

Prevalence of *Listeria monocytogenes* in Beef, Chevron and Chicken in Bangladesh

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Abstract

The present research was conducted for the isolation and characterization of the *Listeria monocytogenes* from meat samples collected from different local markets around Mymensingh town such as Boira, Paglar bazar, Kawatkhali bazar, Kamal-Ranjit (K-R) market and Shes More bazar. A total of 36 meat samples were collected from cattle, goat and chicken. After preparation the samples were inoculated into different selective media such as Oxford Agar, Mannitol Salt Agar and Blood Agar for isolation and identification of *L. monocytogenes*. In Oxford Agar, *Listeria* spp. produced black zone around the colonies. Gram staining, motility test, blood hemolysis, Christie Atkins Munch Peterson (CAMP) test and biochemical test were performed to confirm *L. monocytogenes*. The *L. monocytogenes* fermented dextrose and maltose with the production of only acid and no gas was observed in Durham's tube. The *L. monocytogenes* were found Methyl-Red and Voges-Proskauer (MR-VP) test positive but indole negative. *L. monocytogenes* were catalase, CAMP test positive and motile. Among 36 meat samples, 4 (11.11%) were *L. monocytogenes*. Among animal species, the distribution of *L. monocytogenes* was 8.33% (1/12) in chicken, 16.66% (2/12) in beef and 8.33% (1/12) in chevon. The market wise distribution of *L. monocytogenes* was 0% in Boira, Paglar bazar, 11.11% in KR market, 22.22% in Shesmore bazar and 11.11% in Kawatkhali bazar. The *L. monocytogenes* isolate was resistant to ampicillin and penicillin; sensitive to the ciprofloxacin, vancomycin and gentamicin. This is the first report on isolation and identification of *L. Monocytogenes* from beef, chevon and chicken in Bangladesh. The multidrug resistant *L. monocytogenes* may be transmitted to the human through consumption of contaminated meat and may lead to public health hazard.

Keywords

Beef, Chevron, Chicken, Listeria, Meat, Multidrug-Resistant

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1. Introduction

Listeria monocytogenes, a Gram-positive intracellular bacterium, causes foodborne listeriosis [1]. Infections with this bacterium are currently associated with a fatality rate of approximately 17%, which is the highest rate observed among foodborne pathogens [2]. *L. monocytogenes* is ubiquitous in the environment and can survive even under low temperatures and pH, high concentrations of salt or bile, oxidative stress, carbon starvation, and other adverse conditions [3]. Among the different *Listeria* spp. *L.*

monocytogenes is an emerging zoonotic bacteria in the food industry and causes listeriosis in humans and animals [4]. The infection is generally transmitted through contaminated food [5]. Humans are infected by consuming contaminated food. Generally industrially processed foods such as raw meat, fish, milk, milk-related products have been linked for the listeriosis [6, 7, 8, 9].

Meat is the major source of protein that contains valuable qualities of vitamins for most people in many parts of the world [10]. Due to the chemical composition and biological characteristics, meats are highly perishable foods which

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provide excellent source for growth of many hazardous microorganisms that can cause infection in humans and spoilage of meat [11]. Microbial contaminations which can occur as a result of wrong techniques during slaughtering process, defective cutting practices and insufficient sanitation programmes can pose risk in terms of human health. Regarding microbial contamination of food, researches done for the detection of *L. monocytogenes* in different foods [12, 13, 14] showed that those foods were substantially contaminated with the bacterium which can have clinical courses of meningitis, meningoencephalitis, and septicemia in human body.

Pregnant women, new-born babies, immunocompromised persons and the elderly are at great risk for listeria infections [15]. The listeriosis is a rare but serious food-borne disease as it exhibits 20–30% mortality, 91% hospitalization and 50% neonatal death rates [16]. In human, clinical manifestations of listeriosis range from gastrointestinal disturbances, i.e. non-bloody diarrhoea, nausea, and vomiting, to influenza-like illness with high fever, headache, and myalgia whereas untreated cases may lead to septicemia, meningitis, encephalitis, abortion and occasionally death [11]. Listeria infection in animal shows broad range of symptoms [17].

Heavy use of antibiotics as a growth promoter for farm animals and injudicious use of antibiotics accelerated evolution of bacteria towards antibiotic resistance. *Listeria*

has also evolved towards multiple antibiotics resistance [18]. Ampicillin/penicillin and gentamicin are the primary antibiotics for listeriosis therapy, resistance to these antibiotics has been the focus of many previous studies [19, 20]. In addition, single or multiple antibiotic-resistant *L. monocytogenes* strains isolated from food and the environment have also been frequently reported [21, 22]. There is no study on the prevalence and antibiotic susceptibility of *L. monocytogenes* in beef, chevon and chicken in Bangladesh. Therefore the present research was undertaken with the following specific objectives i) To isolate and identify *Listeria* spp. from beef, chevon and chicken and ii) to determine antibiogram profile of isolated *Listeria* spp. against commonly used antibiotics.

2. Materials and Methods

2.1. Collection and Transportation of Meat Sample

A total of 36 meat samples such as Beef (n=12), Chicken (n=12) and Chevon (n=12) were collected from Boira, Paglar bazar, Kawatkhal bazar, Kamal-Ranjit (K-R) market and Shesh more bazar. Following collection, the meat samples were immediately transported to the Bacteriology Laboratory in the Department of Microbiology and Hygiene, BAU, Mymensingh. The summary of samples collection is presented in Table 1.

Table 1. Summary of different meat samples collected from different areas.

Sl No.	Areas of sample collection	Beef	Chevon	Chicken	Total	Grand total
1	Boira, Paglar bazar	3	3	3	9	36
2	Kamal-Ranjit (K-R) market	3	3	3	9	
3	Kewatkhal bazar	3	3	3	9	
4	Shes more bazar	3	3	3	9	

Legend: BAU=Bangladesh Agricultural University, K-R= Kamal-Ranjit

2.2. Isolation of Bacteria in Pure Culture

The meat samples were homogenized in 0.1% peptone water. Then 0.1 ml of that homogenized sample was streaked onto Listeria selective agar (Oxford formulation, USA) and incubated at 37°C aerobically for 24 hrs. Single colony grown on listeria selective agar was further sub-cultured by spread plate method until pure culture was obtained.

2.3. Identification of Bacteria

Identification of bacteria was performed on the basis of colony morphology (shape, size, margin, elevation and colour), Gram staining reaction, Christie, Atkins, Munch-Petersen (CAMP) test, motility test, hemolytic activity and biochemical tests such as sugar fermentation test (Dextrose, Sucrose, Lactose, Maltose, Mannitol), Indole test, MR-VP test and D xylose test [23].

2.4. Antibiotic Sensitivity Test

Isolate of *Listeria* spp. was tested for antimicrobial drug susceptibility against five commonly used antibiotics such as ampicillin, penicillin, ciprofloxacin, gentamicin and vancomycin by disc diffusion or Kirby Bauer method [24]. Antimicrobial testing results were recorded as susceptible, intermediate and resistant according to zone diameter interpretive standards provided by [25].

3. Results

Among 36 samples, 4 were culture positive. The overall prevalence of *L. monocytogenes* in this study was 11.11%. Among the different samples prevalence of *L. monocytogenes* was 8.33% in chicken, 16.66% in beef and

8.33% in chevon (Table 2). The market wise prevalence was 0% in Boira, Paglar bazar, 11.11% in Kamal-Ranjit (K-R) market, 22.22% in Shes more bazar and 11.11% in

Kawatkhali bazar. The highest of *L. monocytogenes* was recorded in Shes more bazar (22.22%) (Table 3).

Table 2. Results of prevalence of *L. monocytogenes* in meat samples.

Type of sample	No. of meat sample	No. of culture positive sample	Prevalence (%)	Overall prevalence (%)
Chevon	12	1	8.33	
Beef	12	2	16.66	11.11
Chicken	12	1	8.33	

Table 3. Prevalence of *L. monocytogenes* in meat samples according to study areas.

SI No.	Study areas	No. of tested sample	No. of culture positive sample	Prevalence (%)
1	Boira, Paglar bazar	9	0	0
2	Kamal-Ranjit (K-R) market	9	1	11.11
3	Kewatkhali bazar	9	1	11.11
4	Shes more bazar	9	2	22.22

3.1. Cultural Characteristics

The isolated bacteria produced black zone around the colonies in Oxford Agar media due to the formation of black iron phenolic compounds derived from the aglucon, which indicate growth of *L. monocytogenes*.

3.2. Gram's Staining

The Gram's staining revealed Gram-positive, coccobacillary bacteria, arranged in clump, short chain or single organism under the microscopic examination with 400X objectives.

3.3. Motility Profile

The isolates were found motile when examined using hanging drop slide under microscope. It is mobilized by means of peritrichous flagella.

3.4. Sugar Fermentation Test

L. monocytogenes fermented dextrose (DX) and maltose (ML) and only acid was produced. No gas was observed in Durham's tube. Negative reaction was indicated by no change of color.

3.5. MR-VP and Indole Test

All isolates were Methyl-red and Voges-Proskauer positive and indole negative.

3.6. Catalase and D-xylose Test

All the isolates of *Listeria* were catalase positive that was confirmed by the formation of bubble in H₂O₂ within few seconds. The isolates were found D-xylose test negative.

3.7. Hemolytic Activity and CAMP Test

On Blood Agar media the isolates produced zone of hemolysis on that media. When performing the CAMP test on Blood Agar media using *Staphylococcus aureus* and *Listeria* isolates, synergistic aero shape hemolysis was found

near the junction of *Staphylococcus aureus* and *Listeria* spp.

3.8. Results of Antibiotics Sensitivity Tests

On the basis of zone of inhibition *L. monocytogenes* isolates were found sensitive against ciprofloxacin, gentamicin and vancomycin and resistant against ampicillin and penicillin. The results of antibiotic sensitivity assay are presented in Table 4 and Fig. 1.

Table 4. Antimicrobial profile of *L. monocytogenes*.

Name of antibiotic disc	Diameter of zone of inhibition (mm)	Interpretation
Ampicillin	13	R
Ciprofloxacin	28	S
Gentamicin	24	S
Penicillin	0	R
Vancomycin	18	S

R=Resistant, S=Sensitive

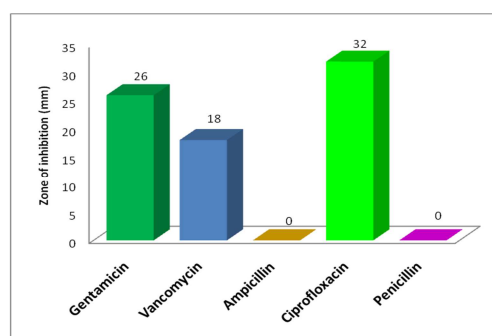


Fig. 1. Summary of antibiogram profile of *L. monocytogenes* against five antibiotics.

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 [26]. In our study, 11.11% meat samples were positive for *L. monocytogenes*. Similar prevalence results were also reported in other countries [26,

27]. It is generally assumed that meat cannot be free from *Listeria* because of slaughtering, evisceration and food processing methods that allow greater chance for contamination. Furthermore, *Listeria* species are ubiquitous in the environment [28]. People handling food at different levels can also be sources of contamination. In this study, *L. monocytogenes* isolated from meat samples were identified on the basis of biochemical and carbohydrate fermentation results. All *L. monocytogenes* in this study showed positive results for the catalase, Voges-Proskauer (VP) and methyl red (MR) tests and negative results. Morphological and staining characteristics of the isolated bacteria were similar to the findings of the author [29]. The finding of motility property and catalase test of the isolates was similar to the findings of the author [23]. The biochemical characteristic of *Listeria* was similar to the findings of other authors [30].

L. monocytogenes has been isolated from wide environmental sources [31]. The organism possesses ability to survive in harsh conditions and therefore can persist in environment for long time. Because of such persistence *L. monocytogenes* can easily enter in food chain [32]. *L. monocytogenes* is pathogenic to humans and animals. Therefore, meat quality controlling authorities from several developed countries have enforced strict regulations over occurrence of *L. monocytogenes* in meat and meat products [33]. However, such regulations are largely lacking in developing countries because of underestimated listerial scenario. In Bangladesh, due to lack of awareness, burden of other traditional diseases, expertise, and poor reporting, the incidence of listeriosis is unknown. To understand the listeriosis in detail, there is a need of systematic and coordinated studies to estimate the prevalence of *L. monocytogenes* in different habitats, occurrence of listeriosis in humans as well as in animals.

In the present study, the incidence of *L. monocytogenes* in raw meat was 16.66% in beef, 8.33% in chicken and 8.33% in chevon. These results are in close agreement with the findings reported by [34, 35] where the organism was recovered from 8.3% and 12.5% of meat samples, respectively. 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. It has been known that salinity, pH, and water activity (*aw*) play an important role in regulating the growth of *L. monocytogenes*. The bacteria grow better (short generation and lag time) at the alkaline pH and high *aw* [36]. At pH of <4.3 and *aw* of <0.930, *L. monocytogenes* survive but do not grow [37]. The values of these parameters are not controlled for meats sold in open markets. Unfortunately, the pH and *aw* of the food samples of both of our studies were not determined.

The prevalence of *Listeria* in raw meat samples in the present study was lower as compared to raw meat sold at the open markets in Greece and Spain, which were 27.5% (beef 20% and chicken 35%) and 34.9% respectively [38, 39]. Beef samples were more contaminated with *L. monocytogenes* in this study as compared to chicken and chevon which are similar to the findings reported from elsewhere [40, 41].

The antibiotic resistance of the pathogen is a significant public health concern. Recent reports suggest the evolution of *L. monocytogenes* towards antibiotic resistance [42, 43]. It is suggested that the increased use of antibiotics for therapeutic purposes in animals and humans may lead to the development of antibiotic resistance [44, 45]. Depending upon different geographical area, antibiotic resistance patterns of *L. monocytogenes* in food and environmental sources may change [45]. We tested *L. monocytogenes* isolates for their antibiotic sensitivity.

Until recently, the *Listeria* genus was thought to be uniformly susceptible to antibiotics active against Gram-positive bacteria including ampicillin or penicillin (combined with aminoglycosides). But these are resistant. Hence, these antibiotics were used treatment of human listeriosis and veterinary medicine [46, 47]. However, the first antibiotic-resistant *L. monocytogenes* was described in 1988 [48] and many more resistant strains have been detected in food and sporadic cases of listeriosis since then [49, 50].

In our study, *L. monocytogenes* was resistant to ampicillin and penicillin. The result of Antibiotic sensitivity test was similar to the findings of the author [24]. Multidrug resistant *L. monocytogenes* might be resulted from incorrect use of these antimicrobial agents for therapeutically purposes in veterinary medicine.

5. Conclusion

The presence of multidrug-resistant *Listeria* sp. in raw meat is a significant public health concern and frequent use of antibiotics in human and animals may be the cause of this resistance. So, the study underscores the need for implementation of proper hygienic and sanitary measures during slaughtering, skinning and evisceration operations to prevent contamination of meat with *Listeria* species.

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