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Prevalence, molecular detection and antimicrobial susceptibility of *Listeria monocytogenes* isolated from milk, poultry meat and meat products

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Abstract

Listeria monocytogenes is a food borne zoonotic pathogen that causes listeriosis in people and animals. It has a low incidence but a high case fatality rate. The goal of this study was to assess the prevalence of *L. monocytogene*s in raw and frozen milk, raw and frozen poultry meat and meat products as well as their molecular identification and antimicrobial susceptibility. A total of 100 samples were collected from four different locations in Mymensingh town, including raw milk (n = 20), frozen milk (n = 20), raw poultry meat (n = 20), frozen poultry meat (n = 20), frozen chicken meat ball (n = 10), and chicken burger (n = 10). Listeria selective PALCAM agar was used to isolate *L. monocytogenes* from the samples. *Listeria* spp. created a black zone around the colonies on this agar. Gram staining, motility testing, Christie, Atkins, Munch-Petersen (CAMP) testing, haemolysis testing, sugar fermentation testing and other biochemical assays were used to confirm the presence of *L. monocytogenes*. The disc diffusion method was used to test antibiotic sensitivity against ten antibiotics: ampicillin, vancomycin, gentamycin, streptomycin, cefixime, cefalexin, norfloxacin, colistin, erythromycin, and amikacin. The prevalence of *L. monocytogenes* in various samples was found to be 30% in raw milk, 0% in frozen milk, 40% in raw poultry meat, 10% in frozen poultry meat and not found in meat products. Market 1 had a prevalence of 13.33%, market 2 had a prevalence of 15%, market 3 had a prevalence of 0%, and market 4 had a prevalence of 0%. The isolates of *L. monocytogenes* were shown to be susceptible to amikacin, gentamycin, vancomycin, streptomycin and norfloxacin but resistant to ampicillin, cefixime, cefalexin, colistin and erythromycin. According to the findings of this study, multidrug-resistant *L. monocytogenes* can be found in milk, poultry meat, and meat products, posing a public health risk.

1. Introduction

Listeria monocytogenes is a Gram positive, facultative anaerobic, non-spore-forming microorganism that is responsible for listeriosis in both animals and humans (Ieren *et al.,* 2013). *Listeria monocytogenes* is available in the environment and has been found in a variety of sources like water, sludge, soil, plants, vegetation, foods and dairy products, food processing plants and infected humans and animals (Liu, 2008; Dhama *et al*., 2013; Skowron *et al.,* 2019). This microorganism can survive in the food-processing and produce-packing environment and equipment (Azizoglu *et al*., 2017). *Listeria monocytogenes* is an important hazard in foods because it has the ability to grow or survive at low temperatures, low pH and low water activities (Conter *et al*., 2009). It is considered that the main route of bacterial transmission is the consumption of contaminated food such as meat and meat products, vegetables, raw and ready-to-eat seafood, unpasteurized milk, ice creams and soft cheese (Milian and Cifre, 2014; Kim *et al.,* 2018).

The detection of *L. monocytogenes* in meat is a particular concern in terms of consumer safety as these organisms are capable of growing on both raw and cooked meat at refrigeration temperatures. It is therefore appropriate to detect such pathogenic micro-organisms from milk and meats to prevent food poisoning on consumption of milk and meats and protect public health (Malek *et al.,* 2010). This pathogen has also been isolated from food products like unpasteurized (raw) milk or foods made from unpasteurized milk, red meats,

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poultry, seafood, vegetables, fruits and ready-to-eat food products (Karakolev, 2009; Malek *et al*., 2010).

A variety of ready-to-eat food products, such as frozen or raw vegetables, raw milk and milk products (soft cheese), meat and meat products (chicken burgers and chicken meat balls) and seafood support the growth of *L. monocytogenes. Listeria* causes severe invasive disease and the manifestations include neurological infections like encephalitis, meningitis, septicaemia and abortion with a mortality rate of up to 20-30% (Boland *et al.,* 2001). During the early stages of infection, human listeriosis often displays non-specific flu like symptoms (e.g. chills, fatigue, headache, and muscular and joint pain) and gastroenteritis (Boland *et al*., 2001).

Antimicrobial resistance of bacteria has become a serious problem worldwide. While much of the resistance observed in human medicine is attributed to the inappropriate use of antibiotics in humans, mass medication of animals with a reserve group of antibiotics for humans and the long-term use of antimicrobials in feed for growth promotion (Collignon and McEwen, 2019), there is increasing evidence that antimicrobial use in animals selects for resistant foodborne pathogens that may be transmitted to humans as food contaminants (Liu *et al.,* 2007; Collignon *et al.,* 2009).

Antimicrobial resistance is currently the greatest challenge worldwide. It decreases the effectiveness of drugs that decrease morbidity and mortality associated with serious and life-threatening infections. Thus, compromising human health (Collignon *et al.,* 2009). Multidrug resistance among *L. monocytogenes* isolated from food or the environment has also been described, which imposes an additional risk to public health (Conter *et al*., 2009; Jamali *et al.,* 2013; Gomez *et al.,* 2014). The first multidrug-resistant strain of *L. monocytogenes* was isolated in France in 1988 (Salmeron *et al.,* 1990) and since then multidrug resistant *L. monocytogenes* strains have been recovered from food, the environment and sporadic cases of human listeriosis (Charpentier *et al*., 1995). Some strains of *L. monocytogenes* are resistant to antibiotics such as tetracycline, gentamycin, penicillin, ampicillin, streptomycin, erythromycin, kanamycin, sulfonamide, trimethoprim, and rifampicin (Charpentier and Courvalin, 1999; Jamali *et al.,* 2015).

Our previous study reported the prevalence of *L. monocytogenes* in beef, chevon and chicken in Bangladesh based on morphological and biochemical identification (Islam *et al.*, 2016). Information on the prevalence, molecular detection and antimicrobial susceptibility of *L. monocytogens* in raw and frozen milk, raw and frozen poultry meat and meat products is very limited both in the veterinary and public health sectors. Therefore, this study aimed to determine the prevalence of *L. monocytogenes* in raw and frozen milk, poultry meat and meat products, isolate and identify *L. monocytogenes* from raw and frozen milk, poultry meat and meat products by PCR, based on *prs* and *hlyA* gene sequence and to know the antimicrobial susceptibility of isolated *L. monocytogenes* against commonly used antibiotics

2. Materials and methods

2.1 Collection of samples

A total of 100 samples such as raw milk samples (n = 20), frozen milk samples (n = 20), raw poultry meat samples (n = 20), frozen poultry meat samples (n = 20), frozen chicken meat balls (n = 10) and chicken burgers (n = 10) were collected from different markets and shops located at Mymensingh Sadar. Each of the samples was collected aseptically using sterile instruments and transferred carefully to appropriate containers and levelled with an identification mark. The samples were carefully handled, kept in an ice box and immediately transported to the Bacteriology laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for enrichment.

2.2 Processing of milk, poultry meat and meat products

The processing and inoculation of the samples were conducted as described by previous studies with some modifications (Gebretsadik *et al.,* 2011). Approximately 25 mL of cow raw milk was weighed out and mixed thoroughly with 225 mL Listeria Enrichment Broth (Oxoid Ltd., Hampshire, UK) by shaking to ensure the homogeneity of its contents and the sample was incubated at 30°C for 48 hrs in an incubator. Before inoculation, the Listeria Enrichment Broth was mixed with the Listeria Selective Enrichment Supplement (Oxoid Ltd., Hampshire, UK) according to the instructions provided by the manufacturer. The role of this supplement was to restrict the growth of other organisms apart from *Listeria* spp. Approximately 25 g of each poultry meat sample and meat product samples (chicken burgers and chicken meat balls) were collected aseptically, ground using the mortar and pestle in 225 mL of 0.1% peptone water and incubated at 37˚C for 24-48 hrs. Enriched samples were streaked onto *Listeria* selective PALCAM agar and incubated at 37˚C aerobically for 24-48 hrs. The plates were examined for typical *Listeria* colonies (black colonies with black sunken centers). Presumptive *L. monocytogenes* colonies grown on *Listeria* selective agar were further sub-cultured until pure culture was obtained.

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2.3 Isolation of bacterial isolates

From the Listeria Enrichment Broth, a loop-full of the culture was streaked onto Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) agar (Oxoid Ltd., Hampshire, UK) plates and incubated at 37°C for 24-48 hrs followed by the observation for bacterial growth. Typical *Listeria* colonies surfaced as black or black-green, having a black halo and sunken centre with a growth diameter of 1-2 mm (Scotter *et al*., 2001; Molla *et al.*, 2004). The colonies on primary culture were repeatedly sub-cultured until the pure culture was obtained.

2.4 Identification of bacteria

The identification of bacteria was performed by colony characteristics such as colour, shape and size, Gram's staining, catalase test, biochemical tests such as sugar fermentation test, indole formation, Methyl-Red and Voges-Proskauer (MR-VP) test, D-Xylose test, haemolytic activity and CAMP(Christie-Atkins-Munch-Peterson) test following standard methods (USFDA/ CFSAN, Bacteriological Analytical Manual (USFDA/ CFSAN, 2003). The motility test was performed according to the method described by Cowan (1985) to differentiate motile bacteria from the non-motile ones. For all the biochemical tests, *L. monocytogenes* ATCC 19117 was used as reference strain (positive control) in order to compare with the results of the isolates under study.

2.4.1 Gram staining

Gram staining method was used to study the morphological and staining characteristics of bacteria isolated from milk, poultry meat and meat products. Gram staining was performed according to the method described by USFDA/CFSAN (2003). Briefly, a thin smear was prepared from a single colony of *L. monocytogenes* grown on *Listeria* selective PALCAM agar. The smear was stained by Gram's staining reagent and then examined under a microscope with a high power objective (400x) using immersion oil.

2.4.2 Biochemical characterization

For the carbohydrate utilization test, isolated colonies were transferred into test tubes containing different sugar media (five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol), xylose, indole, methyl red, Voges-Proskauer and incubated at 37°C for up to 7 days. Acid production was indicated by the color change from pink to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Cheesbrough, 1984).

2.5 Molecular characterization of bacteria by polymerase chain reaction

2.5.1 DNA extraction

DNA was extracted from bacteria using Wizard® Genomic DNA purification kit, (Promega, USA). The extraction procedure was performed according to the protocol provided by the kit.

2.5.2 Amplification of prs and hlyA genes by PCR

In this study, specific primers were used to detect *prs* gene for genus identification, and *hlyA* gene for *L. monocytogenes*. For *Listeria* spp. and *L. monocytogenes*, primer sequences used in the study were 5′-GCTGAAGAGATTGCGAAAGAAG-3′, 5′ CAAAGAAACCTTGGATTTGCGG-3′ (Doijad *et al*., 2011) and 5′-GAATGTAAACTTCGGCGCAATCAG-3′, 5′-GCCGTC GATGATTTGAACTTCATC-3′ (Bohnert *et al*., 1992) respectively. The PCR amplification was carried out in a 25 µL reaction mixture that consisted of 5 µL of 5x PCR buffer, dNTPs (0.5 µL), $MgCl_2$ (2 µL), *Taq* DNA polymerase (0.5 µL), 0.5 µL of each 20 pM primer, 13 µL of distilled water, and 2 µL of DNA template.

For the *prs* gene, amplification phases carried out were initial denaturation at 98°C for 60 s, and then 30 cycles of denaturation at 98°C for 30 s, annealing at 58° C for 30 s, extension at 72°C for 30 s, and then the final extension at 72°C for 7 mins. For the *hlyA* gene, amplification phases carried out were initial denaturation at 98°C for 60 s, then 30 cycles of denaturation at 98°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 60 s, and then the final extension at 72°C for 7 min.

Following PCR amplification, about 5 µL of the PCR product was mixed with DNA loading dye (6×) and electrophoresed in 1.5% Agarose gel in TAE buffer using a mini tank at 80 V for 30 min and visualized under UV transillumination. The electrophoresed product on the gel was stained with ethidium bromide for 30 mins, destained for 20 mins, and visualized under UV illuminator (SYNGENE, Biosystems, UK). A 100 bp DNA ladder (Promega Corporation, USA) was included to estimate the size of the amplified products.

2.6 Antibiotic sensitivity test

Antimicrobial drug susceptibility against ten commonly used antibiotics was performed by disc diffusion or Kirby-Bauer method (Bauer *et al.*, 1966). Briefly, antibiotic sensitivity was tested using 0.5 McFarland turbidity standard inoculum and freshly prepared dried Mueller Hinton agar (Oxoid, UK) against 10 common antibiotics: ampicillin (25 µg), vancomycin (30 µg), gentamycin (10 µg), streptomycin (10 µg),

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cefixime (5 µg), cefalexin (30 µg), norfloxacin (10 µg), colistin (10 µg), erythromycin (15 µg) and amikacin (30 µg) (Oxoid, UK). Antimicrobial testing results were recorded as resistant, intermediate and sensitive according to zone diameter interpretive standards provided by Clinical and Laboratory Standards Institute (CLSI) (2018).

3. Results

3.1 Isolation of Listeria monocytogenes from milk, poultry meat and meat products

The cultural characteristics of bacteria isolated from milk, poultry meat and meat products (chicken burgers and chicken meat balls) were observed for the determination of size, shape, colony characteristics, pigment and enzyme production in various solid media after 48 hrs of inoculation. *Listeria* spp. produced a black zone around the colonies due to the formation of black iron phenolic compounds derived from the aglucon which indicates the growth of *L. monocytogenes* (Figure 1).

Figure 1. Culture positive sample of the genus *Listeria* produced black zone on the PALCAM agar.

3.2 Identification of bacteria

3.2.1 Gram staining

Microscopic examination (100×) found Gram-positive, coccobacillary bacteria, arranged in clumps, short chains or single organisms (Figure 2).

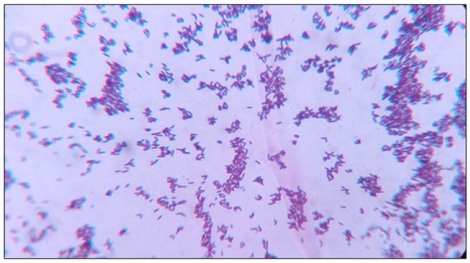

Figure 2. Gram staining of *Listeria monocytogenes*

3.2.2 Haemolysis test

Listeria monocytogenes produces a narrow zone of α -haemolysis on blood agar. All the *Listeria* isolates were tested for α or β haemolysis on 5% sheep blood agar. A narrow zone of α-haemolysis was seen on blood agar which is the characteristic of *L. monocytogenes* (Figure 3).

Figure 3. Results of haemolysis test.

3.2.3 Christie, Atkins, Munch-Petersen test

Synergistic haemolysis found near the junction of *S. aureus* and *Listeria* spp. It is a typical aero-shaped or extended area of haemolysis which indicates the organism is *L. monocytogenes* (Figure 4).

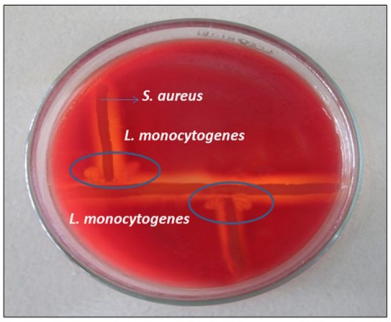

Figure 4. Results of Christie, Atkins, Munch-Petersen (CAMP) test.

3.2.4 Biochemical characterization

Listeria monocytogenes fermented dextrose (DX) and maltose (ML) and only acid was produced. No gas was observed in Durham's tube. All *L. monocytogenes* were Catalase and MR-VP positive but Oxidase, Indole and D-xylose negative. Table 1 presents the biochemical differentiation and characteristics of *L. monocytogenes.*

3.2.5 Molecular characterization of bacteria by polymerase chain reaction

DNA extracted from *Listeria* spp. was used in the PCR assay. PCR primers targeting the *prs* gene of *Listeria* spp. amplified 370 bp fragments of DNA confirmed the identity of *Listeria* spp. (Figure 5). On the other hand, PCR primers targeting the *hlyA* gene of *Listeria* spp. amplified 388 bp fragments of DNA confirmed the identity of *L. monocytogenes* (Figure 6).

3.3 Prevalence of Listeria monocytogenes

Sixteen samples out of 100 samples were found

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Table 1. Identification test results of the *Listeria monocytogenes* isolates.

Sample Type	Sample ID	Biochemical Test							
		Gram stain	Catalase	Oxidase	Motility	Indole	D-Xylose	Methyl red	Voges Proskauer
Milk	M2	+	+	-	+	-	-	+	+
	M4	+	+	-	+	-	-	+	+
	M5	+	+	-	+	-	-	+	+
	M7	+	+	-	+	-	-	+	+
Poultry Meat	PM1	+	+	-	+	-	-	+	+
	PM3	+	+	-	+	-	-	+	+
	PM4	+	+	-	+	-	-	+	+
	PM6	+	+	-	+	-	-	+	+
Chicken meat ball	CMB1	+	+	-	+	-	-	+	+
	CMB3	+	+	-	+	-	-	+	+
Chicken burger	CB2	+	+	-	+	-	-	+	+
	CB4	+	+	-	+	-	-	+	+

M: Milk, PM: Poultry meat, CMB: Chicken meat ball, CB: Chicken burger

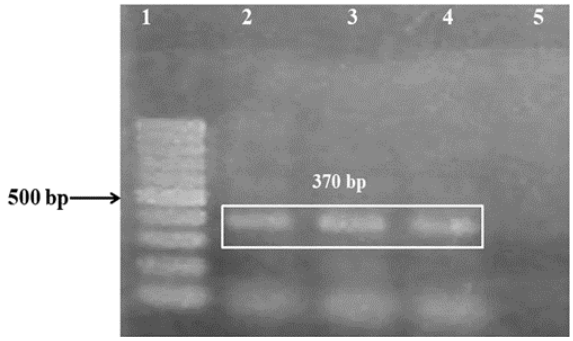

Figure 5. Results of PCR for amplification of *Listeria* spp. by *prs* gene (370 bp). Lane 1: 100 bp DNA marker (Thermo Scientific, USA), Lane 2 and 3: DNA of *Listeria* spp. obtained from milk samples, Lane 4: DNA of *Listeria* spp. obtained from meat samples, Lane 5: Negative control.

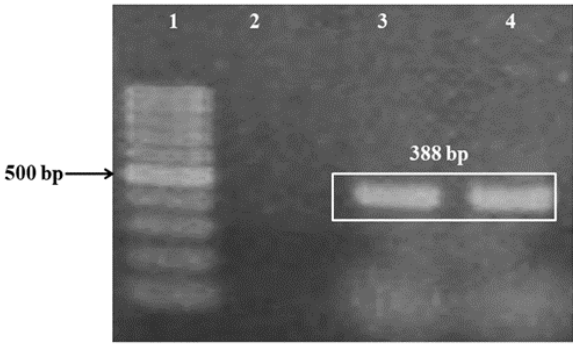

Figure 6. Results of PCR for amplification of *L. monocytogenes* by *hlyA* gene (388 bp). Lane 1: 100 bp DNA marker (Thermo Scientific, USA), Lane 2: Negative control, Lane 3: DNA of *L. monocytogenes* obtained from milk sample, Lane 4: DNA of *L. monocytogenes* obtained from meat sample.

positive for *L. monocytogenes.* Prevalence of *L. monocytogenes* was 30% in raw milk, 0% in frozen milk, 40% in raw poultry meat, 10% in frozen poultry meat and not detected in frozen chicken meat ball and chicken burger (Table 2). Area wise prevalence of *L. monocytogenes* was 13.33% in market 1, 15% in market 2, 0% in market 3 and market 4. The highest percentage of *L. monocytogenes* was recorded in market 2 (15%) and no *L. monocytogenes* was isolated from market 3 and 4 (Figure 7).

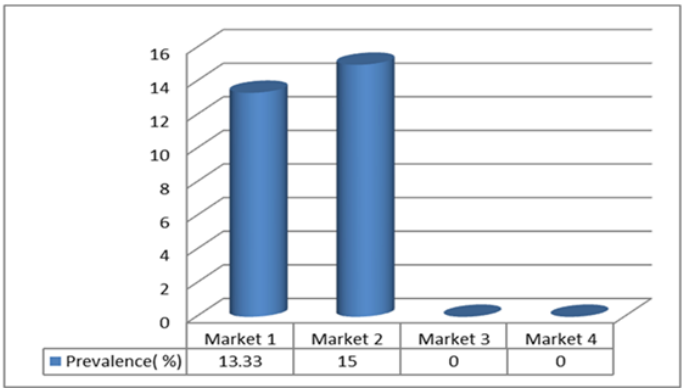

Figure 7. Area wise prevalence of *L. monocytogenes.*

3.4 Results of antibiotic sensitivity test

Listeria monocytogenes isolates were found to be sensitive to amikacin, gentamycin, vancomycin, streptomycin, norfloxacin and resistant to ampicillin, cefixime, cefalexin, colistin, erythromycin on the basis of zone of inhibition. The results of the antibiotic sensitivity test are presented in Table 3.

4. Discussion

Listeriosis has been recognized to be one of the emerging zoonotic diseases during the last two decades and is contracted mainly from the consumption of contaminated foods and food products. Increasing evidence suggests that substantial portions of cases of human listeriosis are attributable to the food borne transmission of *L. monocytogenes* (Uyttendaele *et al.,* 1999). It is generally assumed that milk, poultry meat and meat products cannot be free from *Listeria* spp. because of handling, slaughtering, evisceration and food processing methods that allow a greater chance for contamination. People handling food at different levels can also be sources of contamination. Sporadic cases of

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Table 2. Prevalence of *L. monocytogenes* in milk, poultry meat and meat products

Type of samples		No. of	Total	No. of culture positive and	Prevalence (%) of culture positive
Milk	Raw milk	20	100	6 8	30
	Frozen milk	20		0	0
Poultry meat	Raw poultry meat	20		8	40
	Frozen poultry meat	20		2	10
Meat products	Frozen chicken meat ball	10		0	0
	Chicken burger	10		0	0

Table 3. Summary of antibiogram profile of sixteen *L. monocytogenes* isolates against ten antibiotics.

Antibiotics	Concentration (µg)	Susceptibility of *Listeria* spp. isolates (n = 16)		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin (AMP)	25	16 (100)	0 (0)	0 (0)
Vancomycin (VA)	30	0 (0)	0 (0)	16 (100)
Gentamycin (GEN)	10	0 (0)	0 (0)	16 (100)
Streptomycin (S)	10	0 (0)	16 (100)	0 (0)
Cefixime (CFM)	5	16 (100)	0 (0)	0 (0)
Cefalexin (CN)	30	16 (100)	0 (0)	0 (0)
Norfloxacin (NX)	10	0 (0)	16 (100)	0 (0)
Colistin (CL)	10	12 (75)	0 (0)	4 (25)
Erythromycin (E)	15	16 (100)	0 (0)	0 (0)
Amikacin (AK)	30	0 (0)	0 (0)	16 (100)

listeriosis occur worldwide. The findings of the present study also showed that *L. monocytogenes* are prevalent in different foods in Bangladesh.

Listeriosis is a highly severe and widespread disease caused by *L. monocytogenes.* Ready-to-eat (RTE) food, meat, poultry, vegetables and dairy products act as vehicles for listeriosis transmission (Orsi *et al.,* 2011). *Listeria* spp. is ubiquitous in nature and has been isolated from wide environmental sources (Vitas *et al.,* 2004; Liu, 2008). The organism has the ability to survive in harsh conditions and therefore can persist in the environment. Due to such persistence, *Listeria* spp. can easily enter into the food chain (Carpentier and Cerf, 2011). Common sources of *L. monocytogenes* in raw milk have been reported to be faecal (Husu, 1990) and environmental contamination during milking, storage and transport, infected cows in dairy farms and poor silage quality (Bemrah *et al.,* 1998).

Listeria monocytogenes isolated from milk, poultry meat samples which were identified on the basis of cultural characteristics, Gram's staining, biochemical tests such as sugar fermentation test, motility test, catalase test and haemolytic activity on blood agar and CAMP test in this study. All *L. monocytogenes* showed positive results for the catalase, Voges-Proskauer (VP) and Methyl Red (MR) tests in this study. Morphological, staining and biochemical characteristics of the isolated bacteria were similar to the findings of Islam *et al.* (2016). The finding of the motility property and catalase test of the isolates was similar to the findings of Sarker and Ahmed (2015). The CAMP test is used for the identification of *L. monocytogenes* which gives positive results in CAMP test. Quinn *et al.* (2001) found CAMP test positive in raw milk.

The prevalence of *L. monocytogenes* was 30% in the raw milk sample in this study. In meat samples obtained from cattle, goats and chickens, Islam *et al.* (2016) found 11.11% prevalence of *L. monocytogenes. Listeria monocytogenes* was found in 11.4% of ready-to-eat (RTE) foods, according to Jamali *et al.* (2013). Effimia (2015) who recorded 14.4% prevalence of *L. monocytogenes* in ready-to-eat meals, salads, desserts and ice cream in Greece. In Mexico, Ruelas *et al.* (2013) reported 14.4% prevalence in ready-to-eat meals, salads, desserts and ice cream. Alsheikh *et al.* (2013) reported 13.6% prevalence in ready-to-eat meat products. In raw milk, Sreeja *et al.* (2016) reported 16.6% prevalence. Usman and Mukhtar (2014) observed 2.25% prevalence in raw milk. In ice cream, Rahimi *et al.* (2012) found 16.6% prevalence of *L. monocytogenes.* In desserts, Effimia *et al.* (2015) discovered 13.2% prevalence of *L. monocytogenes*. In milk filters, Pantoja *et al.* (2012) found 66% *L. monocytogenes*. In milk, Lakhanpal *et al.* (2016) reported 2.5% prevalence of *L. monocytogenes*. Al-Mariri *et al.* (2013) reported the prevalence of *Listeria* spp. was 16.2% in raw milk in Syria and the highest prevalence of *Listeria* spp. was found in raw milk which is consistent with this study. *Listeria monocytogenes* was detected in 6% raw milk samples

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(Abdeen *et al.,* 2021).

The prevalence of *L. monocytogenes* was 40% in raw poultry meat samples. Indrawattana *et al*. (2011) reported 15.4% prevalence of *L. monocytogenes* in meat. According to Islam *et al.* (2016), *L. monocytogenes* was found 8.33% in chicken, 16.66% in beef, and 8.33% in chevon. *Listeria monocytogenes* was found in 13.2% of chicken meat and chicken meat products, according to Jamali *et al.* (2013). In broiler wing meat samples, Elmali *et al.* (2015) discovered a high prevalence of *Listeria* spp. (47.5%) and *L. monocytogenes*. In numerous types of ready-to-eat (RTE) beef products, Gomez *et al.* (2015) found 17.14% *L. monocytogenes* in cooked products, 36.84% in raw-cured products and 24.32% in dry-cured, salted products. Ruelas *et al.* (2013) reported 23.3% *L. monocytogenes* in raw chicken breast and 8.3% in raw ground beef. The organism possesses the ability to survive in harsh conditions and therefore can persist in the environment for a long time. Because of such persistence, *L. monocytogenes* can easily enter in food chain (Carpentier and Cerf, 2011). *Listeria monocytogenes* is pathogenic to humans and animals. Therefore, meat quality controlling authorities from several developed countries have enforced strict regulations over the occurrence of *L. monocytogenes* in meat and meat products (CDC, 2012).

The area-wise prevalence of *L. monocytogenes* was determined to be 13.33% in market 1, 15% in market 2, 0% in market 3 and market 4, according to this study. From above it is recommended that the highest prevalence of *L. monocytogenes* was found in market 2. Meat sold at different markets in Mymensingh is likely to have different degrees of bacterial contamination due to their different sources and standards of meat processing and handling in individual markets (Islam *et al.,* 2016). Jang *et al.* (2021) discovered that *L. monocytogenes* contamination in raw meat could develop in slaughterhouse conditions due to contact with gloves, splitting saws and drains.

Antibiotic resistance to *L. monocytogenes* has emerged over the last few years (Charpentier and Courvalin, 1999). Antibiotic resistance not only interferes with effective treatment measure, the antibiotic resistance gene pool in bacteria also facilitates the horizontal transfer of these genes among different bacterial strain that poses a huge threat to the human being. It was observed that the pathogenic *L. monocytogenes* was resistant against ampicillin and erythromycin and had intermediate susceptibility to ciprofloxacin (Arslan and Ozdemir*,* 2008). Resistance to ampicillin is significant, as it is the first choice of antibiotic in listeriosis treatment for humans (Conter *et al*., 2009).

In this investigation, *L. monocytogenes* was 100% susceptible to amikacin, gentamycin, vancomycin, streptomycin, norfloxacin and resistant to ampicillin, cefixime, cefalexin, colistin and erythromycin. These results were similar to the findings of Al-Nabulsia *et al.* (2014) who discovered that *L. monocytogenes* was sensitive to gentamycin and vancomycin but resistant to erythromycin. *Listeria monocytogenes* was sensitive to gentamycin and vancomycin but resistant to ampicillin, according to Islam *et al.* (2016). *Listeria monocytogenes* was found to be resistant to ampicillin and erythromycin by Sarker and Ahmed (2015). *Listeria monocytogenes* was found to be sensitive to streptomycin but resistant to ampicillin by Issa *et al.* (2011). According to Chin *et al*. (2018), the detection of multidrug-resistant *Listeria* strains from various food sources and locations is noteworthy because these strains could serve as reservoirs for antimicrobial resistance genes, facilitating the spread and development of other drug-resistant strains.

5. Conclusion

This research represents some findings about *L. monocytogenes* found in milk and poultry meat and meat products (chicken burgers and chicken meat balls). Listeriosis can be spread if the milk, poultry meat and meat products are not properly cooked, packaged and preserved. The findings of this study suggested that those who consume raw and unpasteurized milk as well as poultry meat, may be at risk of infection with multidrug-resistant Listeria. There is a need for continuous screening of foods of animal origin, such as milk, chicken meat and meat products, must be continuously screened for listeria contamination, and suitable measures must be taken to reduce such contamination. Preventing infections, reducing over-prescribing and over-use of antimicrobials, and stopping the spread of antimicrobial resistance are all necessary steps in combating antimicrobial resistance.

Conflict of interest

The authors declare no conflict of interest.

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