

## Research Article

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# Prevalence, molecular characterization and antibiogram study of *Listeria monocytogenes* isolated from raw milk and milk products

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### Citation

Nishat Zafar, Zeeshan Nawaz, Azka Qadeer, Sidra Anam, Rabia Kanwar, Ashiq Ali, Muhammad Mudassar, Muhammad Tariq Javid, Abdullah Zafar and Asima Tariq. Prevalence, molecular characterization and antibiogram study of *Listeria monocytogenes* isolated from raw milk and milk products. Pure and Applied Biology. Vol. 9, Issue 3, pp1982-1987. <http://dx.doi.org/10.19045/bspab.2020.90211>

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Received: 01/02/2020

Revised: 08/05/2020

Accepted: 04/06/2020

Online First: 15/06/2020

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### Abstract

Foodborne diseases are the primary cause of infections and mortality, especially in developing countries like Pakistan. *Listeria monocytogenes* is an emerging foodborne infection and a public health threat globally. The present study was designed to detect the prevalence and antimicrobial susceptibility pattern of *Listeria monocytogenes* in raw milk and milk products. A total of (n= 90) samples were collected from different dairy shops of Faisalabad comprising of 40 raw milk, 25 cheese, and 25 yoghurt samples. The samples were collected in sterile plastic bags and shifted to the laboratory in an icebox. The isolation and identification of *Listeria monocytogenes* was made by standard microbiological and biochemical procedures, while confirmation was done by polymerase chain reaction (PCR) by amplifying the *hlyA* gene. Antibiotic susceptibility profiling was performed by Kirby Bauer disk diffusion method. The results of the present study showed that among the total 90 samples, 24 (26.66%) were detected positive for *L. monocytogenes* with the highest prevalence in raw milk (16) followed by cheese (05) and yoghurt (03). The highest resistance was observed for Penicillin (63.15%) among the isolates followed by Clindamycin (57.89%), Ciprofloxacin (26.31%) and Rifampicin (26.31%) while 100% isolates were found susceptible to Ampicillin, Cephalothin, Chloramphenicol, and Vancomycin. It is concluded that the frequency of *Listeria monocytogenes* is at an increasing trend in our dairy products, and stringent screening in transportation, processing and storage of dairy products should be needed to reduce the burden of *L. monocytogenes* infections.

**Keywords:** Antibiogram; *Listeria monocytogenes*; Prevalence; Raw milk

### Introduction

*Listeria monocytogenes* (*L. monocytogenes*) has been recognized as an emerging foodborne pathogen. The severe

life-threatening disease known as listeriosis is caused by the intake of food contaminated with this pathogenic microorganism [1]. Listeriosis is an

emerging and significant global public health issue. The first reported case of listeriosis was associated with consumption of dairy products, whereas raw cow milk was considered as the carrier of the lethal listeriosis [2]. Pregnant women, children, and old age people are at higher risk of *L. monocytogenes* infection [3]. Prominent clinical outcomes caused by *Listeria monocytogenes* are sepsis, febrile gastroenteritis, meningoencephalitis, and abortions [4]. Invasive listeriosis could be produced in the babies due to unhygienic conditions in the mother through her placenta. Perinatal listeriosis can cause different defects in neonates like early birth, the conception of a stillborn hatchling and sepsis, or meningitis [5].

*Listeria monocytogenes* is usually found in compost and silage (2-8%) within the dairy farm. Some other prominent areas for *Listeria* presence are steady water in channels, surfaces, and washing areas [6]. Mostly the milk becomes contaminated with *Listeria* species from the unhygienic environment of farm [7].

Antimicrobial resistance has been increasing day by day due to the emergence of resistant bacteria [8]. Numerous outbreaks of listeriosis were confirmed to be related to the intake of raw milk and initiating immense attention in the milk product manufacturing [9]. Keeping in view the facts mentioned above and the increasing trend of antibiotic resistance, the present study was designed to determine the prevalence and antibiotic susceptibility testing of *L. monocytogenes* in raw milk and milk products.

## Materials and methods

### Sample collection and transportation

A total of 90 samples (40 raw milk, 25 cheese, and 25 yoghurt) were collected from different milk shops of Faisalabad. These samples were collected in sterile plastic bags and immediately transported to the laboratory under the aseptic condition in ice boxes. Each sample was mixed in peptone water and incubated at 30°C overnight followed by inoculation of (5 ml)

suspension in Fraser Broth (95 ml) and incubation at 30°C for 48 hrs for enrichment [10].

### Isolation and identification of *Listeria monocytogenes*

A loopful of each enriched sample was then streaked on Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) agar medium plates and incubated overnight at 37°C aerobically. The identification was made based on colony characters, Gram's staining, and biochemical characters. The pattern of hemolysis was detected by streaking the isolated colonies on blood agar [11, 12].

### Confirmation of *L. monocytogenes*

The isolated *Listeria* colonies were subjected to DNA extraction with the help of a commercially available kit (Thermo Scientific, USA). Amplification of hemolysin (*hly A*) gene was done by PCR with the help of specific primers (F: 5'-CGGAGGTTCCGCAAAGATG-3') and (R: 5'-CCTCCAGAGTGATCGATGTT-3'). The PCR products were analyzed by electrophoresis [13].

### Antibiotic susceptibility profiling

Kirby Bauer disk diffusion technique was used for the detection of antibiotic susceptibility of isolates. Antibiotic disks used were; Penicillin, Ampicillin, Trimethoprim-sulfamethoxazole, Vancomycin, Clindamycin, Rifampicin chloramphenicol, tetracycline and ciprofloxacin, cephalothin and gentamicin. The resistance and susceptibility pattern was determined and compared through clinical laboratory standard institute (CLSI) standard guidelines [14].

## Results

### Frequency of *Listeria monocytogenes*

The results of the present study showed that out of a total 90 samples collected, 24 (26.66%) were found positive for *L. monocytogenes* based on standard microbiological. The highest percentage *L. monocytogenes* was found in raw milk samples 40% (16/40), while cheese and yogurt showed 20% (5/25) and 12% (3/25), respectively, as shown in (Table 1).

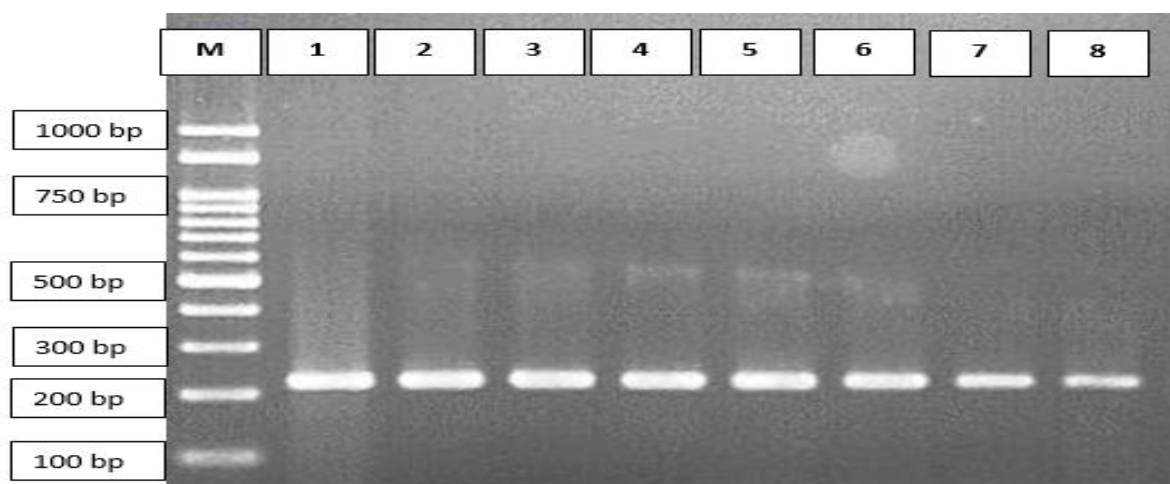
### Molecular detection of *Listeria monocytogenes*

All the isolates were subjected to molecular detection of an *L. monocytogenes* virulence factor Listeriolysin O, which is encoded by

the *hlyA* gene. It was recorded that all the (n=24) isolates showed positive amplification of the *hlyA* gene with a 234bp band, as shown in (Fig. 1).

**Table 1. Occurrence of *Listeria monocytogenes* in raw milk and milk products**

Type of Sample	No. of Samples	Positive Samples	Positive Percentage
Raw Milk	40	16	40%
Cheese	25	05	20%
Yogurt	25	03	12%
Total	90	24	26.66%



**Figure 1. DNA marker in lane M and amplified product of the *hlyA* gene of 234bp from lane 1-8**

### Antibiotic susceptibility test

The antibiotic susceptibility profiling of *L. monocytogenes* was performed by Kirby Bauer disk diffusion method, and it was observed that the highest resistance was found for Penicillin (63.15%) among the isolates followed by Clindamycin

(57.89%), Ciprofloxacin (26.31%) and Rifampicin (26.31%). All the isolates were found sensitive to Ampicillin (100%), chloramphenicol (100%), vancomycin (100%), and cephalothin (100%), as shown in (Table 2).

**Table 2. Percentage resistance pattern of *L. monocytogenes* against different antibiotics**

Antibiotics	Susceptible	Intermediate	Resistant
Ampicillin	19 (100%)	00 (0%)	00 (0%)
Ciprofloxacin	12 (63.15%)	02 (10.52%)	05 (26.31%)
Cephalothin	19 (100%)	00 (0%)	00 (0%)
Clindamycin	07 (36.84%)	01 (5.26%)	11 (57.89%)
Chloramphenicol	19 (100%)	00 (0%)	00 (0%)
Gentamycin	15 (78.94%)	02 (10.52%)	02 (10.52%)
Penicillin	06 (31.57%)	01 (5.26%)	12 (63.15%)
Rifampicin	14 (73.68%)	00 (0%)	05 (26.31%)
Trimethoprim-sulfamethoxazole	16 (84.21%)	01 (5.26%)	02 (10.52%)
Tetracycline	13 (68.42%)	04 (21.05%)	02 (10.52%)
Vancomycin	19 (100%)	00 (0%)	00 (0%)

## Discussion

Today, food safety is everybody's concern, and it is tough to find someone who has not encountered an unpleasant moment of foodborne illness at least once in a year. The majority of the foodborne diseases are self-limiting and mild, but severe cases can occur in high-risk groups, including infants, young children, the elderly, and the immunocompromised persons [15]. Listeriosis is caused by *Listeria monocytogenes*, a highly fatal opportunistic pathogen resulting in abortions, sepsis, and meningoenzephalitis [16]. Listeriosis is a zoonotic problem generally caused by the utilization of milk and milk products [8]. The results of the present study showed that out of a total of 90 samples collected, 24 (26.66%) were found positive for *L. monocytogenes*. The findings of Gohar *et al.* [17] showed a 13.6% prevalence from Pakistan, Hosseini *et al.* [18] showed 19.04% from Iran, and Yakubu *et al.* [19] showed 22.4% from Nigeria. This increased prevalence of *Listeria monocytogenes* in the present study is due to improper milking, unhygienic handling, poor transportation, and storage facilities. It was recorded that raw milk had the highest percentage of contamination 40%, while yoghurt had at least 12%. A similar trend in the results was achieved by Gohar *et al.* [17] in Pakistan, Sheela, and Shrinithiviahshini, [20] in India and Hosseini *et al.* [18] in Iran. This low prevalence of *Listeria monocytogenes* in yoghurt is due to the production of lactic acid during yoghurt manufacturing by *Lactobacillus* species, which suppresses the growth of harmful bacteria. This fact is also supported previously by scientists that lactic acid bacteria produce some compounds which reduce the levels of *L. monocytogenes* in yoghurt [21, 22]. The results of the present study also showed that Penicillin (63.15%) and clindamycin (57.89%) were found resistant to isolates of *L. monocytogenes*, which is also under the findings of (Akrami-Mohajeri *et al.* [23] and Harakeh *et al.* [24]. The resistance of *L.*

*monocytogenes* isolates from different dairy products to common antibiotics. Penicillin, tetracycline, and gentamicin is evident [19, 25]. In contrast, the current study revealed a 100% resistance to Ampicillin, chloramphenicol, and Vancomycin. A similar type of result was previously mentioned in Nigeria [19] and Ethiopia [26]. The emergence of multidrug-resistant (MDR) *Listeria monocytogenes* is also reported in humans and animals, which is due to the irrational use of antibiotics among the food animals [27, 28].

## Conclusion

The results of this study conclude that increasing the trend of *Listeria monocytogenes* in milk and dairy products is an alarming situation and a public health threat that can cause morbidity and mortality outbreaks. The milk for human consumption and production of milk products must be pasteurized or at least boiled to protect young, pregnant women and immune-compromised individuals from this infection.

## Authors' contributions

Conceived and designed the experiments: MZ Nawaz, Performed the experiments: N Zafar, Analyzed the data: S Anam, R Kanwar, MT Javid & A Tariq, Contributed materials/ analysis/ tools: MZ Nawaz, Wrote the paper: A, Ali, A Qadeer, N Zafar, A Zafar & M Mudassar.

## Acknowledgement

The author would like to thanks all the co-authors and laboratory staff during the research.

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