Original Research Article
DOI: https://doi.org/10.62418/ijvph.9.3.2023.42-46

Antibiogram Assay of *Listeria monocytogenes* Isolates from Milk Samples in and Around Kolkata

Monmon Saha [(1)], Saurabh Majumder [(2)*], Chanchal Debnath [(3)], Amiya Kumar Pramanik [(3)], Debasish Murmu [(3)]

[(1)]Department of Veterinary Public Health & Epidemiology, C.V.Sc. & A.H., R.K. Nagar, Tripura (W), [(2)] Department of Veterinary Microbiology, C.V.Sc.& A.H., R.K. Nagar, Tripura (W), [(3)]Department of Veterinary Public Health & Epidemiology, WBUAFS, 37, K.B. Sarani, Kolkata, WB

(Received: 4th December 2023 | Accepted: 29th December 2023)

Abstract

Listeria monocytogenes is well-known globally as one of the most significant foodborne bacterial pathogens. *L*isteriosis may trigger life-threatening illnesses such as severe sepsis and meningitis, sometimes resulting in lifelong harm and even death. This study aimed to determine the occurrence and antibiotic resistance pattern of *L. monocytogenes* in a milk sample collected from in and around Kolkata. For this, a total of 104 milk samples [from individual cow udders (n = 36) and pooled can milk collected from farms (n = 20) as well as from the market (n = 48)] were examined for a period of 6 months, starting from January 2014 to June 2014**.** For the isolation of *L. monocytogenes*, samples were cultured on selective media and tested for their susceptibility to common antibiotics by disk diffusion assay. The results revealed that the overall occurrence of *Listeria species*in unpasteurized raw milk was 14 (13.46%), and *L. monocytogenes* was 5 (4.81%). All five *L. monocytogenes* isolates were subjected to an antibiotic sensitivity test using the Kirby-Bauer disc diffusion method. In this method, three antibiotics tetracycline (100%), gentamycin (100%), and penicillin (100%) exhibited complete sensitivity. However, the isolates showed variable resistance against ampicillin (16.21%), vancomycin (21.62%), and penicillin (43.24%).

Keywords: Occurrence, *Listeria monocytogenes*, Antibiogram, Milk samples, Characterization.

Introduction:

India is the world's largest producer of dairy products by volume and has the world's largest dairy herd. The country accounts for more than 13% of the world's total milk production and is also the world's largest consumer of dairy products, consuming almost all of its milk production (Singh, 2010). There are many organisms secreted through milk; one of them is *Listeria monocytogenes*, which causes significant public health problems. *L. monocytogenes* has been called an "emerging food-borne pathogen" because only recently we have recognized that it can be transmitted through food. *Listeria monocytogenes* is a ubiquitous bacterium. It causes Listeriosis, a serious infectious disease that occurs as a consequence of consumption of food contaminated with this pathogenic bacterium. Listeriosis is a significant public health problem (Rocourt and Catimel, 1985). The first communications/ reports of the presence of *Listeria* in food associated with dairy products, where cow milk was mentioned as a carrier of the fatal Listeriosis (Farber and Peterkin, 1991). According to many communications, consumption of milk and dairy products contaminated with *L. monocytogenes* can lead to individual cases of Listeriosis or a true outbreak of this disease. Of all dairy products, soft cheeses and non-pasteurized milk are the most common causes of Listeriosis. In the process of production of milk and dairy products, it most commonly occurs as a consequence of post-pasteurization contamination. Listeriosis is a serious disease of humans, occurring sporadically or in the form of an epidemic, with a mortality rate of over 25**%** (USDA, 1999).

Materials and Methods:

Isolation and identification of *Listeria monocytogenes* strains: ISO 11290 method was employed to isolate the organisms, whereby pre-enrichment of 10ml sample was done in 20 ml half-strength Fraser broth containing selective supplements (HiMedia) for 24 h at 30°C, which was followed by second enrichment of 0.1 ml of pre-enriched Fraser broth content in 10 ml full strength Fraser broth containing selective supplements (HiMedia) for 48 h at 37°C incubation temperature. After the enrichment procedure, the inoculum was plated on PALCAM agar (HiMedia) and incubated for 48 h at 37°C. The gray-green colonies are surrounded by a diffuse black zone on PALCAM agar. Subsequently, pinpoint colonies of PALCAM were subjected to identification procedures which included Gram's staining followed by a microscopic examination, catalase test, and oxidase test. The characteristic Gram-positive, coccobacillus or short rod-shaped organisms which were catalase positive and oxidase negative, were sub-cultured in Brain heart

infusion (BHI) broth at 25°C for 12-18 h. Subsequently, "presumptive" Listeria isolates were in turn subjected to detailed biochemical tests viz.; methyl red, Voges-Proskauer, nitrate etc. for confirmation of *L. monocytogenes* strains (Farber and Peterkin, 1991).

Antibiotic sensitivity testing of *Listeria monocytogenes* isolates: In the present study, *Listeria monocytogenes* isolates were tested for their susceptibility to antimicrobial agents by the standard Kirby-Bauer disc diffusion method (Bauer et al., 1966) following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines, 1997. All positive *L. monocytogenes* isolates were grown in BHI broth overnight at 37^{o}C. The culture suspension was adjusted to 0.5 McFarland Standard (approximately 1.5 x 108 cells). Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times, pressing firmly on the inner wall of the tube above the fluid level to remove excess inoculum from the swab. Mueller-Hinton Agar (Hi- media®) was used as a medium to study the susceptibility to antibiotics. Then cultured was spread on the entire surface of a dried Muller Hinton agar plate with the sterile culture containing a swab. The culture-inoculated plates were held at room temperature for 10 minutes to allow the evaporation of free surface liquid as adopted by Anon (1997). Commercially available following antibiotics octa disks (Hi-Media®) were used: (D033) Ampicillin (10 mcg), Tetracycline (30 mcg), Cotrimoxazole (25 mcg), Ciprofloxacin (5 mcg), Gentamicin (10 mcg), Erythromycin (15 mcg), Chloramphenicol (30 mcg), Cefalexin (30 mcg). (D034): Ceftrixone (30 mcg), Ceftazine (30 mcg), Cefotaxime (30 mcg), Lincomycin (2 mcg), Netilmycin (30 mcg), Ofloxacin (2 mcg), Vancomycin (30 mcg), Amikacin (30 mcg). (D0286) Penicillin (10 unit), Erythromycin (15 mcg), Vancomycin (30 mcg), Telecoplanin (30 mcg), Clindamycin (2 mcg), Ofloxacin (5 mcg), Azithromycin (15 mcg), Tetracycline (30 mcg) were placed on the surface of each inoculated plate using a sterile forceps. After incubation for 24 hours at 37^{0}C, the diameter of the zone around each disc was measured, and interpreted by the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

Results and Discussion:

A total of 14 (13.46%) *Listeria* spp. isolates were obtained in this study within which only 5 (4.81%) were found to be *L. monocytogenes* isolates. All isolates were Gram-positive, and coccobacilli, 0.5µm in diameter and 1-5µm in length that do not form spores or capsules, which were catalase positive and oxidase negative. These were MR positive, Nitrate reduction negative, and VP test positive which confirmed these as *L. monocytogenes* (Farber and Peterkin, 1991).

All five *L. monocytogenes* isolates showed different results in the antibiotic sensitivity test. In this study, 3 antibiotics, tetracycline (100%), gentamicin (100%), and penicillin G (100%), exhibited complete sensitivity. However, the isolates showed variable resistance against ampicillin (16.21%), vancomycin (21.62%), and penicillin (43.24%) shownin Table 1 and Table 2 and zone of inhibition of different antibiotics shown in Figures 1, 2 and 3.

Table 1: Antimicrobial drug resistance and sensitivity pattern of *L. monocytogenes* strains

Antimicrobial agents	No. of isolates tested	*L. monocytogenes* isolates frommilk					
		Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Ampicillin	5	1	20	-	-	4	80
Tetracycline	5	0	0	-	-	5	100
Cotrimoxazole	5	4	80	-	-	1	20
Gentamicin	5	0	0	-	-	5	100
Ciprofloxacin	5	5	100	-	-	0	0
Erythromycin	5	3	60	-	-	2	40
Chloramphenicol	5	4	80	1	20	0	0
Cefalexin	5	1	20	1	20	3	60
Ceftriaxone	5	1	20	-	-	4	80
Ceftazidime	5	1	20	-	-	4	80
Cefotaxime	5	1	20	-	40	2	40

Table 2: Antimicrobial drug resistance and sensitivity pattern of *L. monocytogenes* isolates

Antimicrobial agents	No. of isolatestested	*L. monocytogenes* isolates frommilk					
		Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Lincomycin	5	2	40	-	-	3	60
Netilmycin	5	1	20	1	20	3	60
Ofloxacin	5	1	20	-	_	4	80
Vancomycin	5	1	20	1	20	3	60
Amikacin	5	2	40	-	-	3	60
Penicillin	5	0	0	-	-	5	100
Erythromycin	5	4	80	-	-	1	20
Clindamycin	5	2	40	-	-	3	60
Azithromycin	5	1	20	1	20	2	60

Figure 1: Antibiotic disc sensitivity test of *L. monocytogenes* strains using antibiotics like Ampicillin, Tetracycline, Cotrimoxazole, Ciprofloxacin, Gentamicin, Erythromycin, Chloramphenicol and Cefalexin

Figure 2: Antibiotic discsensitivity test of *L. monocytogenes* strains using antibiotics like Ceftriaxone, Ceftazidime, Cefotaxime, Lincomycin, Netilmycin, Ofloxacin Vancomycin and Amikacin

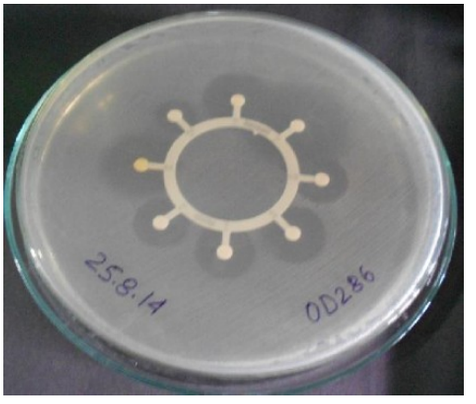

Figure 3: Antibiotic discsensitivity test of *L. monocytogenes* strains using antibioticslike Penicillin, Erythromycin, Vancomycin, Telecoplanin, Clindamycin Ofloxacin, Azithromycin and Tetracycline

From Table 1and Table 2, it was found that the highest resistance was recorded against Ciprofloxacin (100%), moderate resistance was found against cotrimoxazole, chloramphenicol, and erythromycin and the highest sensitivity was observed against tetracycline, gentamicin, and penicillin (100%). Zone of inhibition of different antibiotics was shown in Figure 1, 2 and 3 against different antibiotics.

The above results were partially correlated with Altunta et al. (2012) who reported a susceptibility pattern of *L. monocytogenes* isolates to antibiotics, such as penicillin G, vancomycin, tetracycline, chloramphenicol, rifampicin, erythromycin, gentamicin, and trimethoprim. However, the percentages of fosfomycin and streptomycin resistances were 92.9% and 7.1%, respectively.

The above result was partially correlated with Sharif et al. (2010) who reported a susceptibility pattern of *L. monocytogenes* isolates to gentamicin, doxycycline, ampicillin, tetracycline, and penicillin G and resistance to ciprofloxacin, cotrimoxazole, nalidixic acid, and erythromycin. Shu Bing et al. (2004) reported the sensitivity of *L. monocytogenes* to 12 antibiotics including gentamicin, vancomycin, kanamycin B, norfloxacin, ofloxacin, erythromycin, chloramphenicol, tetracycline, cephalothin, and cefazolin, were carried out. The study revealed that *L. monocytogenes* was resistant to enrofloxacin and nitrofurantoin. Enurah et al. (2013) reported chloramphenicol was the most effective antibiotic against the *L. monocytogenes* isolates with the least resistance (3.70%) while nalidixic acid proved to be least effective with resistance of 90.74%.

Conclusion:

*L. monocytogenes*isa psychrophilic bacteria recognized as a pathogen of great importance of food. It is accepted that Listeriosis in humans is a disease that is transmitted mainly through food. The series of outbreaks of the 1980s showed that *L. monocytogenes* causes very serious invasion and often life-threatening disease, constituting an economic burden for both public health services and the food industry. Infection with *L. monocytogenes* is a wide spread zoonosis, affecting mainly cattle, sheep, and goat herds. *Listeria* species are ubiquitous bacteria widely distributed in the natural environment. The ubiquitous character of the bacteria inevitably results in the contamination of numerous food products. All *Listeria* species are small, regular rods, 0.5µm in diameter and 1-5µm in length that do not form spores or capsules. They produce catalase but not oxidase. It is a Gram-positive, facultative anaerobic bacterium with both psychotropic and mesophilic features.

The prevalence of organisms in raw milk, meat, fish, vegetables, and ready-to-eat food is documented in Western as well as Asian countries.The possible causes of the emergence of listeriosis include major changes in food production, processing, and distribution, increased use of refrigeration as a primary means for the preservation of food, and changes, in the habits of the people.

Drug sensitivity test of *L. monocytogenes* with different antimicrobial agents revealed that all the isolates were highest resistant to ciprofloxacin (100%), moderately resistant to cotrimoxazole (80%), chloramphenicol (80%), and erythromycin (60%) and the highest sensitivity was observed against tetracycline, gentamicin, and penicillin (100%). These high resistances to commonly used antimicrobials may be due to indiscriminate use of these drugs.

Acknowledgments:

The authors thank the Hon'ble Vice-Chancellor and DREF, WBUAFS for providing necessary funds and the Faculties of the Department of Veterinary Public Health, F/VAS, WBUAFS, West Bengal for the research facilities and support for this study. The field staff/ shop owners also deserve special thanks for their help during sampling.

Conflict of Interest:

No competing interest exists among the authors.

References:

Altunta EG, Kocan D, Cosansu S, Ayhan K, Juneja, VK, Materon L. Antibiotic and bacteriocin sensitivity of *Listeria monocytogenes* strains isolated from different foods. Journal of Food and Nutrition Sciences. 2012; 3(3): 363-68.

Anon S. Performance standards for antimicrobial disk susceptibility Test. 6th ed. Approved Standard document. 1997. Wayne, PA: NCCLS.

Bauer AW, Kirby WM, Sherris JC. Antibiotic susceptibility testing by a standard single disc method. American Journal of Clinical Pathology. 1966; 45: 493–96.

Enurah LU, Aboaba OO, Nwachukwu SCU, Nwosuh, CI. Antibiotic-resistant profiles of food (fresh raw milk) and environmental (abattoir effluents) isolates of *Listeria monocytogenes* from the six zones of Nigeria. African Journal of Microbiology Research. 2013; 7(34): 4373-78.

Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiology.1991; Rev. (55): 476-511.

National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility tests, 4th Edition, NCCLS. 1993; 10(7).

Rocourt J, Catimel B. Biochemical characterization of species in the genus *Listeria*. Zentralble Bakteriologika Mikrobiologi Hygiene. 1985; 260: 221-31.

Sharif J, Nillayat MM, Sheikh GN, Roy SS, Bhat SA. Prevalence and antibiogram of *Listeria monocytogenes* in case of abortion and Stillbirth in sheep of Kashmir. Journal of Veterinary Public Health. 2010; 9(1): 43-6.

Shu Bing Y, Jing Tao L, Qiang Zhong Z. Isolation of *Listeria monocytogenes* from food and test of drug

susceptibility. Journal of China Tropical Medicine. 2004; 4(4): 515-34.

Singh R. Indian Dairy and Products Annual Report 2010. USDA Foreign Agricultural Service: Global Agricultural Information Network. Retrieved 16 June 2011, from static.globaltrade.net/files/pdf/20110226231255627.pdf.

USDA. 1999. United States Department of Agriculture. Consumer Information from USDA, Food Safety Education Office, FoodSafety and Inspection service, USDA, Washington D.C. 20250-3700.

***Corresponding author's email ID:** sauravviro@gmail.com

Citation: Saha M, Majumder S, Debnath C, Pramanik AK, Murmu D. Antibiogram Assay of *Listeria monocytogenes* isolates from Milk Samples in and around Kolkata. Indian Journal of Veterinary Public Health. 2023; 9(3): 42-46. DOI: https://doi.org/10.62418/ijvph.9.3.2023.42-46