ISSN: 0067-2904

Study the Antimicrobial Resistance Genes of *Listeria Monocytogenes* Isolated From Industrial and Clinical Samples in Iraq

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Received: 26/3/2022 Accepted: 2/8/2022 Published: 28/2/2023

Abstract

Multi-drug resistance in *Listeria monocytogenes* is considered a major public health problem associated with foodborne outbreaks and causes high hospitalization and mortality rates. This study aimed to investigate the antimicrobial resistant genes among *Listeria monocytogenes* isolated from meat and clinical samples. Phenotypically, the isolates were tested for their susceptibility against the 12 most commonly used antimicrobials in veterinary and human therapy via the disc diffusion method, while conventional PCR was performed to study the presence or absence of 14 resistance genes predicted in *L. monocytogenes* isolates. The study established that 30(66.66%) of *L. monocytogenes* isolates showed phenotypic multi-drug resistance against at least three antimicrobial classes. Furthermore, high resistance frequencies were reported among commonly used antibiotics for listeriosis therapy. The present study revealed that the investigated isolates show resistance against tetracycline 33(73.3%), ampicillin 29(64.4%), penicillin 28(62.2%), erythromycin 26(57.8%), and gentamycin, clindamycin and vancomycin 24(53.3% each). Of the 45 *L. monocytogenes* isolates studied, 37(82.2%) were phenotypically susceptible to meropenem, followed by ciprofloxacin 36(80.0%) and SXT 30(66.7%). PCR amplification of antimicrobial resistance genes established the occurrence of antibiotic resistance genes in all studied *L. monocytogenes* isolates. Notably, 41(91.11%) of these isolates exhibited more than five resistance genes. Surprisingly, penA and ampC were detected in all *L. monocytogenes* strains 45(100%), followed by ermB 44(97.8%), tetA 38(84.4%), tetG 32(71.1%), and vanB 30(66.7%). Moreover, vanA 22(48.9%) and tetB 18(40.0%) were detected less frequently. The lowest incidences of resistance genes were observed in *L. monocytogenes* carrying tetD 4(8.9%) and cmlA 6(13.3%). In conclusion, the study demonstrates that the majority of *L. monocytogenes* from human and meat samples displayed a high index of resistance to a variety of agents used for clinical listeriosis treatment adding further burden to the existing global antibiotic resistance problem.

Keywords: *Listeria monocytogenes*, antimicrobial susceptibility, antimicrobial resistance genes, PCR

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دراسة الجينات المقاومة للمضادات الحيوية في *Listeria monocytogenes* المعزولة من اللحوم و العينات السريرية في العراق

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الخلاصه

تعتبر مقاومة الأدوية المتعددة في *جينات* الليستيريا الأحادية مشكلة صحية رئيسية مرتبطة بالفاشيات المنقولة بالأغذية وتسبب ارتفاع معدلات دخول المستشفيات والوفيات. هدفت هذه الدراسة إلى التحقيق في الجينات المقاومة لمضادات الميكروبات بين جينات الليستيريا الأحادية المعزولة من اللحوم والعينات السريرية. ومن الناحية الظاهرية، تم اختبار العزلات للتأكد من حساسيتها بمضادات الميكروبات ال 12 الأكثر استخداما في العلاج البيطري والبشري عن طريق طريقة نشر القرص، في حين تم إجراء تفاعل البوليميراز المتسلسل التقليدي لدراسة وجود أو عدم وجود 14 جينا مقاوما متوقعا في عزلات الليستيريا الموحدة. أثبتت الدراسة أن (66.66%)30 من عزلات *الليستيريا الموحدة* أظهرت مقاومة مظهرية متعددة الأدوية ضد ثلاث فئات مضادة للميكروبات على الأقل. علاوة على ذلك ، تم الإبلاغ عن نسب مقاومة عالية بين المضادات الحيوية شائعة الاستخدام لعلاج داء الليستريات. كشفت الدراسة الحالية أن العزلات التي تم فحصها تظهر مقاومة ضد التتراسيكلين بنسبة (73.3%)33 ، الأمبيسلين (64.4%)29 ، البنسلين (62.2%)28 ، الإريثروميسين (57.8%)26 ، والجنتاميسين ، الكليندامايسين والفانكومايسين (53.3% لكل منهما) 24. من بين العزلات ال 45 التي تمت دراستها ، كان (82.2%)37 حساسة ظاهريا للميروبينيم ، يليه سيبروفلوكساسين (80.0%)36 و SXT (66.7%)30 .من ناحية أخرى، أثبت تضخيم PCR لجينات مقاومة مضادات الميكروبات وجود جينات مقاومة للمضادات الحيوية في جميع عزلات الليستيريا الموحدة المدروسة. والجدير بالذكر أن (91.11%)41 من هذه العزلات أظهرت أكثر من خمسة جينات مقاومة. والمثير للدهشة أنه تم الكشف عن penA و ampC في جميع سلالات الليستيريا 45 (100%) ، تليها ermB (97.8%)44 ، tetA (84.4%) 38، tetG(71.1%)32 ، وvanB (66.7%) 30 علاوة على ذلك، تم اكتشاف vanA(48.9%)22 و tetB (40.0%) 18 بنسب أقل. لوحظت أدنى حالات جينات المقاومة في عزلات الليستيريا الموحدة التي تحمل tetD (8.9)4 و cmlA(13.3%)6. في الختام ،توضح الدراسة أن غالبية عزلات الليستيريا الموحدة من عينات الإنسان واللحوم أظهرت مؤشرا عاليا لمقاومة مجموعة متنوعة من الادوية المستخدمة في علاج داء الليستريات السريري مما يضيف عبئا إضافيا إلى مشكلة مقاومة المضادات الحيوية العالمية الحالية.

1. Introduction

Listeria monocytogenes is a Gram-positive facultative intracellular foodborne pathogen that poses a significant threat to the food industry. It mostly initiates foodborne illness and represents a major public health concern since it may lead to serious human illness with severe consequences (e.g. meningitis, sepsis, prematurity), with mortality rates of up to 30% and a neonatal mortality rate of 50% [1,2]. The pathogen is broadly distributed in a variety of foods, including aquatic products, meat, dairy products, etc. [2]. Controlling *L. monocytogenes* contamination is a major challenge facing the food industry because the bacterium can survive under various conditions, such as cold storage temperatures, high salt concentrations, and low pH levels [3,4]. In the past, *L. monocytogenes* was considered vulnerable to a wide range of antibiotics that have bactericidal effects against Gram-positive bacteria, including tetracyclines,

erythromycin, ampicillin, penicillin and gentamicin, which were routinely used to treat human cases of listeriosis [5]. Over the past few decades, numerous studies have reported a high frequency of multi-drug resistance in *L. monocytogenes* isolates obtained from the clinical setting and food chain [4,6,7]. Therefore, monitoring modifications in the antimicrobial resistance of *L. monocytogenes* due to the continuous emergence of resistant strains is required. The present study aimed to explore the phenotypic and genotypic characterization of antimicrobial resistance genes in *L. monocytogenes* strains isolated from meat and clinical samples.

2. Materials and methods

2.1 Source bacterial isolates

A total of 45 *L. monocytogenes* isolates were obtained from humans (*n*=14, including high vaginal swabs (HVS)=4, blood=4, placenta=6) and meat (*n*= 31, minced red meat=7, frozen chicken meat= 17, fresh red meat=7) between 2018 and 2020 were included in this study. HVS and placental isolates were collected from women with poor obstetric history (spontaneous abortion and preterm delivery) attending the Duhok Obstetrics and Gynecology Teaching Hospital, while blood strains were isolated from meningitis and septicemia cases at Hevi Pediatric Teaching Hospital and the Duhok Kidney and Diseases Transplantation Center. Meat isolates were obtained from the Food Testing Laboratory of the Directorate of Prevention Affairs in Duhok. Isolates were maintained on PALCAM agar (Neogen®Company-UK) at 37°C for 24 hours. Then 3 to 5 pure colonies were transferred to 10 ml brain heart infusion broth (Neogen®Company-UK) before the examination. All isolates were bacteriologically and biochemically confirmed using Gram staining, a catalase test, a haemolysis test on sheep blood agar, a Harlequin™ Listeria Chromogenic Agar (ISO) subculture (Neogen®Company-UK) (green colonies surrounded by an opaque halo), and API ®Listeria REF 10300 (BioMérieux, Marcy I'Etiole, France). The *L. monocytogenes* strains were further confirmed by amplification of the 938bp primer of 16S rRNA (Macrogen- South Korea), which is considered a species-specific primer for the detection of *L. monocytogenes*. A previously isolated and sequenced *L. monocytogenes* strain (access number: MK968366) was used as a positive control. The utilized primers are listed in (Table 1).

2.2 Antimicrobial susceptibility testing

Listeria monocytogenes isolates were tested for antimicrobial susceptibility to the 12 most frequently used antibiotics (Oxoid-UK) in veterinary and human medicine. This test was performed on Muller- Hinton agar (Oxoid-UK) using the disc diffusion method [3]. Isolates were investigated for susceptibility to glycopeptides (vancomycin 30µg), aminoglycoside (gentamycin 10 µg), lincomycin (clindamycin 2µg), β lactams (cephalexin 30µg, ampicillin 10µg, penicillin 10U), carbapenems (meropenem 10 µg), tetracyclines (tetracycline 30 µg), phenicols (chloramphenicol 10 µg), quinolones (ciprofloxacin 10µg), macrolides (erythromycin 15µg), and sulfonamide (trimethoprim-sulphamethoxazole 1.25/23.75 µg). After incubation for 24 hrs at 37°C, the inhibition zone (in mm) was interpreted according to clinical and laboratory standard institute (CLSI, 2021) guidelines established for *L. monocytogenes* [8] The crucial values of *Staphylococcus spp.* were used for vancomycin and cephalexin because the susceptibility criteria for *L. monocytogenes* are not standardized [4].

2.3 Antimicrobial-resistant gene screening of L. monocytogenes isolates:

To investigate the presence or absence of 14 antibiotic-resistant genes in *L. monocytogenes* isolates, conventional PCR techniques were applied. The primers and predicted base pairs (Macrogen, South Korea) for the PCR amplification of *L. monocytogenes* antimicrobial resistance genes are listed in Table 1. Antimicrobial resistance genes encoding penicillin-binding protein gene (penA), tetracycline efflux pump (tetA, tetB, tetC, tetD, tetE and tetG); chloramphenicol-resistance protein (cmlA), vancomycin resistance genes, vanillate o-demethylase oxygenase subunit (vanA and vanB), adenine methylase (ermA), erythromycin resistance methylase (ermB), erythromycin esterase type II (ereB) conferring resistance to erythromycin and beta-lactamase–ampicillin resistance gene (ampC) were verified. The target genes were amplified using a DNA thermal cycler (Applied Biosystems 9700, USA) and master mix (2X) (Genet-Bio, South Korea) in 0.5 mL 96-well PCR plates (Applied Biosystems 9700, USA). The reaction mixture (25 µL total volume) consisted of 12.5 µL of ready-to-use master mix (1X), 1.0 µL of each primer (stock concentration, 10 µM), and 2 µL of DNA template. Then, 9.5 µL of ddH_2O (Ambion-USA) was added to obtain a 25 µl PCR reaction. Initial denaturation was performed at 94°C for 4 min. Thirty-five PCR cycles were performed as follows: denaturation at 94°C for 45 sec, annealing at 53°C for 45 sec, and DNA extension at 72°C for 45 sec. For the final extension, PCR tubes were incubated at 72°C for 7 min followed by incubation at 4°C (Applied Biosystems 9700, USA). *Salmonella typhii* (Bio Lab Code for the VITEK2 test: 001761044126210) were used as positive controls in all PCR reactions. The positive control was obtained from the Food Testing Laboratory of the Directorate of Prevention Affairs-Duhok.

Table 1: List of used primers in this study.

Antimicrobial resistance associated genes				
Target gene	**Primer**	**Primer sequence (5' →3')**	**bp**	**Reference**
tet A	TetA F	GGCCTCAATTTCCTGACG	372	[9]
	TetA R	AAGCAGGATGTAGCCTGTGC		
	TetB F	GAGACGCAATCGAATTCGG		
tet B	TetB R	TTTAGTGGCTATTCTTCCTGCC	228	
	TetC F	TGCTCAACGGCCTCAACC		
tet C	TetC R	AGCAAGACGTAGCCCAGCG	379	
	TetD F	GGATATCTCACCGCATCTGC		
tet D	TetD R	CATCCATCCGGAAGTGATAGC	436	
	TetE F	TCCATACGCGAGATGATCTCC		
tet E	TetE R	CGATTACAGCTGTCAGGTGGG	442	
tet G	TetG-F	CAGCTTTCGGATTCTTACGG	844	**[6]**
	TetG-R	GATTGGTGAGGCTCGTTAGC		
cmlA	CmlA F	CCGCCACGGTGTTGTTGTTATC	698	**[6]**
	CmlA R	CACCTTGCCTGCCCATCATTAG		
	PenA F	ATCGAACAGGCGACGATGTC	500	**[10]**
penA	PenA R	GATTAAGACGGTGTTTTACGG		
vanA	VanA-F	CATGACGTATCGGTAAAATC	885	**[11]**
	VanA-R	ACCGGGCAGRGTATTGAC		
vanB	VanB-F	CATGATGTGTCGGTAAAATC	882	
	VanB-R	ACCGGGCAGRGTATTGAC		
ampC	AmpC-F	TTCTATCAAMACTGGCARCC	550	
	AmpC-R	CCYTTTTATGTACCCAYGA		

ermB	ErmB-F	GAAAAGGTACTCAACCAAATA	639	**[7]**
	ErmB-R	AGTAACGGTACTTAAATTGTTTAC		
ereA	EreA-F	AACACCCTGAACCCAAGGGACG	420	**[12]**
	EreA-R	CTTCACATCCGGATTCGCTCGA		
ereB	EreB-F	AGAAATGGAGGTTCATACTTACCA	546	
	EreB-R	CATATAATCATCACCAATGGCA		
16S rRNA	F	CAG CAG CCG CGG TAA TAC	938	**[13]**
	R	CTC CAT AAA GGT GAC CCT		

3. RESULTS and DISCUSSION

3.1 Antimicrobial susceptibility testing

Listeria monocytogenes is considered a significant foodborne pathogen linked to high morbidity and mortality [14]. In Iraq, there is a lack of official data on the antimicrobial resistance profile of *L. monocytogenes* [15]. Such data could assist in determining the significance of the resistance rate reported in this and other studies. The present study investigated the phenotypic and genotypic characterization of the antimicrobial susceptibility pattern of 45 *L. monocytogenes* strains isolated from meat (n=31) and various clinical (n=14) specimens. According to the currently accepted standard [16], when a bacterium is resistant to three or more antibiotics of different classes, it is considered a multi-drug resistant bacterium. In this study, 66.66% of multi-drug resistant strains (resistant to >3 classes) were established (Table 2). Since the first report on the antimicrobial resistance profile of *L. monocytogenes* isolates [17], the global prevalence of microbial resistance in *L. monocytogenes* was considered relatively insignificant [18,19]. However recent findings suggest an emerging incidence of antimicrobial resistance in *L. monocytogenes* isolated from different sources [20,21]. A total of 29(64.4%), 28(62.2%), and 24(53.3%) *L. monocytogenes* isolates were resistant to ampicillin, penicillin and gentamycin, respectively. These antimicrobials are traditionally used as first-choice drugs for listeriosis treatment. Resistance to ampicillin and penicillin in *L. monocytogenes* recovered from meningitis cases have been reported anciently by [22]. To corroborate this study's findings, a survey conducted in Iran reported a high resistance rate to a penicillin (100%) and ampicillin (90%) in *L. monocytogenes* isolated from various sources. Furthermore, 50% of *L. monocytogenes* exhibited resistance to gentamycin in a study performed by [23] in Jordan. In contrast, numerous studies have shown *L. monocytogenes* strains that were nearly susceptible to penicillin, ampicillin and gentamycin [24-26]. Regardless of the sample type, this study highlighted an increase in the resistance rate to tetracycline (73.3%), erythromycin (57.8%), SXT (57.1%), vancomycin (53.3%) and ciprofloxacin (50%) (Table 2). These antimicrobials are commonly used as the second choice of treatment in clinical listeriosis and veterinary use. However, vancomycin is considered the last line of defense against most Gram-positive bacteria. Globally, existing studies largely support the results of the present study. In India, *L. monocytogenes* isolates showed 85.7% and 90.5% resistance to erythromycin and tetracycline, respectively. Likewise, in Egypt, high resistance frequencies were also reported for erythromycin (62.5%) and tetracycline (59.4%) [27]. Moreover, a recent study from Iran reported variable results for commonly used antimicrobials, with some of the results being inconsistent with the outcomes of the present study [28]. Swetha et al. noted that 90% of *L. monocytogenes* isolates recovered from animal products were susceptible to erythromycin, while 80% of isolates were

susceptible to either gentamycin and vancomycin, 70% displayed resistance to tetracycline, and 50% presented intermediate resistance to ciprofloxacin [28]. However, Yan et al. observed a low frequency of resistance to ciprofloxacin (17.8%) in a study conducted in China [29]. Furthermore, a fully sensitive *L. monocytogenes* to erythromycin (100%) was reported in Iraq by Zena Khalil which is in contrast to this study's findings [25]. The clinical isolates showed susceptibility to meropenem (85.7%), followed by trimethoprim/sulfamethoxazole (SXT) (57.1%) and ciprofloxacin (50.0%). However, the meat strains showed high susceptibility to ciprofloxacin, meropenem, SXT and chloramphenicol (77.4, 74.2, 64.5 and 58.1% respectively) (Table 2). The present study highlights the high rate of antimicrobial resistance among humans and various types of meat, which is a topic of concern. As such, continuous monitoring is required to establish the resistance profiles of the most common pathogenic bacteria circulating in Iraq. The high prevalence of antimicrobials resistance detected in this study possibly occurred due to the extensive use of these antibiotics in clinical therapy and animal feed complements [30].

Table 2: Phenotypic susceptibility *L. monocytogenes* isolates to different antimicrobials

			Frequency of Resistance isolates No. (%)			
Class	**Antimicrobials**	**Break points (MM)**	**Human (n=14)**	**Meats (n=31)**	**Total**	
			R	**R**		**P value**
Beta lactamse (Cephalosporine)	Ampicillin	≤ 28	8(57.1%)	21(67.7%)	29(64.4%)	0.492
	Cephalexin	≤ 14	9(64.3%)	19(61.3%)	28(62.2%)	0.342
Phenicoles	Chloramphenicol	≤ 12	8(57.1%)	13(41.9%)	21(46.7%)	0.344
Quinolones	Ciprofloxacin	≤ 15	4(28.6%)	5(16.1%)	9(20.0%)	0.154
Lincomycin	Clindamycin	≤ 0.5	9(64.3%)	15(48.4%)	24(53.3%)	0.457
macrolides	Erythromycin	≤ 13	8(57.1%)	18(58.1%)	26(57.8%)	0.665
Tetracyclines	Tetracycline	≤ 19	11(78.6%)	22(71.0%)	33(73.3%)	0.484
Aminoglycoside	Gentamycin	≤ 12	8(57.1%)	16(51.6%)	24(53.3%)	0.925
Carbapenems	Meropenem	≤ 14	2(14.3%)	6(19.4%)	8(17.8%)	0.547
Penicillins	Penicillin	≤ 28	9(64.3%)	19(61.3%)	28(62.2%)	0.848
sulfonamide	SXT	≤ 10	5(35.7%)	10(32.3%)	15(33.3%)	0.798
Glycopeptides	Vancomycin	≤ 14	7(50.0%)	17(54.8%)	24(53.3%)	0.904

3.2 *Prevalence of L. monocytogenes* antimicrobial resistance genes

The occurrence of antibiotic resistance genes in all 45 *L. monocytogenes* isolates from human and meat samples was established with primers (see Table 3). The results of the present study indicate that the genotypic characterizations were quite consistent with the phenotypic resistance profiles of the examined *L. monocytogenes* isolates. Of the 45 *L. monocytogenes* strains, 41 (91.11%) isolates had more than five resistance genes (Table 3). Many pathogens are intrinsically resistant to certain antimicrobials. Notably, this is related to their undefined physiology. Conversely, many other pathogens may develop antimicrobial resistance via gene mutation or other genetic alteration mechanisms [31]. It has been discovered that numerous mechanisms are involved in the transfer of undesirable antimicrobial associated genes into *L. monocytogenes*. For instance, the conjugation transfer of resistance genes from *Streptococcus*

spp. and *Enterococcus* spp. and to some extent from a *Staphylococcus spp*. plasmid or transposons into *L. monocytogenes* to the gastrointestinal tract, may be associated with multi-drug resistance [32,33]. Moreover, a decrease or increase in outer membrane permeability, mutations in ribosomal protein genes, and activation of the efflux pump could also contribute to antimicrobial resistance phenotypes [34, 35]. In this study it was reported that penicillin and ampicillin resistance genes (represented by penA and ampC), were detected in all considered strains, followed by ermB (97.8%), tetA (84.4%), tetG (71.1%), vanB (66.7%) and ereB (64.4%) (Table 3). Moreover, vanA (48.9%) and tetB (40.0%) were detected less frequently (Figure 1). Although the presence of resistance genes alone does not necessarily indicate that the pathogen is phenotypically resistant to the same antibiotic since the gene associated with resistance may not be released by the bacteria, surveying the resistance genes is remain important since many of these bacteria may work as an antimicrobial resistance gene pool for other commensal and pathogenic bacteria [24,36]. Confirming the previous statement, the findings of this study showed that all *L. monocytogenes* isolates possessed ampC and penA resistance-associated genes, whereas only 64.4 and 62.25 of the isolates were phenotypically resistant to ampicillin and penicillin, respectively. Likewise, 97.8% carried ermB, 64.4% carried ereB and 15.6% of the isolates were ereA resistance- gene positive (representing erythromycin resistance genes). However, only 57.8% of the isolates displayed phenotypic resistance to erythromycin. Additionally, tetA and vanB resistance genes were detected in 84.4 and 66.7% of *L. monocytogenes* strains, respectively, whereas 73.3 % of isolates exhibited resistance to tetracycline and 53.3%exhibited resistance to vancomycin. The lowest incidence of resistance genes was observed in *L. monocytogenes* with tetD (8.9%), cmlA (13.3%), ereA (15.6%), tetE (17.8%), and tetC (20.0%) (Table 3). Similarly, a study conducted in Iran reported that 66.7% of studied *L. monocytogenes* isolates harbored ampA and only 33.3% carried vanA resistance genes [37]. These results are also supported by other studies conducted in the UK and the USA [38] and [24], respectively. The results of this study are consistent with those of [24], which involved carrying one resistance gene but not the other representing the same antimicrobial. In contrast to the results of this study, [37] and [39] reported that no tetracycline (tetA,tetB, tetC, tetK, tetL, tetM and tetS), vancomycin (vanA, vanB) or erythromycin (ermA, ermC) resistance determinants were identified in any *L. monocytogenes* strains isolated from food products in Iran and Australia, while we found an ermB resistance gene in a single isolate. Additionally, ermA and ermB, were also not detected in the study by Swetha et al., in Iran [28]. The percentage of resistance genes (tetB , tetC, tetD, cmlA, vanB and ereA) in *L. monocytogenes* isolated from humans was higher than for the *L. monocytogenes* isolates from meat. However, these values were not significant except for the ereA resistance gene (p-value= 0.012). This study established that at least three of the resistance genes examined were found in 75% of the *L. monocytogenes* strains isolated from HVS, while at least five of the resistance genes examined were detected in *L. monocytogenes* recovered from placenta and blood. Regarding the *L. monocytogenes* isolates from meat, this study ascertained that at least six resistance genes were found in all meat samples irrespective of their type (Table 3).

Table 3: Frequency of antimicrobial resistance associated genes in *L. monocytogenes* isolates.

Frequency of antimicrobial resistance associated genes isolates No. (%)				
Genes	**Human (n=14)**	**Meats (n=31)**	**Total**	**P value**
TetA	11(78.6%)	27(87.1%)	38(84.4%)	0.465
TetB	7(50.0%)	11(35.5%)	18(40.0%)	0.357
TetC	4(28.6%)	5(16.1%)	9(20.0%)	0.334
TetD	2(14.3%)	2(6.5%)	4(8.9%)	0.393
TetE	2(14.3%)	6(19.4%)	8(17.8%)	0.681
TetG	9(64.3%)	23(74.2%)	32(71.1%)	0.497
cmlA	3(21.4%)	3(9.7%)	6(13.3%)	0.283
penA	14(100%)	31(100%)	45(100%)	------
Van A	6(42.9%)	16(51.6%)	22(48.9%)	0.586
vanB	11(78.6%)	19(61.3%)	30(66.7%)	0.255
ampA	14(100%)	31(100%)	45(100%)	-------
ereA	5(35.7%)	2(6.5%)	7(15.6%)	0.012
ereB	7(50%)	22(71.0%)	29(64.4%)	0.174
ermB	14(100%)	30(96.8%)	44(97.8%)	0.497

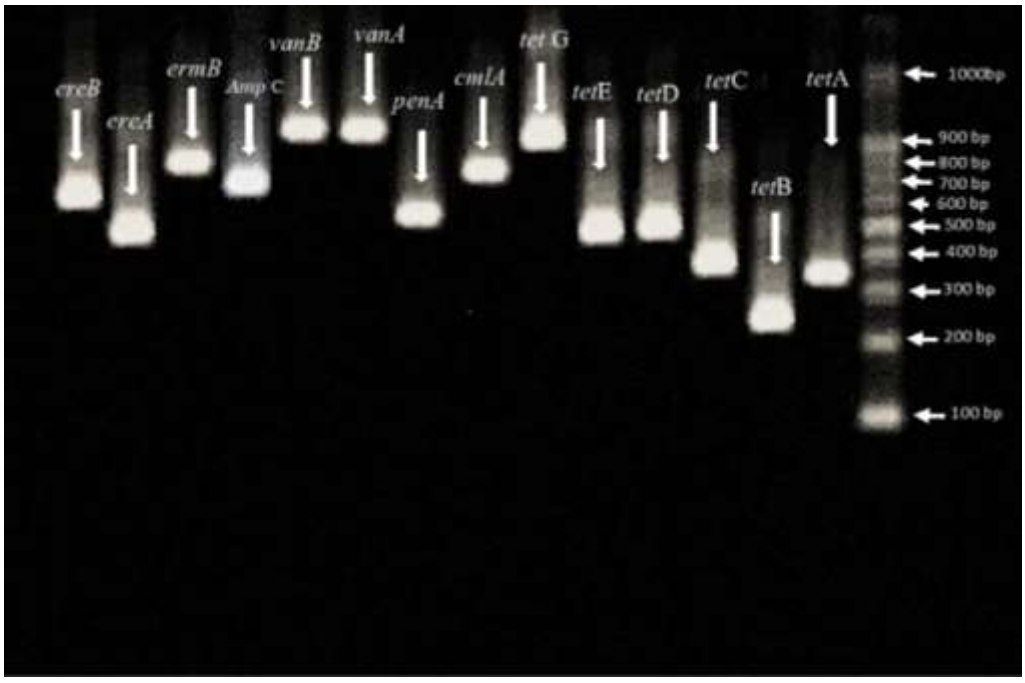

Figure 1: Amplification of tetA, tetB, tetC, tetD, tetE, tetG, camlA, penA, vanA, vanB, ampC, ermB, ereA, and ereB at 372bp, 228bp, 379bp, 436bp, 442bp, 844bp, 698bp, 500bp, 885bp, 882bp, 550bp, 639bp, 420bp, and 546bp, respectively, by conventional PCR. 100bp ladder were used on 1.5% agarose gel at 85V for 45mints.

4. CONCLUSION

In conclusion, this study established the emergence of a major transition in the incidence of antimicrobial resistance in *L. monocytogenes* isolates, which is logically predicted as food manufacturing techniques shift from locally grown foods to a globally connected food supply paradigm. The present study also provides significant information on *L. monocytogenes* susceptibility to commonly used antibiotics. Moreover, the study demonstrates that the majority of *L. monocytogenes* from human and meat samples displayed a high index of resistance to a variety of agents used for clinical listeriosis treatment adding further burden to the existing global antibiotic resistance problem. To minimize the prevalence of antimicrobial resistance pathogens and decrease the potential public health influence, we require a better understanding of how these resistance genes are acquired and transmitted. Furthermore, this study highlights the need for an international approach to the antimicrobial resistance surveillance of clinical settings and food chains.

5. Acknowledgment

The author acknowledges all the members of the Directorate of Prevention Affairs/ Foodstuff Analysis Laboratory, Duhok Obstetrics and Gynecology Hospital, Azadi Teaching Hospital, Hevi Pediatric Hospital, Duhok kidney and Diseases Transplantation Center, and Central Public Health Laboratory for their unlimited help whenever needed.

6. Conflict of Interest

The authors declare that they have no conflicts of interest.

7. Ethical Approval

All strategies of the present study were approved by the local Ethical Committee of College of Health and Medical Technology, Duhok Polytechnic University, Iraq and Duhok Directorate General of Health (Reference No. 100520174)

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