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

Faruk, M.O., Ema, F.A., Islam, M.A. and *Khatun, M.M.

Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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. monocytogenes 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, cefixime, cefalexin, norfloxacin, colistin, erythromycin, and amikacin. 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,

**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

*Corresponding author.
Email: mmkhatun@bau.edu.bd

DOI: https://doi.org/10.26656/fr.2017.7(5).186
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. monocytogenes* 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.
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-Xylene 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 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’-GCTGAAGAGATTCGAAAGAGG-3’, 5’ CAAAAGAACCTTGGATTGCGG-3’ (Doijad et al., 2011) and 5’-GAATGAACTTTCGGCCGCAATCG-3’, 5’-GCCGTC GATGATTGGAATTCATC-3’ (Bohnet 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), MgCl₂ (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 min. 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 30 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), etc.
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).

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).

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).

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).

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...
positive for \textit{L. monocytogenes}. Prevalence of \textit{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 \textit{L. monocytogenes} was 13.33% in market 1, 15% in market 2, 0% in market 3 and market 4. The highest percentage of \textit{L. monocytogenes} was recorded in market 2 (15%) and no \textit{L. monocytogenes} was isolated from market 3 and

Table 1. Identification test results of the \textit{Listeria monocytogenes} isolates.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sample ID</th>
<th>Gram stain</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Motility</th>
<th>Indole</th>
<th>D-Xylose</th>
<th>Methyl red</th>
<th>Voges Proskauer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>M2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>M5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>M7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>PM1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Poultry Meat</td>
<td>PM3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>PM4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>PM6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chicken meat ball</td>
<td>CMB1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CMB3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chicken burger</td>
<td>CB2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CB4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

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

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

3.4 Results of antibiotic sensitivity test

\textit{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 \textit{L. monocytogenes} (Uyttendaele et al., 1999). It is generally assumed that milk, poultry meat and meat products cannot be free from \textit{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
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, Sreem 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, Lachanpal et al. (2016) reported 2.5% prevalence of L. monocytogenes. Al-Mari 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.

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

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No. of</th>
<th>Total</th>
<th>No. of culture positive and Prevalence (%) of culture positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw milk</td>
<td>20</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Frozen milk</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poultry meat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw poultry meat</td>
<td>20</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Frozen poultry meat</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Meat products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen chicken meat ball</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chicken burger</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration (µg)</th>
<th>Susceptibility of Listeria spp. isolates (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (AMP)</td>
<td>25</td>
<td>Resistant (%)  Intermediate (%) Sensitive (%)</td>
</tr>
<tr>
<td>Vancomycin (VA)</td>
<td>30</td>
<td>0 (0) 0 (0) 16 (100)</td>
</tr>
<tr>
<td>Gentamycin (GEN)</td>
<td>10</td>
<td>0 (0) 0 (0) 16 (100)</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>10</td>
<td>0 (0) 16 (100) 0 (0)</td>
</tr>
<tr>
<td>Cefixime (CFM)</td>
<td>5</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Cefalexin (CN)</td>
<td>30</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Norfloxacin (NX)</td>
<td>10</td>
<td>0 (0) 16 (100) 0 (0)</td>
</tr>
<tr>
<td>Colistin (CL)</td>
<td>10</td>
<td>12 (75) 0 (0) 4 (25)</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>15</td>
<td>16 (100) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Amikacin (AK)</td>
<td>30</td>
<td>0 (0) 0 (0) 16 (100)</td>
</tr>
</tbody>
</table>
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 (Carpentier 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, ceftixime, ceftalexin, 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.

Acknowledgements

The authors would like to convey thanks and gratitude to Stephen M. Boyle (Virginia Tech, United States) for editorial comments. This work was supported by the Bangladesh Agricultural University Research System (BAURES) Project No: 2017/36/BAU.
References


Medical Bacteria. 2nd ed. Cambridge, United Kingdom: Cambridge University Press.


