INTERNATIONAL JOURNAL OF
ADVANCED SCIENTIFIC RESEARCH AND INNOVATION

Print ISSN: 2785-9533 - Online ISSN: 2785-9541

VOLUME 6, ISSUE 2, 2023, 92 – 113.

Phenotypic and genotypic of Listeria Monocytogenes isolated from raw milk in Assiut governorate

Dr. Azhar M Hasan

Animal Health Research Institute Head of microbiology Department Assiut Branch

Abstract:

Listeria monocytogenes (*L. monocytogenes*) is a deadly foodborne pathogen affecting populations, although most foodborne pathogens cause considerable illness but little fatality. Raw milk and uncooked dairy products are frequently associated with notable *Listeria* attacks. Microbial virulence traits are potential markers for discriminating the invasive and environmental *L. monocytogenes* variants. So, this study explored the bacteriological and molecular aspects of *L. monocytogenes* isolates, their virulence genes (*hly*A, *inI*B, and *prf*A), and antimicrobial sensitivity in raw milk in Assiut, Egypt. One hundred raw milk samples (40 Cow, 20 markets, 40 Buffalo) were analyzed. *L. monocytogenes* was detected in only 11 (27.5 %), 8 (40 %) and 6 (15 %) of collected samples from cow, market and buffalo raw milk, respectively. These isolates carried the virulence *hly*A, *Inl*B, and *prf*A genes and were sensitive to Sulfamethoxazole trimethoprim (SXT) and Ciprofloxacin (CIP) followed by Chloramphenicol (C), less sensitivity to Gentamicin (CN), and resistant to Erythromycin (E) and Amoxicillin (AX).

Keywords: *L. monocytogenes, raw milk, PCR, hly A, Inl B and prf A genes.*

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

INTRODUCTION:

Listeria monocytogene is a facultative intracellular foodborne pathogen. Its growing prominence in recent years is entirely due to the changes in food preparation and distribution occurring in the so-called "global village" in the last decades of the 20th century and the rising pervasiveness of host variables that raise the infection risk. It has virtually nothing associated with the changing pathogenicity of the organism (**Walter and Schlech, 2000)**. *L. monocytogenes* from a large-scale variety of food categories is linked to outbreaks in people and other animals Listeriosis **(Renato *et al.*, 2015**). **Hilliard *et al.*, (2018)** and **Şanlıbaba and Tezel, (2018)** assigned that listeriosis induces serious clinical manifestations such as meningitis or septicemia in immune-compromised hosts including infants, organ transplant recipients, cancer and AIDS patients, and in pregnant women with the fatality rates of 20 – 30%. It is responsible for about 99 % of cases after consumption of contaminated food. Immunocompromised individuals constitute high-risk populations for infection, especially pregnant women, infants, and the elderly **(EFSA ECDC, 2015).** *L. monocytogenes* was isolated from meat, milk, and milk products around the world **(Ikeh *et al.*, 2010; Khan *et al.*, 2013).** From 100 to 1000 *L. monocytogenes*/g of food is sufficient to produce listeriosis in humans as assigned by most experts **(Ooi and Lorber, 2005). Gray *et al.* (2006)** reported that as just as capable of living in the environment as it is of infecting humans and producing listeriosis, *L. monocytogenes* may also have a saprophytic life cycle. Throughout the past few years, experimental studies have shown that contaminated food is a significant means of bacterial transmission to people **(O'Connor *et al.*, 2010)**. Listeria has been linked to contaminants in food-processing facilities due to its ability to persist for several

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

months or years **(Lianou and Sofos, 2007)**, its ability to colonize abiotic surfaces **(Poimenidou *et al.*, 2016b)**, and its ability to survive under harsh conditions (**Poimenidou *et al.*, 2016a**), expanding the risk to food safety. Additionally, it can lead to miscarriages and nervous disorders in economically significant livestock animals like sheep and cattle. This caused the animals to produce less milk and eventually die. Many foodborne pathogens and opportunistic bacteria reside in the skin and digestive tract of livestock animals, which makes them ideal homes for foodborne pathogens and opportunistic bacteria like *L. monocytogenes*, which can contaminate meat and milk products during slaughter and milking. These bacterial infections can also infiltrate a variety of different natural settings (**Krawczyk *et al.*, 2021**).

According to **Theivagt *et al.* (2006)** and ***Listeria* 2022 from the US Centers for Disease Control and Prevention**, *L. monocytogenes* is a gram-positive, non-capsulated bacterium that phenotypically forms single, short chains. It is a rod-shaped, non-spore-forming, facultative anaerobic bacteria that is 0.5 mm wide and 1-1.5 mm long **(Vazquez-Boland et al., 2001; Vera et al., 2013)**. Taxonomically, it is divided into six species*: Listeria monocytogenes, Listeria ivanovii, Listeria seeligeri, Listeria innocua, Listeria welshimeri,* and *Listeria grayi.* The only pathogenic *L. monocytogenes* are *L. ivanovii.* Genotypic identification of bacterial virulence genes or gene products is the basis for characterizing L. monocytogenes. Listeriolysin O (LLO), according to **Ward *et al.* (2010)**, is a marker needed for the intracellular survival of invasive bacteria in mammalian hosts. L. monocytogenes also generates the virulence proteins PI-PLC and PC-PLC, which are encoded by the hly-A gene.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Due to the large number of cases and the roughly 30% overall death rate of these outbreaks, milk intake may be the primary source of listeriosis, which is raising outstanding worry in the dairy sector **(Amagliani *et al.*, 2004).** For the identification of L. monocytogenes from samples taken from food and the environment using traditional techniques, enrichment cultures followed by selective plating are needed **(Reissbrodt, 2004)**. Numerous novel and quick detection techniques based on molecular and antibody technologies have substantial overlap with methods for selective culture enrichment **(Reissbrodt, 2004; Gasanov et al., 2005).** PCR has been utilized more frequently than the other fast detection techniques for the quick, accurate, and focused detection of foodborne pathogens (**Olsen *et al.*, 1995)**. The purpose of this study was to estimate the prevalence of L. monocytogene in raw milk using a confirmatory PCR test and a traditional culture approach and screening their antimicrobial sensitivity.

MATERIALS AND METHODS

1. Sample collection

One hundred raw milk samples were aseptically collected from random different sources in Assiut City including market (20), buffalo (40), and cow (40) milk. The samples were put in sterile plastic bags in ice box and transported as fast as possible to the laboratory.

2. Microbiological analysis for *Listeria*:

Following the instructions of ISO 11290, *L. monocytogenes* was isolated and identified as described by **Becker *et al.* (2006)**.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

2. 1. Preenrichment: (Fraser and Sperber, 1988)

After spinning the samples, 25 mL were added to 225 mL of half Fraser broth (Merck) as an initial enrichment culture, and the mixture was then homogenized using a stomacher (Lab blender 400, Seward Medical, London, UK) and incubated for 24 h at 30 °C.

2. 2. Selective enrichment:

As a second enrichment culture, the incubated Fraser broth (10 mL) was added to 0.1 mL of half Fraser broth and re-incubated under aerobic conditions 3 hours at 37 °C for 48 hours.

2. 3. Isolation and identification:

On the agar plates ALOA®, Merck agar (Merck), and OXFORD agar, a loopful of enhanced Fraser broth culture (the tubes that exhibit a blackening) was applied for selective plating. At 37 °C, cultures were incubated for 24 hours. Three to five presumed colonies were re-streaked on tryptic soy agar with 0.6% yeast extract (Oxoid, Basingstoke, UK).

Bacteriological analytical manual identification was done according to FDA (**Hitchins, 1995**). Biochemical assays such as the catalase, oxidase, methyl-red, vogues Proskauer, and urease tests were used to confirm colonies and fermentation of the xylose, rhamnose, and mannitol sugars. First, hemolysis on blood agar and the CAMP test were used to confirm isolates. Initially recognized the chosen colonies as Listeria spp. and transferred them to Columbia Agar with 5% sheep blood (bioMérieux) in accordance with the manufacturer's instructions. The kind of hemolysis was then evaluated. Finally, the PCR technique was used.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

2.4. Molecular analysis of the isolated *L. monocytogenes* and their virulence

The isolated *L. monocytogenes* colonies were molecularly analyzed according to **Kaur *et al.* (2007), Shah *et al.* (2009), Daniela *et al.* (2023)** and **Kumar *et al.* (2015)**. Briefly, pure colonies were dispersed in phosphate-buffered saline (5 mL) and centrifuged (3000 rpm for 10 min. at 4°C). This step was repeated 3 times till obtaining pellet. Pellets were used for DNA extraction using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's instructions**.** The extracted DNA was used as a template and amplified by polymerase chain reaction (PCR) using *L. monocytogenes* species-specific primer sets (Metabion, Germany) for 16 rRNA gene [**Forward**: (GGACCGGGGCTAATACCGAATGATAA) and **Reverse**: (TTCATGTAGGCGAGTTGCAGCCTA)] and EmeraldAmp® Master mix (Takara, Japan) in Veriti thermocycler (Applied Biosystems, Germany) following **Kumar *et al.*, (2015)** cycling conditions. Exactly, an initial 94°C hot start for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min, and extension at 72°C for 1 min. then a final extension at 72°C for 12 min. The amplified products (5µl) were loaded in 1.5 % agarose gel stained with ethidium bromide **(**AppliChem, Germany, GmbH**)**, electrophoresed (5V/cm, at room temperature), visualized with UV light in comparison to 100-1.500bp DNA ladder (Qiagen, Germany, GmbH) and photo'd by a gel documentation system (Alpha Innotech, Biometra). An amplicon size of 1200 bp is specific to *L. monocytogenes.*

2.5. Determining virulence of the isolated *L. monocytogenes*

The *L. monocytogenes* DNA was analyzed for the virulence genes; internalin hemolysin (*hly*A), internalin B (*inl*B), and positive regulatory factor A (*prf*A) genes

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

by PCR techniques using gene-specific primer set (**Table 1**). The DNA was amplified with the primer set specific to each gene as described above **(part 2.6.)** following the cycling conditions described in **table 2.** The amplified products were screened as mentioned above.

Table 1: Primers sets specific to the virulence genes intended for determining the virulence of the isolated *L. monocytogenes*

Primer		Sequence	Amplicon size	Reference
***hly*A**	**F**	5'GCATCTGCATTCAATAAAGA 3'	174 bp	**Deneer and Boychuk (1991)**
	R	5'TGTCACTGCATCTCCGTGGT 3'		
***inl*B**	**F**	5'CTGGAAAGTTTGTATTTGGGAAA3'	343 bp	**Liu *et al.* (2007)**
	R	5'TTTCATAATCGCCATCATCACT3'		
***prf*A**	**F**	5'TCTCCGAGCAACCTCGGAACCTGG3'	1052 bp	**Dickinson *et al.* (1995)**
	R	5'ATTGACAAAATGGAACA3'		

Table 2: The cycling conditions followed in the PCR for the different primers intended for determining the virulence of the isolated *L. monocytogenes*

Gene	Initial denaturation	Amplification (35 cycle)			Final extension
		denaturation	Annealing	Extension	
***hly*A**	94˚C (5 min.)	94˚C (30 sec.)	50˚C(30sec.)	72˚C(30 sec.)	72˚C(7min.)
i*nl*B		94˚C (30 sec.)	55˚C(40sec.)	72˚C(40sec.)	72˚C(10min.)
***prf*A**		94˚C (30 sec.)	50˚C(50sec)	72˚C(1min.)	72˚C(10min.)

2.4 *In vitro* anti-microbial sensitivity testing:

As recommended by the European Committee on Antimicrobial Susceptibility test (EUCAST), the disc agar diffusion technique was used to explore the sensitivity of the isolated *L. monocytogenes* strains against 8 antibiotics; Erythromycin (E), ceftriaxone (CRO), Gentamycin (CN), Clindamycin (DA), Amoxicillin (AX), Ciprofloxacin (CIP), Chloramphenicol (C) Sulfamethoxazole-trimethoprim (SXT) and Ciprofloxacin (CIP) **(NCClS, 1999).** In reality, a suspension with 0.5 McFarland turbidity of about 5-6 fresh overnight *Listeria* colonies and 1 mL normal saline solution was prepared. The produced suspension was flooded over Mueller-Hinton

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

agar (HiMedia, India) plates, and the antibiotic discs were then applied using a disc dispenser. The plates were incubated at 37^{o} C for 24 h, and then after zones of bacterial growth inhibition were measured to the nearest mm. Results were interpreted following EUCAST principles.

4. Statistical analysis

The mean values were dependent on describing results and these data were analyzed by applying the Chi-square test using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) to determine the relationship between the true/false positive results and the food categories**.**

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

RESULTS

The results demonstrated in Table 3 showed the detection rate of *L. monocytogenes* in the examined Market, Buffalos, and cow milk samples in Assiut governorate. *L. monocytogenes* was detected in 8 out of 20 Markets, 6 out of 40 Buffalos, and 11 out of 40 cow raw milks with 40, 15, and 27 % detection rates, respectively.

Table (3): *L. monocytogenes* percentage in the examined Market, Buffalos, and cow milk samples in Assiut governorate:

Source	*L. monocytogenes* n/t (%)
Cow	11/40 (27. 5)
Market	8/20 (40.00)
Buffalos	6/40 (15.00)
Total	25/100 (25.00)

Species identification within the tested isolates (PCR)

Molecular characters of the recovered *L. monocytogene* isolates during the molecular analysis showed in **figure 1**. The isolates that positively reacted to biochemical reactions carried the *L. monocytogene* specific16 rRNA gene and its virulence genes**;** *hyl*A, *inl*B, and *prf*A genes.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

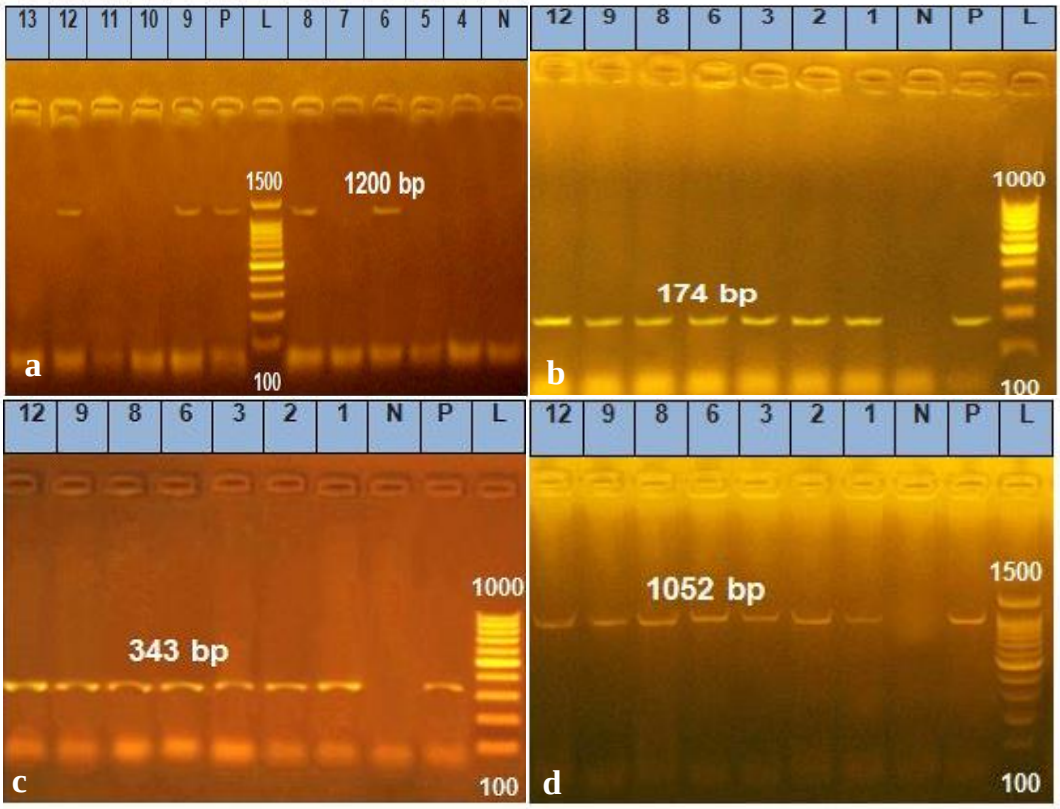

Fig. 1: Agarose gel electrophoresis gel showing (**a)** 16 rRNA gene specific for *Listeria monocytogene*, and its virulence genes **(b)** *hyl*A, **(c)** *inl*B, and **(d)** *prf*A genes. **Lane L**:100-1000 bp DNA Ladder as a standard marker. **N:** Negative control. **P:** Positive control at 1200, 174, 343, and 1052 bp, respectively. **Lanes 1-12:** *L. monocytogenes* positive isolates.

3. Anti-microbial sensitivity of the isolated *L. monocytogenes*

In total, the detected *Listeria* isolates (25 isolates) were resistant to one or more of the tested antibiotics (**table 4**). They were sensitive to Sulfamethoxazole-trimethoprim (SXT) (25 µg /disc) and Ciprofloxacin (CIP) (10 mcg) followed by Chloramphenicol (C) (30 µg /disc), with weak sensitivity to Gentamicin (CN) (120 µg /disc). While they were resistant to Erythromycin (E) (15 µg /disc) and Amoxicillin (AX) (20 / 10 µg /disc)

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Table 4*: In-vitro* antimicrobial sensitivity test for isolated *L. monocytogenes*:

Source	Anti-microbial								
	Sxt	**CN**	**E**	**CRO-30**	**NV**	**D**	**AX**	**CIP**	**C**
Cow milk									
S %	70	30	10	50	zero	20	zero	90	80
R %	20	40	90	10	90	40	100	0	10
I %	10	30	zero	40	10	40	zero	10	10
Market milk									
S %	83.3	16.7	00.0	33.3	00.0	33.3	00.0	83.3	00.0
R %	16.7	66.7	100.0	50	83.3	66.7	100.0	00.0	33.3
I %	00.0	16.7	00.0	16.7	16.7	00.0	00.0	16.7	66.7
Buffalo milk									
S %	100.0	33.3	00.0	33.3	00.0	66.7	00.0	66.7	33.3
R %	00.0	00.0	100.0	00.0	100.0	00.0	100.0	00.0	33.3
I %	00.0	66.7	00.0	66.7	00.0	33.3	00.0	33.3	33.3

S: *sensitivity.* ***R:*** *Resistance.* ***I:*** *Intermediated*

DISCUSSION

Adzitey *et al.* (2013) and **Liao *et al.* (2021)** claimed that more than 200 diseases that are known to be dangerous to human health can spread through food. Among these food-borne bacteria, *L. monocytogenes* was discovered and has a major role due to its ability to flourish in refrigerator temperatures and high mortality rates (**Lida *et al.*, 2014; Anonymous, 2021**). *L. monocytogenes* is an important pathogen in both human and veterinary medicine, inducing a number of illnesses in mammals, birds, and fish, as well as abortion and encephalitis in sheep and cattle (**Kalrey *et al.*, 2008**).

According to **Lundėn *et al.* (2004)**, the consumption of ready-to-eat (RTE) food items is a key contributing factor to human listeriosis infections. The use of contaminated raw milk or post-processing contamination from environmental sources unrelated to raw milk can both lead to *L. monocytogenes* infection of fermented dairy products made from raw milk (**Kells and Gilmour, 2004)**. Controlling *listeria* during

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

food processing is difficult because it is so prevalent in agricultural and food production areas (**Sarfraz *et al.,* 2017**). The expression of virulence factors and the immunological state of people determined the ability of *L. monocytogenes* to cause illness. The immunity mediated by cells of those who are most vulnerable to *L. monocytogenes* is typically compromised (**Lecuit *et al.,* 2004).**

The obtained data in this study revealed that *L. monocytogenes* was detected in 8, 6, and 11 in the cow , market, and buffalo raw milk respectively, samples out of 20,40 and 40 samples respectively, 11 (27.5 %), 8 (40 %) and 6/40 (15.00)) represented as in the market, buffalo, and cow raw milk that disagreed with that recorded by **Zeinali *et al*., (2017**)

The virulence of *L. monocytogenes* was determined by the hemolytic activities of the isolated *Listeria* species (**Maarouf *et al.,* 2007**). Similar results were reported by **Marrouf *et al.,* (2007) and Rahimi *et al.,* (2020)**, the result of virulence tests for isolated *listeria* showed that all *L. monocytogenes* were positive to CAMP test and showed narrow zone of β-hemolysis in sheep agar.

With dissimilar results to that reported by **Cao *et al.,* (2018),** the PCR results for L. monocytogenes isolated showed that 16rRNA, *hly*A, *inl*B, and *perf*A genes were not detected in all isolates regarding the occurrence of 16rRNA genes in *L. monocytogenes* isolates, the obtained result revealed that, it was amplified in all tested isolates, that agreed with those recorded by **Ciolacu *et al.,* (2015) and Abdelhamed *et al.,* (2022)**

Disagreeing with **Shen *et al.,* (2000)**, the PCR result for amplification of the internalin B (*inl*B) gene in *L. monocytogenes* showed that the i*nl*B gene wasn't amplified in all tested isolates.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Similar to **Gelbicova and Karpiskova, (2012); Khan *et al.*, (2014); Ciolacu *et al.*, (2015) and Self *et al.*, (2019**), the result of PCR for amplification of the listeriolysin O (hemolysin,hlyA) gene in *L. monocytogenes* showed that hlyA gene was amplified in all tested isolates giving the product of 174bp.

Dissimilar results with (**Gelbicova and Karpiskova, 2012 and Ciolacu *et al.*, 2015**) the result of PCR for amplification of the positive regulatory factor gene (prfA) in *L. monocytogenes* showed that the *prf*A gene wasn't amplified in all tested isolates.

In the present study, it is postulated that the detection rate of *L monocytogenes* using the conventional method was 7.5 % in raw milk samples, almost similar results were studied by **Aygun and pehlivanlar, (2006)** who reported *L. monocytogenes* in 5% of raw milk samples, while, (**Kasalica and Oljačić 2007**) showed that in 30 samples of raw milk (sheep, goat and cow milk) the presence of *L. monocytogenes* was not established and (**Mohamed, 2010**) reported that listeria monocytogenes was present in 1.7 and 3.3 % in raw milk samples in farm A and b. also, (**Jami *et al.*, 2010**) found listeria monocytogenes in 4% of raw milk samples, cow milk is mentioned as carrier of the fatal listeriosis (**Farber and Peterkin, 1991; Sepahvand *et al.*, (2022)**.

Sick animals on the farm were represented the most common way for transmission of *L. monocytogenes* to raw milk mainly, and healthy animals acted as a carriers of *listeria. monocytogenes* and source of contamination of the surrounded area, or milk. The poor quality of prepared silage was the main source of contamination of animals by these bacteria. *Listeria* was isolated in 1.2-60% samples as a result of poor quality of prepared silage (pH > 5-5.6) according to some literature

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

data (**Vilar *et al.*, 2007**). *Listeria* spp. was isolated in 2-6.1% samples of milk collected from cows fed silage (**Vilar *et al.*, 2007**) **Brown, et al.,** *(2021).*

The presence of *L. monocytogenes* in food that reported by many researchers obtained in developed countries (**Melanie and Siegfried, 2001; Karakolev, 2009**). In Europe the incidence of *L. monocytogenes* that was recorded by microbiological studies has shown that 2.5 - 6 % of samples of raw milk can be contaminated with L. monocytogenes, these results come parallel with those recorded by their article according that there was a potential risk for the human population from dairy products manufactured from raw milk that reported by **Donnelly, (2004)**.

The identification of several molecular virulence determinants plays an important role in cellular infection by *L. monocytogenes* and the detection of their mechanism of action which was made by *L. monocytogenes* acts as one of the most exciting models of host–pathogen interaction at the cellular and molecular levels that reported by **Cabanes *et al.*, (2005)**, **Cossart and Toledo-Arana, (2008)**, **Shourav, *et al.*, (2020)** and **Sleator *et al.*, (2005)**. The most virulence determinants include *Act*A protein, two phospholipases, a metalloprotease, the internalin's, listeriolysin O (LLO), *Vip* protein, a bile exclusion system (BilE), and a bile salt hydrolase. Many articles have been found that a more sensitive and rapid technique for confirmation of the identification of suspect *L. monocytogenes* isolated on selective/differential agar plates was Polymerase chain reaction (PCR) which targeting the *hly* gene (**Gouws and Liedemann, 2005**) Dharshini. and Meera (2023)

The encountered *L. monocytogenes* isolates were sensitive to Sulfamethoxazole-trimethoprim (SXT) and Ciprofloxacin (CIP) followed by Chloramphenicol (C), with weak sensitivity to Gentamicin (CN). While they were

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

resistant to Erythromycin (E) and Amoxicillin (AX) recorded in-vitro antimicrobial sensitivity test, and these results came in accordance with those recorded by **Cabanes *et al.*, (2005)** and disagreed with **Altuntas *et al.* (2012) Jibo. *et al.* (2022)**

Conclusion

The research presented here ultimately leads to the conclusion that L. monocytogenes mainly serves as a food-borne pathogen that can spread through raw milk and cause listeriosis. The isolated *L.monocytogenes* were sensitive to Sulfamethoxazole-trimthoprim (SXT) and Ciprofloxacin (CIP) followed by Chloramphenoicol (C), less sensitivity to Gentamicin (CN). On other hand, the isolates were resistant to Erythromycin(E)and Amoxicllin (AX).

The isolated *L.monocytogenes* were CAMP Positive and produced β-zone of heamolysis. In PCR result assured that strains are *listeria monocytogenes* and have hlyA, inlB and prfA virulence genes genes.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

REFERENCES:

Abdelhamed, H., Ramachandran, R., Narayanan, L., Islam, S., Ozan, O., Freitag, N., et al. (2022). Role of FruR transcriptional regulator in virulence of Listeria monocytogenes and identification of its regulon. PLoS One 17:e0274005. doi: 10.1371/journal. pone.0274005

Adzitey, F.; Huda, N. and Ahmat, A. (2013): Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. Biotech. pp. 97-107.

Altuntas, E.G.; Kocan, D.; Zeinali, T.; Jamshidi, A.; Bassami, M. and M. Rad, (2017): Isolation and identification of *Listeria* spp. in chicken carcasses marketed in northeast of Iran. International Food Research Journal, 24(2): 881-887.

Anonymous, "Centers of disease control and prevention," 2021, https://www.cdc.gov/listeria/index.html**.**

Aygun, O. and Pehlivanlar, S. (2006): *Listeria* spp. in the raw milk and dairy products in Antakya, Turkey. Food Control. 17: 676-679.

Becker, B.; Schuler, S.; Lohneis, M.; Sabrowski, A.; Curtis, G. D. W. and Holzapfel, W. H. (2006). Comparison of two chromogenic media for the detection of *Listeria monocytogenes* with the plating media recommended by EN/DIN 11290-1. Inter. J. Food Microbiol, 109: 127-131.

Borucki, M. K.; Gay, C. C.; Reynolds, J.; Katherine, L.; McElwain, S.; Kim, H.; Call, D. R. and Knowles, D. P. (2005): Genetic diversity of *Listeria monocytogenes* strains from a high-prevalence dairy farm. Appl Environ Microbiol. 71: 5893-5899.

Brown, B., Allard, M., Bazaco, M. C., Blankenship, J., and Minor, T. (2021). An economic evaluation of the whole genome sequencing source tracking program in the U.S. PLoS One 16:e0258262. doi: 10.1371/journal.pone.0258262

Cabanes, D.; Sousa, S.; Cebria, A.; Lecuit, M.; Garcia-del; Portillo, F. and Cossart, P. (2005): Gp96 is a receptor for a novel Listeria monocytogenes virulence factor, Vip, a surface protein. The EMBO Journal, 24(15): 2827-2838.

Ciolacu, L.; nicolau, A.I.; Wagner, m. and Rychli, K. (2015): *Listeria monocytogenes* isolated from food samples from a Romanian Black market show distinct virulence profiles. Int . J. Food Microbial., 209:44-51

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Cosansu E, Ayhan K, JunejaVK, Materon2012 L.monocytogenes Antibiotic and bacteriocin sensitivity of Listeria monocytogenes strains isolated from different foods. JFNS. 2012 Mar;3(3):363-8. DOI: 10.4236/fns.2012.33052

Cossart, P. and Toledo-arana, A. (2008): Listeria monocytogenes, a unique model in infection biology: an overview. Microbes infection, 10, 1041–1050.

Curtis, G.D.; Mictchell, R.G.; King, A.F. and Griffin, E.J. (1989): A selective differential medium for the isolation of Listeria monocytogenes. Lett. Appl. Microbiol. 8:95–98.

Dharshini,S Sai and Meera M.(2023)

Effect of popping and milling on physical, chemical, structural, thermal properties and angle of repose of amaranth seed (Amaranthus cruentus L.) and finger millet (Eleusine coracana L. Gaertn) from Udhagamandalam Contents lists available at ScienceDirect Applied Food Research

Donnely, C.W. (2004): Growth and survival of microbial pathogens in cheese. In: Fox, P.F., McSweeney, P.L., Cogan, T.M. and Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology, Volume 1, General Aspects (3rd Ed.), Elsevier Academic Press, 541-559.

EFSA ECDC (2015). EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J. 13:4329. doi: 10.2903/j.efsa.2015.4329

Farber, J.M. and Peterkin, P.I. (1991): Listeria monocytogenes, a food-bornepathogen. Microbiol. Rev., 55, 476-511.

Fraser, J.A. and Sperber, W.H. (1988): ISO NORMATIVE 11290-1 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method.

Gasanov, U.; Hughes, D. and Hansbro, P.H. (2005): Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol. Rev., 29: 851- 875

Gelbicova,T., Karpiskova, R. 2012. Outdoor environment as a source of L.monocytogenes in food chain Czech J .Food Sci.30 (1):83-88.

Gouws, P.A. and Liedemann, I. (2005): Evaluation of diagnostic PCR for the detection of Listeria monocytogenes in food products. Food Technol. Biotechnol., 43: 201–205.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Gray, M. J., Zadoks, R. N., Fortes, E. D., Dogan, B., Cai, S., Chen, Y., et al. (2004). Listeria monocytogenes isolates from foods and humans form distinct but overlapping populations. Appl. Environ. Microbiol. 70, 5833–5841. doi: 10.1128/AEM.70.10.5833-5841.2004.

Halberg Larsen, M., Dalmasso, M., Ingmer, H., Langsrud, S., Malakauskas, M., Mader, A., et al. (2014). Persistence of foodborne pathogens and their control in primary and secondary food production chains. Food Control 44, 92–109. doi: 10.1016/j.foodcont.2014.03.039

Hindawi Veterinary Medicine International Volume 2022, Article ID 3206172, 5 pages

Hitchins, A.D. (1995): Listeria monocytogenes. In 8th ed. Food and Drug Administration. Bacteriological Analytical Manual. AOAC International Pub. Co., Gaithersburg, MD, USA

Ikeh, M.A.C.; Obi, S.K.C.; Ezeasor, D.N.; Ezeonu, I.M. and Moneke, A.N. (2010): Incidence and pathogenicity profile of Listeria spp. isolated from food and environmental samples in Nsukka, Nigeria. Afr J Biotechnol 9:4776-4782.

Jalali, M. and Abedi, D. (2008): Prevalence of Listeria species in food products in Isfahan, Iran. Int . J. Food Microbiol. 122:336-340.

Jibo, G.G. Raji,Y.E. Salawudeen,A. Amin S.
Nordin, Mansor,R. T.Z.M.T. Jamaluddin (2022) A systematic review and meta-analysis of the prevalence of Listeria monocytogenes in South-East Asia; a one-health approach of human-animal-food-environment
One health, 15 (2022), 10.1016/j.onehlt..100417

Kalrey, D.R.; Warke, S.R.; Kurkur, N.V.; Rawool, D.B. and Barbuddhe, S.B. (2008): Listeria species in bovine raw milk: A large survey of central India. J.food Control, 19: 109-112. DOI:101016/j.foodcont.2007.02.006.

Karakolev, R. (2009): Incidence of Listeria monocytogenes in beef, pork, rawdried and raw-smoked sausages in Bulgaria. Food Control., 20, 10, 953-955.

Kells, S., and A. Gilmour. 2004. Incidence of Listeria monocytogenes in two milk processing environments and assessment of Listeria monocytogenes blood agar for isolation. Int. J. Food Microbiol. 91:167–174.

Khan, J.A.,; Rathore, R.S.; Khan, S. and Ahmad, I. (2013): In vitro detection of pathogenic Listeria monocytogenes from food sources by conventional, molecular and cell culture method Braz. J. Microbiol. vol.44 no.3 São Paulo July/Sept.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Khan, J.A., Rathore, R.S., Khan, S., Ahmad , I. (2014)In vitro detection of pathogenic listeria monocytogenes from food sources by conventional, molecular and cell culture method . Braz.J. Microbial., 44(3):751-759

Krawczyk, B., Wityk, P., Gałęcka, M. and Michalik, M., 2021. The many faces of Enterococcus spp.—commensal, probiotic and opportunistic pathogen. *Microorganisms*, *9*(9), p.1900.

Kumar V, et al. (2015) Expression of RCK2 MAPKAP (MAPK-activated protein kinase) rescues yeast cells sensitivity to osmotic stress. Microb Cell Fact 14:85

Liao, J.; Guo, X.; Weller, D.L.; Pollak, S.; Buckley, D.H.; Wiedmann, M.; Cordero, O.X. Nationwide Genomic Atlas of Soil-Dwelling Listeria Reveals Effects of Selection and Population Ecology on Pangenome Evolution. Nat. Microbiol. 2021, 6, 1021–1030.

Lorena Lamas a,⁎ , Liesel Brenda Gende b (2023)Valorisation of brewers' spent grain for the development of novel beverage and food products Applied Food Research journal homepage: 11(1): 59-63.

Lecuit, M., Nelson, D.M., Smith, S.D., Khun, H., Huerre, M., Vacher-Lavenu, M.C., Kells, j. and Gilmour, A. (2004): Incidence of Listeria monocytogenes in two milk processing environments and assessments of Listeria monocytogenes blood agar for isolation. Int.J. Food Microbiol., 91: 167-174. DOI: 10.1016/S0168-1605(03)00367-7.

Lianou, A., and Sofos, J. N. (2007). A review of the incidence and transmission of Listeria monocytogenes in ready-to-eat products in retail and food service environments. J. Food Prot. 70, 2172–2198. doi: 10.4315/0362-028X-70.9.2172

Lida, L.; Abazar, P.; Gholamreza, I. and Jamileh, N. (2014): Polymerase chain reaction (PCR4)-based detection of hly and plc-A genes in Listeria monocytogenes isolated from dairy and meat products in Iran. African Journal of Microbiology Research. Vol.8(10),pp.1098-1101

Marrouf, A.A., El-Bealawy, M.A., Moubarak, M.G. 2007 . Some studies of listeriosis in sheep atEl-Kaliobia Phenotypic and genotypic characterization of listeria species isolated from chicken and milk products 104 government.J.Egypt.Vet.Med.Assoc.67 (4):27-40.

Melanie, R. and Siegfried, S. (2001): High incidence of Listeria monocytogenes in European red smear cheese. Int. J. Food Microbiol., 63, 1-2, 91-98.

Mohamed, M.M. (2010): Occurrence of Listeria monocytogenes in raw milk and dairy products in Noorabad, Iran. Journal of animal and Veterinary advances 9 (1):16-19.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

NCCLS.1999 .National Committee For Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria isolated from animals .Approved Standard M31-A. NCCLS, Wayne,USA.

O'Connor, L.; O'Leary, M.; Leonard, N. ; Godinho, M.; O'Reilly, C.; Egan, J. and O'Mahony, R. (2010):The characterization of Listeria spp.isolated from food products and the food-processing environment. Letters in Appl. Microbiol. 51:490-498.

Olsen, J.E.; Aabo, S.; Hill, W.; Notermans, S.; Wernars, K.; Granum, P.E.; Popovic, T.; Rasmussen, H.N. and Olsvik, F. (1995): Probes and polymerase chain reaction for detection of food-borne bacterial pathogens. Int. J. Food Microbiol., 28: 1-78.

Ooi, S.T. and Lorber, B. (2005): Gastroenteritis due to Listeria monocytogenes. Clin. Infect. Dis., 40, 1327-1332.

Poimenidou, S. V., Chatzithoma, D.-N., Nychas, G.-J., and Skandamis, P. N. (2016a). Adaptive response of Listeria monocytogenes to heat, salinity and low pH, after habituation on cherry tomatoes and lettuce leaves. PLoS ONE 11:e0165746. doi: 10.1371/journal.pone.01657

Poimenidou, S. V., Chrysadakou, M., Tzakoniati, A., Bikouli, V. C., Nychas, G.-J., and Skandamis, P. N. (2016b). Variability of Listeria monocytogenes strains in biofilm formation on stainless steel and polystyrene materials and resistance to peracetic acid and quaternary ammonium compounds. Int. J. Food Microbiol. 237, 164–171. doi: 10.1016/j.ijfoodmicro.2016.08.029

Rahimi, A. T. and Rashidian, E. (2020): "Use of touchdown polymerase chain reaction to enhance the sensitivity of *Brucella melitensis* detection in raw milk," Animal Biotechnology, 10: 1–6.

Reissbrodt, R. (2004): New chromogenic plating media for detection and enumeration of pathogenic Listeria spp. an overview. Int. J. Food Microbiol., 95: 1-9.

Renato, H.; Orsi, T.; Bergholz, M.; Martin, W. and Kathryn, J. B. (2015): The Listeria monocytogenes strain 10403S BioCyc database Database, 2015, 1–11.

Robinson, R. K., Batt, C. A. & Patel, P. D. (editors) (2000). Encyclopedia of Food Microbiology. San Diego, CA: Academic Press.

Sarfraz, Y. Ashraf, and S. Ashraf, "A review: prevalence and antimicrobial susceptibility profile of listeria species in milk products," Matrix Science Medica, vol. 1, no. 1, pp. 3–9, 2017.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Şanlıbaba, P. and B. U. Tezel. 2018. Prevalence and characterization of Listeria species from raw milk and dairy products from Çanakkale province. Turk. J. Agric. For. 6: 61-64.

Sepahvand,F ; Rashidian ,E ; Jaydari, A and Rahimi ,H (2022) Short Communication: Prevalence of Listeria monocytogenes in Raw Milk of Healthy Sheep and Goats

Self, J.L.; Conrad, A.; Stroika, S.; Jackson, A.; Whitlock, L.; Jackson, K.A.; Beal, J.; Wellman, A.; Fatica, M.K.; Bidol, S.; et al. Multistate Outbreak of Listeriosis Associated with Packaged Leafy Green Salads, United States and Canada, 2015–2016. Emerg. Infect. Dis. 2019, 25, 1461–1468

Shen Y, Naujokas M, Park M, Ireton K (2000) InIB-dependent internalization of Listeria is mediated by the Met receptor tyrosine kinase. Cell 103: 501–510.

Shourav, A.H. Hasan M., Ahmed. S (2020 Antibiotic susceptibility pattern of Listeria spp. isolated from cattle farm environment in Bangladesh Journal of Agriculture and Food Research, 2), pp.

Sleator, R.D.; Wemekamp-Kamphuis, H.H.; Gahan, G.M., Abee, T. and Hill, C. (2005): A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in Listeria monocytogenes. Mol. Microbiol., 55, 1183–1195.

Theivagt, A.E., Friesen, J.A. (2006). "Purification and characterization of Listeria monocytogenes HMG-CoA reductase" FASEB Journal 20 (4, Part 1): A472 MAR 6

Todar, K. (2009): "Listeria monocytogenes". Todar/s Online Textbook of Bacteriology. n page, htt:// text book of bacteriology.net/Listeria.html.

U.S. Centers for Disease Control and Prevention Listeria (Listeriosis). Available online: https://www.cdc.gov/listeria/outbreaks/enoki-mushrooms-03-20/index.html (accessed on 19 March 2022).

Vazquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Gonzalez Zorn, B.,2001

Vera A, Gonzalez G, Domınguez M, Bello H. 2013. Main virulence factors of Listeria monocytogenes and its regulation.RevChilenaInfectol. 30:407-416

Vilar, M.J.; Yus, E.; Sanjuan, M.L.; Dieguez, J.L.; Rodriguez-Otero, F.J. (2007): Prevalence of and risk factors for Listeria species on dairy farms. J Dairy Sci, 90, 5083-5088.

Print ISSN: 2785-9533 - Online ISSN: 2785-9541 VOLUME 6, ISSUE 2, 2023, 92 – 113.

Ward, T.J.; Evans, P.; Wiedmann, M.; Usgaard, T.; Roof, S.E. and Strokia,S.G. (2010): Molecular and Phenotypic Characterization of Listeria monocytogenes from US Department of Agriculture Food Safety and Inspection Service Surveillance of Ready-to-Eat Foods and Processing Facilities. J. Food Protection. 73:861-869.

Wehlan, J. &Kreft, J. (2001). Listeria pathogenesis and molecular virulence determinants. ClinMicrobiol Rev 14, 584–640

Cao, X.; Wang, Y.; Wang, Y. and Ye, C. (2018): Isolation and characterization of Listeria monocytogenes from the black-headed gull feces in Kunming, China. *J*ournal of Infection and Public Health, 11(1): 59-63.

Yao L., Yiping LuBo Ning Xiaomin Su Binru Yang Haiqing Dong Bo Yin Zhiqing Pang and Shun Shen (2022) Intravenous Delivery of Living Listeria monocytogenes Elicits Gasdmermin-Dependent Tumor Pyroptosis and Motivates Anti-Tumor Immune ResponseACS Nano 2022, 16, 3, 4102–4115