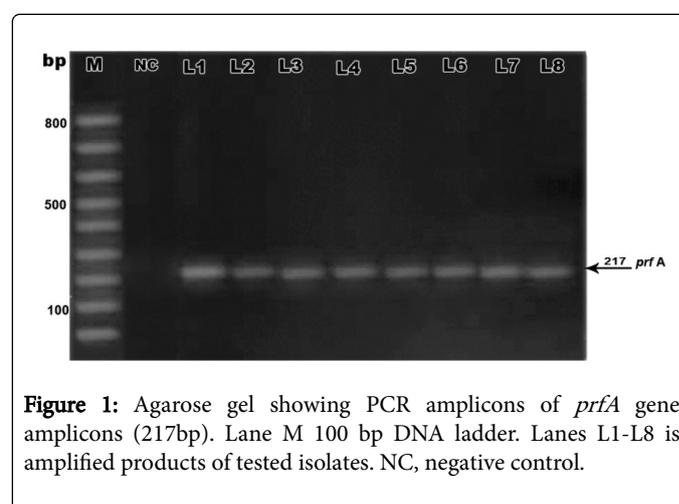


Figure 1: Agarose gel showing PCR amplicons of *prfA* gene amplicons (217bp). Lane M 100 bp DNA ladder. Lanes L1-L8 is amplified products of tested isolates. NC, negative control.

Type of sample	No. & % of samples	No. & % of isolated <i>L. monocytogenes</i>
Peritoneal fluids	200 (22.9%)	15 (30%)
Gastric aspirate	270 (31%)	11 (22%)
Lettuce	42 (4.8%)	2 (4%)
Carrot	49 (5.6%)	3(6%)
ice cream	39 (4.4%)	0
soft cheese	62 (11.6%)	6 (12%)
Frozen meat, live.hamburgers	40 (7.1%)	0
Raw poultry	55 (6.3%)	5 (10%)
Ground raw sausages	53 (6%)	3 (6%)
Raw milk	60 (6.8%)	5 (10%)
Total	870 (100%)	50 (5.74%)

Table 3: *L. monocytogenes* assessments in clinical and environmental samples.

No. of <i>L.monocytogenes</i> isolates	No. & % of β -lactamase producers
Clinical isolates 26	14 (53%)
Non clinical isolats 24	18 (75%)
Total <i>L.monocytogenes</i> isolates 50	32 (64%)

Table 5: Production of β -lactamase enzymes by tested *L.monocytogenes* isolates.

Prevalence of β -lactamase production

β -lactamase production was detected in 32 (64%) / 50 *Listeria* isolates. The rate of production of β -lactamase was higher in environmental samples compared to the clinical one Table 5.

Occurrence of antimicrobial resistance genes *tetM*, *tetA*, *strA* and *strB* genes

Twenty eight of 50 *L. monocytogenes* isolates (56%) possessed more than one antimicrobial resistance gene. A higher incidence of antimicrobial resistance genes was observed in environmental samples (55%), compared to the clinical ones (45%). A highest incidence of antimicrobial resistance genes were recorded for the tetracycline resistance genes, *tetM* and *tetA* which reached respectively to 77% and 53.8%.

Chloramphenicol-resistant *cmlA* gene reached to 22%. The streptomycin-resistant genes reached to 42 and 17% respectively for *strA* and *strB*. The average percentages of erythromycin resistant genes *ermA* and *ermB* attained to 20 and 30 % respectively of the 15 erythromycin resistant isolates. Also *penA* and *ampC* resistant genes were detected in 46% and 18% of the resistant isolates Table 6 and Figures 3-8.

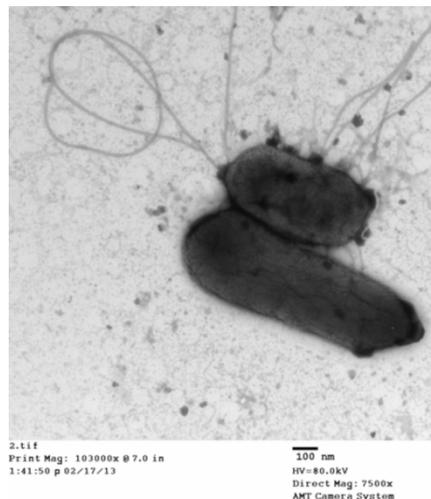


Figure 2: Electron micrograph of *L. monocytogenes* with negative stain showing rod-shaped structure with pattern of flagellar formations.

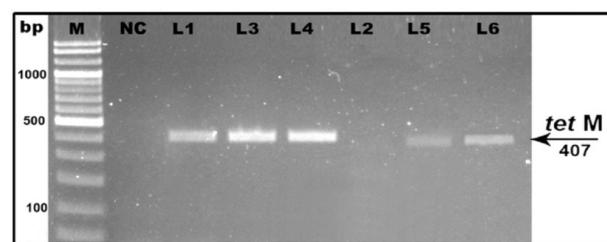


Figure 3: Agarose gel showing PCR amplicons of antimicrobial resistant gene of *tetM* gene of representative *L.monocytogenes* isolates (lanes 1,3,4,5,2 & 6), M: 100 pb molecular marker and lane Nc is negative control.

Antimicrobial agent	Resistance	Sensitive
Ampicillin	28 (56%)	22 (44%)
Amoxicillin	37 (74%)	13 (26%)
Amoxicillin-calvulanate	7 (14%)	43 (86%)
Cefotaxime	47 (94%)	3 (6%)
Ceftazidime	50 (100%)	0
Ceftriaxone	48 (96%)	2 (4%)
Cefipime	50 (100%)	0
Streptomycin	45 (90%)	5 (10%)
Gentamycin	24 (48%)	26 (52%)
Amikacin	23 (46%)	27 (54%)
Erythromycin	15 (30%)	35 (70%)

Ciprofloxacin	30 (60%)	20 (40%)
Norfloxacin	42 (84%)	8 (16%)
Chloramphenicol	9 (18%)	41 (82%)
Ctrimoxazole	12 (24%)	38 (76%)
Tetracycline	13 (26%)	37 (74%)
Vancomycin	12 (24%)	38 (76%)

Table 4: Number and percentages of antimicrobial susceptibility pattern of tested isolates of *L. monocytogenes*.

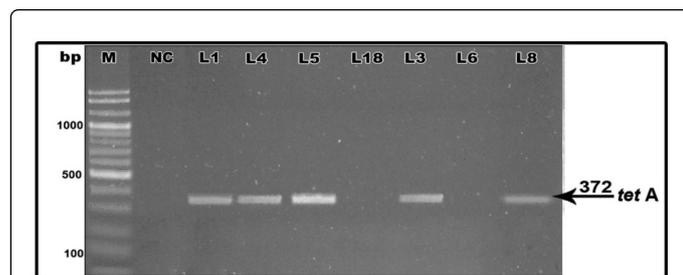


Figure 4: Agarose gel showing PCR amplicons of antimicrobial resistant gene of the *cml* & *tet A* gene of representative *L. monocytogenes* isolates (lanes 1,4,5,18,3,6 &8), M: 100 pb molecular marker and lane NC is negative control.

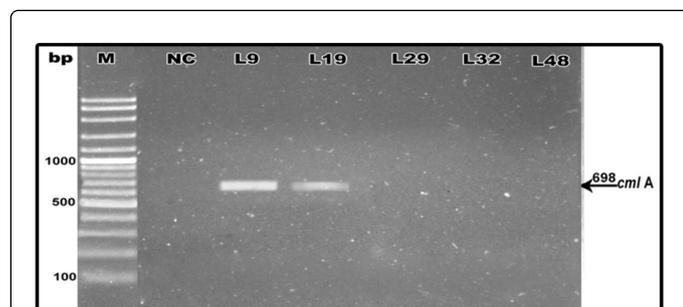


Figure 5: Agarose gel showing PCR amplicons of antimicrobial resistant gene of the *cml* & *tet A* gene of representative *L. monocytogenes* isolates (lanes 9,19,29,12 &48), M: 100 pb molecular marker and lane Nc is negative control.

Antibiotic resistance gene	No. of <i>L. monocytogenes</i> isolates	No & % of resistant genes of <i>L. monocytogenes</i> isolates
<i>tet M</i>	13	10 (20%)
<i>tet A</i>	13	7 (14%)
<i>cml A</i>	9	2 (4%)
<i>erm A</i>	15	3 (6%)
<i>erm B</i>	15	5 (10%)
<i>amp C</i>	50	9 (18%)
<i>pen A</i>	50	23 (46%)

<i>str A</i>	45	19 (38%)
<i>str B</i>	45	8 (16%)

Table 6: Prevalence rate of antimicrobial resistance genes in *L. monocytogenes* isolates.

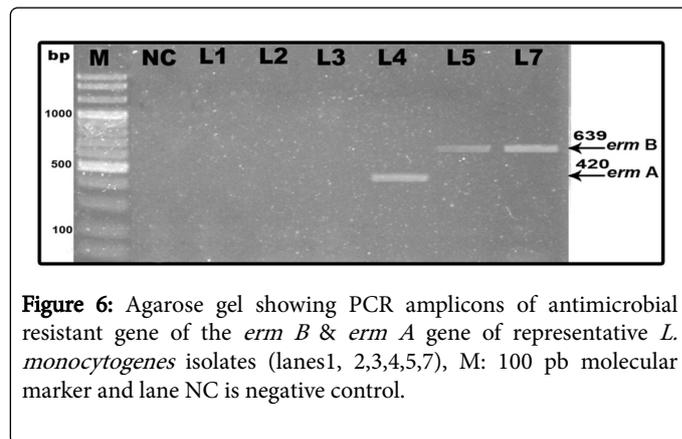


Figure 6: Agarose gel showing PCR amplicons of antimicrobial resistant gene of the *erm B* & *erm A* gene of representative *L. monocytogenes* isolates (lanes1, 2,3,4,5,7), M: 100 pb molecular marker and lane NC is negative control.

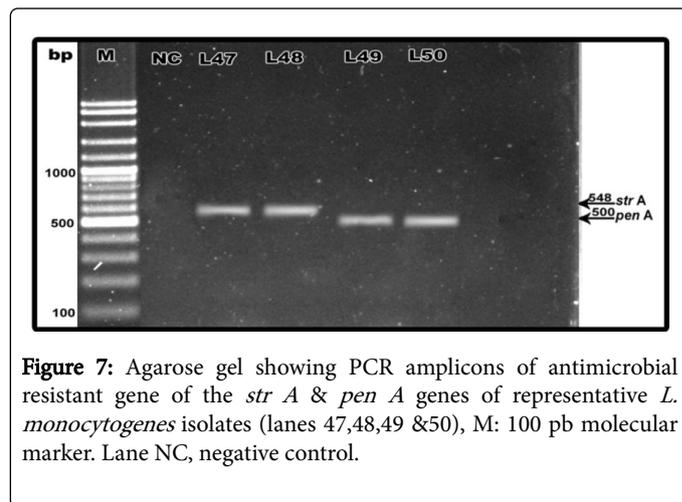


Figure 7: Agarose gel showing PCR amplicons of antimicrobial resistant gene of the *str A* & *pen A* genes of representative *L. monocytogenes* isolates (lanes 47,48,49 &50), M: 100 pb molecular marker. Lane NC, negative control.

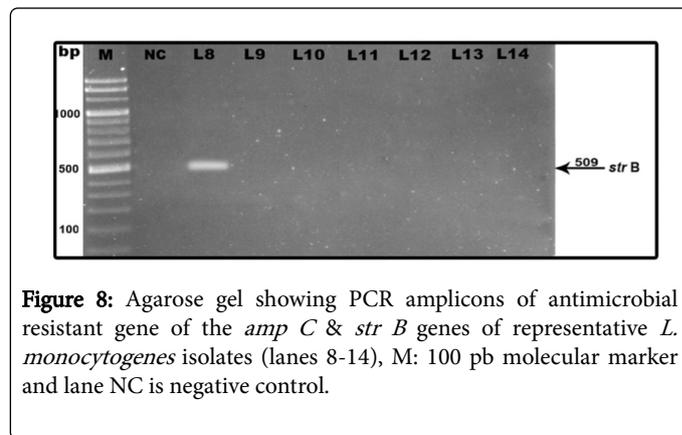


Figure 8: Agarose gel showing PCR amplicons of antimicrobial resistant gene of the *amp C* & *str B* genes of representative *L. monocytogenes* isolates (lanes 8-14), M: 100 pb molecular marker and lane NC is negative control.

Discussion

In the present study, the incidence rate of *L. monocytogenes* reached to 50/870 (5.74%) and varied markedly between clinical and

environmental samples. In clinical samples the rate of listerial infection in ascitic fluid attained to 15/200 (7.5%) compared to 11/270 (4%) in gastric lavages of diseased infants. These findings supported the work by Zaki et al. [21], whom reported increased rate of *L. monocytogenes* in ascitic fluid of cirrhotic patients to 24.4%. Toyoshima et al. [22] reported two cases *L. monocytogenes* related to peritonitis in cirrhotic patients. Concerning infants, there was no reported incidence of Listeriosis, however Mokta et al. [23] reported Listeriosis in a two-day-old full term male baby with fever, skin rash, gastritis and vomiting. Listeriosis was found to be increased in patients with physiologic and pathologic defects related to immune diseases [24].

Furthermore, 24/400 (6%) infection rate of *Listeria* were detected in environmental samples. These include 6/62 (9.6%) of soft cheese and 5/60 (8.3%) of raw milk. Similar finding was reported in soft cheese [25,26] and raw milk [27]. The prevalence of *L. monocytogenes* in raw milk attained to 21.7% in Iran [28] and 6.3% in Ireland [29].

Also, the observed findings showed rates of listerial infection in carrot being 3/49 (6.1%) and 2/42 (4.7%) in lettuce. Similar findings were reported by Ieren et al. [30] whom reported *L. monocytogenes* infection in 1/50(2%) of carrot and 3/49(6.2%) in lettuce. Ding et al. [31]. Detected that the incidence of *L. monocytogenes* infection ranged from 11.9 to 17.4 cases per million persons in Korea and considered lettuce as the most contaminated vegetables. These infections may result from contamination during harvesting during human handling, equipment's, transport containers, wild and domestic animals [32].

The present data reported 5/55 (9%) *L. monocytogenes* isolates from raw poultry (egg shells) and this agreed with Sayed et al. [33] and Shaker et al. [34] whom reported similar rates which may result from contamination or listerial infection [35].

Furthermore, the observed findings revealed the presence of listerial infection at 3/53 (5.6%) in raw sausage raw sausage samples, Similar results were reported by Yucel et al. [36] and Ennaji et al. [37]. Nonetheless, the presence of *L. monocytogenes* in processed meats is a more serious public health issue because the organism can often grow in products having extended shelf-lives during refrigerated storage and reach levels that facilitate the establishment of invasive infections. Problems of infection may arise from subsequent cross-contamination that occurs through using raw materials, by humans, rodents, insects and even birds. Listerial infection in infants seemed to be a result of cross-contamination from the uncooked vegetables as well as from soft cheese made from raw milk is the possible source of *Listeria* infection [38].

Analyzing the PCR Profiles of the 50 *L. monocytogenes* isolates using forward and reverse virulence associated gene *prfA* exhibited detection of 217 bp region. Similar findings were achieved in Iran [39] and India [40] following assessments of the *prfA* gene in *L. monocytogenes* isolates recovered from the food stuffs.

Following assessments of antimicrobial sensitivity of 17 antibiotics toward environmental and clinical isolates, resistance of the isolates was detected against ampicillin, amoxicillin, aminoglycosides and flouroquinolones. The present findings supported the work of AL-Ashmawy et al. [27] and Odjadjare et al. [41]

According to Srinivasan et al. [13], all the *L. monocytogenes* isolates were susceptible to gentamicin. Also, combination of amoxicillin with a B-lactamase inhibitor, clavulanic acid reduced the incidence of resistance by 14%.

Furthermore, all the isolates showed a high resistance toward the third generation cephalosporins and completely resistant to the fourth generation cephalosporins cefepime. The present findings agree with the work of Ennaji et al. [37].

As a result of the weak activity or complete resistance of the second and third generation cephalosporins against *L. monocytogenes*, Cormican and Jones [42] confirmed that it is not used clinically for treating listeriosis.

Also, 30% and 34% of *L. monocytogenes* isolates appeared resistant to erythromycin and tetracycline and the resistance rate reached to 24% for vancomycin and cotrimoxazole however it decreased to 18% for chloramphenicol. Although Odjadjare et al. [41] reported similar findings; Srinivasan et al. [13] mentioned a higher resistance rate reaching approximately to 100% for cotrimoxazole, erythromycin and vancomycin and to 32% for chloramphenicol.

Following B-Lactamase screening test, 32/50 *Listeria* isolates were B-lactamase producers compared to 18 negative isolates. This confirmed that ampicillin is the drug of choice for listeriosis. Resistance of *L. monocytogenes* to antimicrobials is due to production of enzyme like b-lactamases, which are chromosomally encoded or most often plasmid mediated [43].

Also, the present findings revealed that the listerial isolates exhibited average incidence of antimicrobial genes of *tetM*, *tetA*, *strA* and *strB*. These findings were similar to Jamali et al. [44] and contradicted with Srinivasan et al. [13] who reported that 34% of the isolates carried *strA* and missing of the other streptomycin resistance genes (*strB* and *aadA*).

In addition, screening of 15 erythromycin resistant isolates, 3 (20%) and 5 (33%) of isolates contained *ermA* and *ermB* genes respectively. These findings agree with the work of Morvan et al. [45] who found *ermB* gene in three erythromycin-resistant strains. However, Odjadjare et al. [41] did not detect *ermA* and *ermB* in 19 erythromycin resistant isolates.

Although, the B-lactams showed the highest phenotypic resistance, the detected genes responsible for resistance to these antibiotics were not equally detected in the *Listeria* isolates. The *ampC* and *penA* resistance genes were detected in 9 (18%) and 21 (42%) of the B-lactams resistant isolates, respectively. These findings agree with the work of Jamali et al. [44] and Srinivasan et al. [13] whom observed incidence resistance rates of 71.4% and 37% of the isolates containing *penA* gene respectively.

The authors concluded that due to high resistance rate to ampicillin, it should be used in combination with gentamicin to insure synergistic activity against listeriosis and so ampicillin plus gentamicin is often recommended for therapy while sulfamethoxazole-trimethoprim is recommended for secondary prophylaxis.

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