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Original Article

Antibiotic resistance profiling in *Listeria monocytogenes* isolates from white meat available in Pakistani retail markets

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Abstract

Background: Listeriosis is a disease that occurs in immunocompromised people, caused by a zoonotic bacterium, *Listeria monocytogenes*. The significance of its prevalence in raw meat lies in its potential to cause illness if the meat is undercooked, fails to reach the recommended internal temperature, or through cross-contamination. **Aims:** This study aims to assess the presence of *L. monocytogenes* in fresh chicken, fish, and frozen/ready-to-eat (RTE) meat sourced from the retail markets of two major cities in Pakistan. **Methods:** The identification was done by biochemical and molecular methods targeting two genes of *Listeria* Pathogenicity Island (LIP-1) i.e., *prfa* and *hly* genes by PCR and was sequence analyzed, isolates sequences were submitted to NCBI GenBank to get accession number (ON859912, ON933793, ON933794, ON933795, ON933790, ON933791, and ON933792). The antibiotic resistance of these isolates was confirmed against seven antibiotics using the Disk Diffusion Method. **Results:** The antibiotic susceptibility profile of these isolates shows that most of the isolates were resistant to Vancomycin (43.2%), Gentamycin (37.8%), and Erythromycin (18.9%). **Conclusion:** The study reveals a significant presence of *L. monocytogenes* in meat samples, accompanied by antibiotic resistance to commonly used antibiotics for listeriosis in Pakistan. This alarming situation poses serious hazards to public health.

Key words: Meat-borne pathogens, Poultry, Poultry-borne pathogens

Introduction

As per the World Health Organization (WHO) guidelines 2007-2015, foodborne diseases contribute to one-third of deaths in children under the age of five, resulting in an annual toll of 420,000 fatalities. *Listeria monocytogenes* is one such major zoonotic food-borne pathogens of the 21st century. It is a Gram-positive, microaerophilic, intracellular pathogen that causes listeriosis in immuno-compromised individuals (Meghdadi *et al*., 2019) with a fatality rate of 20-30% (Moreno *et al*., 2014). In humans, listeriosis manifests with symptoms like encephalitis, septicemia, and meningitis. Furthermore, listeriosis may induce gastroenteric symptoms in humans and lead to stillbirths or spontaneous abortions in pregnant women. In animals, it can be linked to clinical listeriosis, marked by symptoms such as abortion, encephalitis, and septicemia (Matle *et al*., 2020). Studies have confirmed its presence in foods such as meat, milk, cheese, fish, fruits, and vegetables (O'Grady *et al*., 2009). Distinguishing itself from many other foodborne pathogens, *L. monocytogenes* exhibits the unique ability to replicate and thrive in low temperatures (Goh *et al*., 2012). It can endure extended periods under challenging environmental conditions and create biofilms on food-processing equipment (Kayode and Okoh, 2022). The average mortality of *L. monocytogenes* is higher than other foodborne pathogens like *Campylobacter* species. (0.02-0.1%), *Salmonella enteritidis* (0.38%), and *Vibrio* spp. (0.005-0.01%) in terms of disease severity (Swetha *et al*., 2012). Its pathogenicity is linked with two key virulence factors; *hly A* and *prfA* genes encode listeriolysin O (LLO) and transcriptional proteins for virulence genes, respectively. These virulence genes are responsible for listeriosis upon consumption of contaminated food (Elsayed *et al*., 2022).

After the isolation of the first multi-drug resistant (MDR) strain in 1988, the *L. monocytogenes* antibiotic resistance profile has constantly increased in different environmental and food sources (Wieczorek and Osek, 2017). Several studies from different countries showed

that the prevalence of antibiotic resistance in *L. monocytogenes* varied from 0.6% to 59% based on the sources from which they were isolated (Shamloo *et al*., 2019). These antibiotic resistance levels are influenced due to geographical differences and selective pressure due to the irrational use of antibiotics in agriculture, humans, and cattle (Elsayed *et al*., 2022).

A concerning situation is unfolding in Pakistan due to the emergence of this pathogen that poses significant health risks to a population where strict adherence to hygiene procedures is lacking. It is also increasingly developing resistance to treatment, and the overuse of antibiotics is exacerbating this issue. In Pakistan, several studies on the isolation and identification of *L. monocytogenes* from dairy, meat products, and other foods have been conducted (Chandio *et al*., 2007; Samad *et al*., 2020; Zafar *et al*., 2020), but none of the literature shows the isolation, and antibiotic profiling of *L. monocytogenes* from the meat found in markets of Rawalpindi and Lahore. Considering these factors, the study aimed to assess the microbiological quality, with a specific focus on *L. monocytogenes*, in various meat varieties available in the markets of said cities. The objectives included isolating and identifying the bacterium using molecular techniques and profiling antibiotic resistance to gauge the resistance levels in isolates sourced from different outlets.

Materials and Methods

Collection of samples

A total of 287 samples including fresh fish (n=126), fresh chicken (n=142), and frozen and processed chicken meat (n=19) were gathered from September 2021 to March 2022 from different retail markets in Lahore and Rawalpindi. The meat samples were only collected from healthy-looking animals. The samples (25 gm) were taken aseptically in sterilized PBS solution and transported to the laboratory immediately for further processing.

Reference strain

L. monocytogenes ATCC culture (ATCC 13932) was obtained as the reference strain for isolating and identifying the bacterium.

Isolation of *L. monocytogenes*

Bacterial isolation was done according to the guidelines given by the Drug Administration Bacteriological Analytical Manual (March 2017) and U.S Food. The samples were firstly enriched in *Listeria* Enrichment Broth containing Enrichment Supplement (SR0141 OXOID, UK) and incubated at 37°C for 48 h. After the incubation, a loop full culture was streaked on PALCAM agar (CM0877 OXOID, UK) containing PALCAM (Polymyxin-Acriflavine-Lithium-Chloride-Ceftazidime-Aesculin-Mannitol) selective supplement (SR0150 OXOID, UK) was incubated at 37°C for 48 h. Grey-green colored colonies with black halo zones were purified from the plate and further identified by Gram Staining, Catalase, VP (Voges Proskauer), and CAMP tests.

Molecular characterization

PCR was used to confirm suspected *L. monocytogenes* isolates using a molecular technique. Using the QIAamp DNA Mini Kit (Qiagen, Cat# 51306, USA), DNA was extracted from purified cultures following the manufacturer's instructions. Until further processing, the extracted DNA was stored at -20°C. Two genes were targeted for molecular identification, a Listeriolysin Positive regulatory protein *prfa* and a Listeriolysin O *hly* gene (Jung *et al*., 2013) which are part of *Listeria* Pathogenicity Island called LIP-1. Primer sequences and the expected base pair size of both genes are listed in Table 1.

12.5 µL of Dream Taq Green PCR master mix, 8.5 µL of nuclease-free water, 1 µL of forward primer, 1 µL of reverse primer, and 2 µL of extracted genomic DNA were mixed to make 25 µL total volume of each reaction. Positive and negative controls were also used for test validity. Thermal cycler "T100" (BioRad, USA) was used under the following thermal conditions: an initial denaturation for 7 min at 95°C, 35 X cycles of denaturation for 45 s at 94°C, annealing for 45 s at 50°C for *prfa* and 49°C for 45 s for the *hly* gene, extension at 72°C for 45 s, and a final extension at 72°C for 10 min. The PCR product was resolved on a 1.2% agarose gel containing ethidium bromide at 100 V for 30 min. Gel was visualized by Bio-Rad in a Gel Documentation System.

Phylogenetic analysis

PCR-positive isolates (selected isolated) were sequenced via base-Asia Singapore by single pass genome analyzer. The results were analyzed using BioEdit and MEGAX Softwares and sequences were searched at NCBI server using BLAST suite. Amino acid codes for sequences were obtained using the EXPASY online tool. Sequences of these isolates were submitted to NCBI Banklt and accession numbers were obtained. The phylogenetic tree of the peptide chain release factor

Table 1: Primers sequences of *prfa* and *hly* gene for identification of *L. monocytogenes*

Primer sequence (5´-3´)	Target genes	Species	Expected amplicon size (bp)
F: AACCAATGGGATCCACAAG R: ATTCTGCTAACAGCTGAGC	*prfa*	*Listeria monocytogenes*	479 bp
F: CAAACTGAAGCAAAGGATGCA R: CAAACTGAAGCAAAGGATGCA	*hly*		496 bp

Table 2: Details of antibiotic resistance genes for the confirmation of resistance in *L. monocytogenes* isolates

Antibiotic	Gene	Primer sequence	Target size	Reference
Tetracycline	*tet(M)*	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360 bp	Duran *et al*. (2012)
Erythromycin	*msr(A)*	F: GCAAATGGTGTAGGTAAGACAACT R: ATCATGTGATGTAAACAAAAT	400 bp	Schlegelova *et al*. (2008)
Vancomycin	*vanA*	F: CATGGCAAGTCAGGTGAAGA R: CCGGCTTAACAAAAACAGGA	233 bp	AbdulRazzaq *et al*. (2022)

protein of isolates and their homologs were constructed using the Neighbor-Joining method at a bootstrap value of 100 by using MEGAX software.

Antibiotic susceptibility

The antibiotic susceptibility was tested using the Disk Diffusion method against seven antibiotics including Ampicillin (10 µg), Tetracycline (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Vancomycin (30 µg) and Trimethoprim (1.25 µg). Fresh bacterial inoculum isolates were prepared by mixing purified colonies into sterile normal saline. The inoculum was standardized to McFarland standard 0.5 and a uniform bacterial lawn of this culture was made on Muller-Hinton (MH) agar plates. Antibiotic disks were applied on these plates followed by incubation for 24 h at 37°C. Zone of inhibition (ZOI) was calculated in millimeters and compared with Clinical Laboratory Standards Institute (CLSI) 2017 to categorize the isolates as sensitive, resistant, and intermediate (Doumith *et al*., 2004).

Antibiotic resistance via molecular identification

All the isolates of *L. monocytogenes* either exhibiting susceptibility or resistance against antibiotics were molecularly screened for three different genes responsible for resistance. The details are given in Table 2. A combination that reacts to make 25 µL, 12.5 µL of Dream Taq Green PCR master mix, 8.5 µL of nuclease-free water, 1 µL of forward primer, 1 µL of reverse primer, and 2 µL of extracted genomic DNA were mixed. Positive and negative controls were also used for test validity. The positive controls (DNA) were acquired from the reference laboratory whereas negative controls had no template DNA. Thermal cycler "T100" (BioRad, USA) was used to carry out the PCR reaction. The conditions of the thermocycler were according to the literature, for *tet (K)* (Duran *et al*., 2012), *msr (A)* (Schlegelova *et al*., 2008), and *van A* (AbdulRazzaq *et al*., 2022) were consulted. The PCR product was resolved on a 1% agarose gel containing ethidium bromide at 90 V for 40 min and visualization was performed by Bio-Rad in a Gel Documentation System.

Results

Isolation of *Listeria monocytogenes*

Out of 287 samples, 44 were biochemically positive for *L. monocytogenes*. Molecular identification confirmed 37 isolates as *L. monocytogenes* out of 44 (84.09%). These 37 isolates were positive for both *prfa* and *hly* genes. The distribution of *L. monocytogenes* in meat samples was 12.89% based on molecular detection. Figure 1 presents *L. monocytogenes* prevalence in different types of meat samples. The pathogen is most prevalent in fresh fish meat (19%) followed by frozen and processed meat (15.7%), and fresh chicken meat (9.5%).

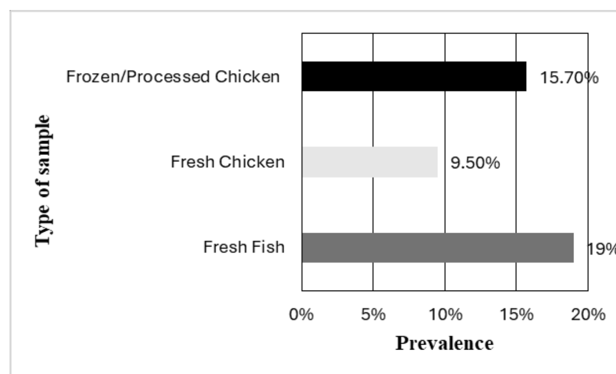

Fig. 1: *Listeria monocytogenes* prevalence in different kinds of meat

Phylogenetic analysis

The accession numbers of isolates of *L. monocytogenes* from chicken meat submitted sequences are 2CHN-RWP-NL-F (accession number; ON859912), 10-CHN-RWP-NL-F (accession number; ON933793), 32-CHN-RWP-NL-F (accession number; ON933794), and 35-CHN-RWP-NL-F (accession number; ON933795). The accession numbers of *L. monocytogenes* isolated from fish meat are 5-FISH-RWP-NL-F (accession number; ON933790), 9-FISH-RWP-NL-F (accession number; ON933791), 17-FISH-LHR-NL-F (accession number; ON933792). The release date is December 30, 2022 (Fig. 2).

Antibiotic susceptibility profile

L. monocytogenes antibiotic resistance profile was determined by disk diffusion assay. Antibiotic zones were grouped as sensitive (S), intermediate (I), and resistant (R) according to CLSI (2017) and EUCAST (European Committee on Antimicrobial Susceptibility Testing 2017). The antibiotic susceptibility profile of *L. monocytogenes* revealed that the highest resistance was found against Vancomycin (43.2%) followed by 37.8% for Gentamicin, 18.9% for Erythromycin and 10.8% for

Trimethoprim and Tetracycline. All isolates were found sensitive to chloramphenicol (Table 3).

Table 3: Antibiotic susceptibility profile of *L. monocytogenes* isolates

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	35 (94.59%)	0 (0%)	2 (5.4%)
Vancomycin	21 (56.7%)	0 (0%)	16 (43.24%)
Gentamicin	18 (48.64%)	5 (13.5%)	14 (37.83%)
Tetracycline	30 (83.78%)	2 (5.4%)	5 (10.81%)
Erythromycin	10 (27.02%)	20 (54.05%)	7 (18.91%)
Chloramphenicol	31 (83.7%)	6 (16.21%)	0 (0%)
Trimethoprim	33 (89.1%)	0 (0%)	4 (10.81%)

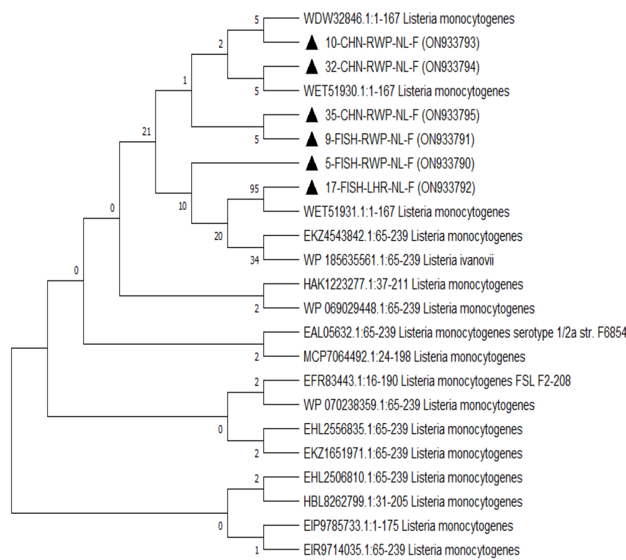

Fig. 2: Phylogenetic tree of the peptide chain release factor protein of *L. monocytogenes* isolates. The isolates are shown as black-cloured arrowheads

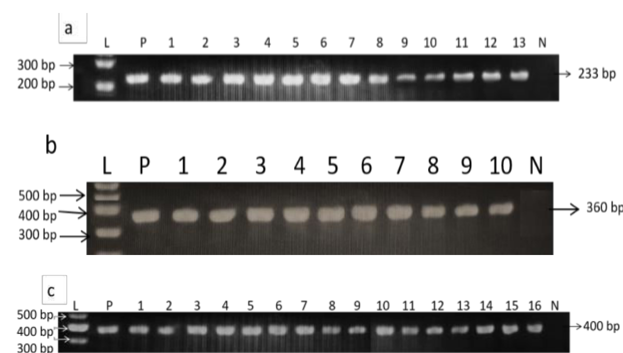

Fig. 3: Results of molecular screening of the isolates for antibiotic resistance genes on agarose gel under UV. (**a**) *Van A* gene- shows the presence of L (100 bp ladder), P (positive control), 233 bp band for *L. monocytogenes* isolates, and N (negative control), (**b**) *tet(M)* gene, represents L, P, and 360 bp band size in bacterial isolates with N, and (**c**) *msr (A)* gene, shows L, P, and bands of 400 bp in bacterial isolates

Antibiotic resistance via molecular identification

The isolates exhibiting resistance against Vancomycin, Tetracycline, and Erythromycin were confirmed via molecular characterization. Of all the bacterial isolates, 13 isolates were found to be positive for the *VanA* gene, and 10 isolates were positive for the *tet(M)* gene. Similarly, of all the *L. monocytogenes* isolates 16 were found to be positive for the *msr(A)* gene (Figs. 3a-c).

Discussion

L. monocytogenes is a food-borne pathogen of public health significance due to its great fatality rate. Several outbreak investigations suggest its transmission via food contact with contaminated surfaces, packing processes, and unhygienic processing units. To our knowledge, this is the first report on the isolation and antibiotic susceptibility analysis of *L. monocytogenes* from three different kinds of meat samples taken from retail markets in Lahore and Rawalpindi. Fresh fish and chicken samples were collected from various markets of both cities, whereas frozen meat was purchased from superstores of Lahore.

In Pakistan, *L. monocytogenes* is found to be prevalent in poultry (Garcia *et al.*, 2003), milk and dairy products (Chandio *et al.*, 2007), and raw vegetables (Samad *et al.*, 2020). In this study, this pathogen was isolated from 12.8% of the samples. These findings are closely related to a study that demonstrated a 12.3% prevalence from food samples in Ireland (O'Grady *et al.*, 2009), another study reported a 13.7% prevalence of the pathogen in raw meat from retail markets of Belgium (Uyttendaele *et al.*, 2009). A study in Bangkok reported 15.6% *L. monocytogenes* prevalence in raw meat products which is very close to our findings (Indrawattana *et al.*, 2011). All these studies signify our findings on the prevalence of *L. monocytogenes* in meat.

Multiple studies have reported the high prevalence of *L. monocytogenes* in fish and its products as compared to other food items (Skowron *et al.*, 2019; Zakrzewski *et al.*, 2023). In our study, 19% of the pathogenic isolates were also from fresh fish meat samples. Based on data from the European Food Safety Authority (EFSA) in 2016, fish and fishery products were the most commonly associated sources of *L. monocytogenes* infections (Authority, 2017) which signifies the role played by seafood in the transmission of this food-borne pathogen. The reason could be that *Listeria* spp. is part of the indigenous microflora in surface water, which can contaminate the fish swimming in that water (Jami *et al.*, 2014).

The rising popularity of lightly preserved and ready-to-eat (RTE) food products has led to an elevated occurrence of *L. monocytogenes*, posing a notable public health risk (Jami *et al.*, 2014). In our study findings, 15.7% of the bacterial isolates were from frozen and processed meat samples, which is alarming. Its prevalence in frozen meat samples was reported to be 17.5% by a study conducted in Faisalabad which is very close to our results (Mahmood *et al.*, 2003). Another study has also reported the presence of said pathogen (26%) in frozen cooked shrimp, cooked crabmeat, and raw seafood (Kataoka *et al.*, 2017). The entry of *L.*

monocytogenes into the food production chain is mainly facilitated through cross-contamination in manufacturing plants. Also, the high prevalence in frozen meat is associated with the nature of the bacterium to grow and survive at lower temperatures for a long time (Jami *et al*., 2014). The food industry is alarmed by *L. monocytogenes'* capacity to acclimate to refrigerated conditions and its capability to create biofilms (Hoffmann *et al*., 2015).

Poultry is considered a cheap source of proteins owing to which it is the most consumed form of meat around the globe, which also makes poultry a vital element in the transmission of several food-borne pathogens. In this study, 9.5% of the *L. monocytogenes* isolates were from fresh chicken meat samples. The reduced presence of the pathogen in fresh chicken meat can be ascribed to the practice in Pakistan where poultry meat is not refrigerated after slaughter. At room temperature, the competitive flora on the meat inhibits significant growth of the bacterium (Oliveira *et al*., 2018). Numerous other studies have also documented the presence of *L. monocytogenes* in poultry meat, showing varying levels of prevalence (Mahmood *et al*., 2003; Kalorey *et al*., 2005; Indrawattana *et al*., 2011). Variations in the prevalence of the bacterium across different countries may stem from distinct slaughtering practices, insufficient training of handlers, the absence of modern techniques, and the adoption of unhygienic measures in those regions (Sohaib and Jamil, 2017).

The isolation of *L. monocytogenes* from fresh meat samples suggests inadequate hygienic conditions, lack of training, and potential cross-contamination at retail shops. It is rendered inactive through processes like pasteurization and thorough cooking thus preventing the spread of pathogens through raw meat. However, this bacterium is frequently detected in food processing plants, where it can establish residency within the facilities and develop biofilms on surfaces used in the processing and ends up in frozen/RTE food (Matle *et al*., 2020). Hence, it is imperative to provide training for meat handlers to ensure the maintenance of hygienic conditions in processing units and retail shops (Hoffmann *et al*., 2015).

The sole recourse for treating listeriosis is antibiotics, yet the antimicrobial resistance exhibited by *L. monocytogenes* is recognized as a significant health concern, posing challenges for the management of outbreaks and human illnesses. The resistance of *L. monocytogenes* to various antibiotics, coupled with limited available data in Pakistan, presents a challenging situation for its control. Results of this study show that 43%, 37%, 18%, and 10% of isolates were resistant to vancomycin, gentamicin, erythromycin, tetracycline, and trimethoprim, respectively. In contrast to current data, a Zambian study of 2022 documented the highest resistance percentages to Tetracycline (30%), Clindamycin (61%), and Erythromycin (13%) (Mpundu *et al*., 2022). Disparities in the antibiotic susceptibility patterns among *L. monocytogenes* isolates from various countries could be attributed to factors such as poultry and livestock management, agricultural practices (Yan *et al*., 2010), inappropriate or excessive antibiotic usage, and variations in strains influenced by geographical factors (Abdollahzadeh *et al*., 2016).

L. monocytogenes antibiotic resistance is acquired and transferred by plasmids and transposons (Osaili *et al*., 2011). Exposure to pH, cold, and salt stress could enhance this resistance properties. The capacity of *Listeria* spp. to form biofilms, express efflux pump and carry mobile genetic elements are among the primary known factors contributing to antibiotic resistance (Olaimat *et al*., 2018). To provide additional confirmation, molecular characterization of three antibiotic resistance genes was also conducted. Tetracycline resistance is conferred by the *tet(M)* gene, which is carried by mobile genetic elements. This gene was identified in ten bacterial isolates. Literature has also reported the presence of *tet(M)* in *L. monocytogenes* isolates (Ji *et al*., 2023). *vanA* gene is responsible for vancomycin resistance and is generally located on a plasmid (Al-Brefkani, 2023). Results of this study show that 13 bacterial isolates were positive for this gene. The presence of *vanA* in *L. monocytogenes* is also reported by other studies (Fugaban *et al*., 2021; Al-Brefkani, 2023). Another gene, *msr(A)* which is responsible for providing resistance against erythromycin was also screened. Results showed that 16 of the isolates were positive for this gene. Studies have also reported the presence of the *msr(A)* gene (Swetha *et al*., 2021). This antibiotic resistance pattern is concerning, and the resistance against multiple classes of antibiotics indicates multi-drug resistance (MDR), making it even more perilous. Addressing antibiotic resistance in food requires coordinated efforts from government bodies, regulatory agencies, the food industry, healthcare professionals, farmers, consumers, and international organizations to ensure responsible antibiotic use and promote public awareness (Bøtner *et al*., 2010).

In the current study, the antibiotic susceptibility profile of the bacterial isolates revealed that most isolates were sensitive to different antibiotics; Ampicillin (94.5%), Tetracycline (83.7%), Chloramphenicol (83.7%) and Trimethoprim (89.1%). This sensitivity pattern of Ampicillin, Tetracycline, Chloramphenicol, and Trimethoprim follows the studies of Du *et al*. (2017) and Osaili *et al*. (2011) who demonstrated a sensitivity of 81%, 88.2%, 100%, and 100%, respectively. Our findings show Ampicillin and Tetracycline to be most effective against controlling this pathogen which is also supported by others (Swaminathan and Gerner-Smidt, 2007; Shamloo *et al*., 2019).

In conclusion, the findings underscore the importance of conducting broader-scale screening to gain a nuanced understanding of *L. monocytogenes* prevalence in Pakistan's meat market. These insights not only enhance our comprehension of the current scenario but also provide a foundation for developing targeted strategies to mitigate the risks associated with *L. monocytogenes* and antibiotic resistance in the food supply chain. Furthermore, the genotyping of isolates and molecular

characterization of other resistance-related genes are crucial steps for accurately assessing the potential threat posed by antibiotic resistance. Future research and surveillance efforts should continue to explore innovative approaches for effective control and prevention.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

Abdollahzadeh, E; Ojagh, SM; Hosseini, H; Irajian, G and Ghaemi, EA (2016). Prevalence and molecular characterization of *Listeria* spp. and *Listeria monocytogenes* isolated from fish, shrimp, and cooked ready-to-eat (RTE) aquatic products in Iran. LWT. 73: 205-211.

AbdulRazzaq, A; Shami, AM and Ghaima, KK (2022). Detection of *vanA* and *vanB* genes among vancomycin resistant *Staphylococcus aureus* isolated from clinical samples in Baghdad hospitals. Iraqi J. Biotechnol., 21: 3-4.

Al-Brefkani, AMT (2023). Study the antimicrobial resistance genes of *Listeria monocytogenes* isolated from industrial and clinical samples in Iraq. Iraqi J. Sci., 64: 594-604.

Authority, EFS (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J., 15: 12-19.

Bøtner, A; Brown, I; Capua, I; Doherr, MG; Domingo, M; Koch, G; Koenen, F; Mumford, E; Pensaert, M and Sharp, M (2010). EFSA panel animal health and welfare (AHAW); Scientific opinion on the pandemic (H1N1) 2009 influenza and its potential implications for animal health: EFSA-Q-2009-00935.

Chandio, TH; Soomro, AH; Bhutto, MB; Dewani, P and Shah, G (2007). Occurrence of *Listeria monocytogenes* in bovine milk in Hyderabad, Pakistan. Ann. Microbiol., 57: 341-344.

Clinical and Laboratory Standards Institute (CLSI) (2017) Performance Standards for Antimicrobial Susceptibility Testing. 27th Edition, Clinical and Laboratory Standards Institute, Wayne. Performance Standards for Antimicrobial Susceptibility Testing., 11(27th Edition). https://www.nih.org.pk/wp-content/uploads/02/CLSI-2017.pdf

Doumith, M; Buchrieser, C; Glaser, P; Jacquet, C and Martin, P (2004). Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J. Clin. Microbiol., 42: 3819-3822.

Du, X; Zhang, X; Wang, X; Su, Y; Li, P and Wang, S (2017). Isolation and characterization of *Listeria monocytogenes* in Chinese food obtained from the central area of China. Food Control. 74: 9-16.

Duran, N; Ozer, B; Duran, GG; Onlen, Y and Demir, C (2012). Antibiotic resistance genes and susceptibility patterns in *Staphylococci*. Indian J. Med. Res., 135: 389-420.

Elsayed, MM; Elkenany, RM; Zakaria, AI and Badawy, BM (2022). Epidemiological study on *Listeria monocytogenes* in Egyptian dairy cattle farms' insights into genetic diversity of multi-antibiotic-resistant strains by ERIC-PCR. Environ. Sci. Pollut. Res. Int., 29: 54359-54377.

Fugaban, JII; Holzapfel, WH and Todorov, SD (2021). Probiotic potential and safety assessment of bacteriocinogenic *Enterococcus faecium* strains with antibacterial activity against *Listeria* and vancomycin-resistant *Enterococci*. Curr. Res. Microb. Sci., 2: 100070-1000101.

Garcia, O; Mahmood, K and Hemme, T (2003). A review of milk production in Pakistan with particular emphasis on small-scale producers, PPLPI Working Papers 23782, Food and Agriculture Organization of the United Nations, Pro-Poor livestock policy initiative.

Goh, SG; Kuan, CH; Loo, YY; Chang, WS; Lye, YL; Soopna, P; Tang, JYH; Nakaguchi, Y; Nishibuchi, M and Afsah-Hejri, L (2012). *Listeria monocytogenes* in retailed raw chicken meat in Malaysia. Poult. Sci., 91: 2686-2690.

Hoffmann, SA; Maculloch, B and Batz, M (2015). *Economic burden of major foodborne illnesses acquired in the United States*. United States Department of Agriculture, Economic Research Service.

Indrawattana, N; Nibaddhasobon, T; Sookrung, N; Chongsa-Nguan, M; Tungtrongchitr, A; Makino, S; Tungyong, W and Chaicumpa, W (2011). Prevalence of *Listeria monocytogenes* in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods. J. Health Popul. Nutr., 29: 26-29.

Jami, M; Ghanbari, M; Zunabovic, M; Domig, KJ and Kneifel, W (2014). *Listeria monocytogenes* in aquatic food products—a review. 13: 798-813.

Ji, S; Song, Z; Luo, L; Wang, Y; Li, L; Mao, P; Ye, C and Wang, Y (2023). Whole-genome sequencing reveals genomic characterization of *Listeria monocytogenes* from food in China. Front. Microbiol., 13: 1049843-1049854.

Jung, KS; Heu, SG; Roh, EJ; Kim, MH; Gil, HJ; Choi, NY; Lee, DH; Lim, JA; Ryu, JG and Kim, KH (2013). Survival of *Salmonella enterica* and *Listeria monocytogenes* in chicken and pig manure compost. Korean J. Soil Sci. Fert., 46: 469-473.

Kalorey, DR; Barbuddhe, SB; Kurkure, NV and Gunjal, PS (2005). Prevalence of *Listeria monocytogenes* in poultry meat in Vidharba region of India. XVIIth European Symposium on the Quality of Poultry Meat. Doorwerth. The Netherlands.

Kataoka, A; Wang, H; Elliott, PH; Whiting, RC and Hayman, MM (2017). Growth of *Listeria monocytogenes* in thawed frozen foods. J. Food Prot., 80: 447-453.

Kayode, AJ and Okoh, AI (2022). Assessment of multidrug-resistant *Listeria monocytogenes* in milk and milk product and one health perspective. PLoS One. 17: e0270993-0271009.

Mahmood, MS; Ahmed, AN and Hussain, I (2003). Prevalence of *Listeria monocytogenes* in poultry meat, poultry meat products and other related inanimates at Faisalabad. Pak. J. Nutr., 2: 346-349.

Matle, I; Mbatha, KR and Madoroba, E (2020). A review of *Listeria monocytogenes* from meat and meat products: Epidemiology, virulence factors, antimicrobial resistance and diagnosis. Onderstepoort J. Vet. Res., 87: 1-20.

Meghdadi, H; Khosravi, AD; Sheikh, AF; Alami, A and Nassirabady, N (2019). Isolation and characterization of *Listeria monocytogenes* from environmental and clinical

sources by culture and PCR-RFLP methods. Iran J. Microbiol., 11: 7-17.

Moreno, LZ; Paixao, R; Sena de Gobbi, DD; Raimundo, DC; Porfida Ferreira, TS; Micke Moreno, A; Hofer, E; dos Reis, CMF; Matté, GR and Matte, MH (2014). Phenotypic and genotypic characterization of atypical *Listeria monocytogenes* and *Listeria innocua* isolated from swine slaughterhouses and meat markets. Biomed Res. Int., 2014: 129-148.

Mpundu, P; Muma, JB; Mukubesa, AN; Kainga, H; Mudenda, S; Bumbangi, FN; Muleya, W; Katemangwe, P and Munyeme, M (2022). Antibiotic resistance patterns of *Listeria* species isolated from broiler abattoirs in Lusaka, Zambia. Antibiotics. 11: 591-610.

O'Grady, J; Ruttledge, M; Sedano-Balbas, S; Smith, TJ; Barry, T and Maher, M (2009). Rapid detection of *Listeria monocytogenes* in food using culture enrichment combined with real-time PCR. Food Microbiol., 26: 4-7.

Olaimat, AN; Al-Holy, MA; Shahbaz, HM; Al-Nabulsi, AA; Abu Ghoush, MH; Osaili, TM; Ayyash, MM and Holley, RA (2018). Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: a comprehensive review. Compr. Rev. Food Sci. Food Saf., 17: 1277-1292.

Oliveira, TS; Varjao, LM; da Silva, LNN; de C. L. Pereira, R; Hofer, E; Vallim, DC and de Castro Almeida, RC (2018). *Listeria monocytogenes* at chicken slaughterhouse: Occurrence, genetic relationship among isolates and evaluation of antimicrobial susceptibility. Food Control. 88: 131-138.

Osaili, TM; Alaboudi, AR and Nesiar, EA (2011). Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. Food Control. 22: 586-590.

Samad, A; Asmat, R; Naeem, M; Ali, H; Mustafa, MZ; Abbas, F; Raza, J and Asmat, MT (2020). Isolation and identification of *Listeria monocytogenes* from raw vegetables and meat sold in Quetta, Pakistan. Pak. J. Zool., 52: 817-820.

Schlegelova, J; Vlkova, H; Babak, V; Holasova, M; Jaglic, Z; Stosova, T and Sauer, P (2008). Resistance to erythromycin of *Staphylococcus* spp. isolates from the food chain. Vet. Med. (Praha), 53: 307-315.

Shamloo, E; Hosseini, H; Moghadam, ZA; Larsen, MH; Haslberger, A and Alebouyeh, M (2019). Importance of *Listeria monocytogenes* in food safety: A review of its prevalence, detection, and antibiotic resistance. Iran. J. Vet. Res., 20: 241-253.

Skowron, K; Wiktorczyk, N; Grudlewska, K; Wałecka-Zacharska, E; Paluszak, Z; Kruszewski, S and Gospodarek-Komkowska, E (2019). Phenotypic and genotypic evaluation of *Listeria monocytogenes* strains isolated from fish and fish processing plants. Ann. Microbiol., 69: 469-482.

Sohaib, M and Jamil, F (2017). An insight of meat industry in Pakistan with special reference to halal meat: a comprehensive review. Korean J. Food Sci. Anim. Resour., 37: 329-345.

Swaminathan, B and Gerner-Smidt, P (2007). The epidemiology of human listeriosis. Microbes Infect., 9: 1236-1243.

Swetha, CS; Madhava Rao, T; Krishnaiah, N and Vijaya Kumar, A (2012). Detection of *Listeria monocytogenes* in fish samples by PCR assay. Ann. Biol. Res., 3: 1880-1884.

Swetha, CS; Porteen, K; Elango, A; Ronald, BSM; Kumar, TMAS; Milton, AP and Sureshkannan, S (2021). Genetic diversity, virulence and distribution of antimicrobial resistance among *Listeria monocytogenes* isolated from milk, beef, and bovine farm environment. Iran. J. Vet. Res., 22: 1-25.

Uyttendaele, M; Busschaert, P; Valero, A; Geeraerd, AH; Vermeulen, A; Jacxsens, L; Goh, KK; De Loy, A; Van Impe, JF and Devlieghere, F (2009). Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. Int. J. Food Microbiol., 133: 94-104.

Wieczorek, K and Osek, J (2017). Prevalence, genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from fresh and smoked fish in Poland. Food Microbiol., 64: 164-171.

Yan, H; Neogi, SB; Mo, Z; Guan, W; Shen, Z; Zhang, S; Li, L; Yamasaki, S; Shi, L and Zhong, N (2010). Prevalence and characterization of antimicrobial resistance of foodborne *Listeria monocytogenes* isolates in Hebei province of Northern China, 2005-2007. Int. J. Food Microbiol., 144: 310-316.

Zafar, N; Nawaz, Z; Anam, S; Kanwar, R; Ali, A; Mudassar, M; Javid, MT; Zafar, A and Tariq, A (2020). Prevalence, molecular characterization and antibiogram study of *Listeria monocytogenes* isolated from raw milk and milk products. PAB (Pure and Applied Biology). 9: 1982-1987.

Zakrzewski, AJ; Gajewska, J; Chajęcka-Wierzchowska, W; Załuski, D and Zadernowska, A (2023). Prevalence of *Listeria monocytogenes* and other *Listeria* species in fish, fish products and fish processing environment: A systematic review and meta-analysis. Sci. Total Environ., 907: 167912-167920.