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Isolation, Identification and Antibiotic Susceptibility Patterns of Listeria Monocytogens from Pregnant Women

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ABSTRACT

Introduction

The present study is made to determine the prevalence rate, isolation, identification and antibiotic susceptibility pattern of *L. monocytogens* isolated from pregnant women**.** This research not only guides to the clinician for treatment but also help laboratory worker to monitor susceptibility pattern among the *Listeria* species.

Methods

The samples were collected from Gynecology department from January 2017 to December 2019 and processed in the Department of Microbiology at College of Medical Sciences, Bharatpur Nepal. Two hundred thirty four antenatal mothers having age between 17 and 39 years of gestation period between 7th weeks and 36th weeks patient with a flue like sign and symptoms were screened for *L. monocytogens*. Bacterial isolation identification and antibiotic sensitivity tests were done as per standard microbiological guidelines.

Results

In this present research, the prevalence of *L. monocytogens* was found 39/234 (16.7%), where high rate of *Listeria* infection was found 21(53.1%) among the patients of age between 25 and 32 who were mostly from urban area. We found eating meat 38(97.4%) and fish 39(100.0%), non-pasteurized simple boiled milk 39(100.0%) and vegetables like salad eater 32(82.1%) has highest rate of listeriosis. The results showed most of the listeriosis were occurring in a 3rd trimester 17(43.6%) compared with the 2nd and 1st trimester. Overall, the antibiotics resistance pattern found low in *L. monocytogens*. This investigation also showed less resistance in erythromycin 9(23.1%), ciprofloxacin 8(20.5%), chloramphenicol 16(41.1%). The increased resistance pattern among *Listeria* species were found in penicillin 31(79.5%), cotrimoxazole 20(51.3%), amoxicillin 21(55.1%) and vancomycin 29(74.4%).

Conclusion

It is very important to consider listeriosis in a clinical practice now a day. Maternal Listeriosis should be considered in all age group of pregnant women. Regular surveillance of antibiotic susceptibility test is very much important for clinician to select best medicine to treat different cases of listeriosis.

Key words: *Listeria monocytogens,* pregnant women, drugs resistance.

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INTRODUCTION

Incidence of *Listeria monocytogens* infection has been significantly increasing over the past few years, mainly affecting pregnant women and immunocompromised patients. The emergence of Zoophilic and Geophilic bacterial infections as the world wide health care problems may be attributed to the extensive use of antibiotics and immunosuppressive agents, as well as an increase in the population of immunocompromised people.[1] During 1980s a number of large scale outbreaks of Listeriosis occurred, in which contaminated ready to eat foods, uncooked meats, fish, cabbage, milk and cheese were implicated as major source for the disease. In United States listeriosis caused approximately 2500 serious illnesses and 500 deaths annually and 17% of cases were associated with pregnancy.[2,3] Infection in pregnant women recognized in third trimester, it may lead to intrauterine fetal infection with a mortality of 30-35%.[4] In adult *Listeria monocytogens* causes meningitis, menigoencephalitis, rhomboencephalitis, brain abscess and spinal cord abscess. It also causes bacteremia and infective endocarditis. Listeriosis represents a significant public health problem and accounts for almost 5-10% of carriage rate in diseases.[5]

It is a Gram positive bacilli (GPB) belongs to the genus *Listeria*, occurs worldwide in soil, surface water, vegetation and throughout the food chain. It is recently placed between Lactobacillus and Bacillus, it is closely related to Brocothrix (Seeliger, 1986).[6] The GPB has six species but only *Listeria monocytogens* is most likely to be encountered as a human pathogens. In the country like Nepal even it is a potential threat to human beings caused by this ignored gram positive bacilli, there is very less data available on listeriosis among pregnant women in Nepal. Therefore, the aim of present research is to determine the prevalence, isolation, identification and antibiotic susceptibility pattern of *L. monocytogens* among pregnant women attending antenatal check-up in College of Medical Sciences, Teaching Hospital, Bharatpur, Nepal.

METHODS

Study area and design

It is a tertiary care center, hospital based; cross-sectional study was conducted from January 2017 to December 2019 at Collage of Medical Sciences, Bharatpur Nepal. Two hundred thirty four antenatal mothers in between 7th weeks to 36th weeks of gestation period patient with a flue like sign and symptoms were screened for *L. monocytogens*. The subjects selected were having age between 17 and 39 years.

Eligibility criteria

Pregnant women having influenza like prodorm with chills, coryzal symptoms, myalgia, backache, headache, vomiting/ diarrhea and urinary frequency was included in the present research.

Sample size and sampling technique

A total of 234 antenatal mothers who were clinically diagnosed as influenza like illness, selectively recruited from January 2017 to December 2019 for the present study.

Dependent variables

Prevalence of *L. monocytogens* in antenatal mother and its antimicrobial susceptibility test.

Independent variables

Socio-demographic variables.

Sample collection

The clinical specimen and demographic data were collected using ethically approved questionnaire. After taking informed written consent from patients or patient attendant guardian, 5 ml of blood sample was aseptically collected and transferred into a sterile Tryptose soy broth in a screw caped test tube, transported immediately to the laboratory without delay within 1 hour. It was incubated 37°C for overnight and turbidity in the broth was observed in the next day.

Culture and identification

The turbid Tryptose soy broth were further subcultured on Palkam agar media and incubated at 37°C for 24 hr. the specific selective growth of green shiny colonies having diffuse black shadow on Palkam agar were taken as a *Listeria* species. If there was no growth found on palkam agar, those plates were further incubated for another 24 hours. Those *Listeria* spp. were further identified using conventional methods, viz gram stain, catalase test, motility test, hemolysis on blood agar, CAMP test, IMViC tests and sugar fermentation tests (xylose, mannito, rhamnose and methyl D-mannopyranoside).[7]

Antibiotic susceptibility tests

Antibiotic sensitivity tests were done as per standard microbiological guide line by Kirby-Bauer disc diffusion method.[8] the *Listeria monocytogens* bacterial isolates inoculum suspension was prepared in a sterile saline and turbidity were compared using 0.5 McFarland standard solution. The Mueller Hinton agar (MHA) plates were inoculated with bacterial saline suspension dipped sterile cotton swab uniformly. Those inoculated MHA plates were incubated for 10 minutes for drying, than antibiotic discs were further placed on it. The antibiotic discs used were Amoxycillin (30mcg/disc), Co-trimoxazole (25mcg/disc), Ciprofloxacin (5mcg/disc), Chloramphenicol (30mcg/disc), Erythromycin (15mcg/disc), Penicillin (10units/disc), Clindamycin (2mcg/disc) and Vancomycin (30mcg/disc), HiMedia, India.

Quality assurance and data analysis

The standard microbiological procedure were followed using reference strain of *L. monocytogens* (ATCC 7644) was used as quality control strain which were obtained from PGI Chandigarh, India. All the data were analyzed using SPSS version 20. Comparisons of species distribution and antifungal susceptibility test were determined by the chi-square test for categorical variables and the Wilcoxon rank sum test for ordinal variables. Binary logistic regression was used to show the association of each variable with the dependent variable. A p-value <0.05 indicated statistical significance between any compared mean groups.

RESULTS

In the present study, A total number of 234 samples of suspected cases of Listeriosis were processed where the prevalence of *L. monocytogens* was found 39/234 (16.7%). The high rate of *Listeria* infection was found 21(53.1%) among the patients of age between 25 and 32 who were mostly from urban area 26(66.7%). Patients with mud house 26(66.7%), farmer 20(51.3%), house wife 12(30.8%) and cat as pet animals 24(53.8%) in their house

were using pipe water 14(35.9%) has more incidence of listeriosis compared to other group of people in the community as shown in Table -1.

Table 1. Association of socio-demographic characters in a *L. monocytogens* isolation from pregnant women visited to the hospital with clinical features of listeriosis.

Variable	Number	Percentage	*L. monocytogens*	
			Positive	Negative
Age				
17-24	46	19.7	11	35
25-32	161	68.8	21	140
33-39	27	11.5	7	20
Residence				
Urban	45	19.2	26	19
Rural	189	80.7	13	176
Knowledge of Hygiene				
Yes	31	13.3	5	26
No	203	86.7	34	169
Occupation				
House wife	86	36.7	12	74
Farmer	85	36.3	20	65
Office worker	63	29.9	7	56
Source of water use				
Piped	98	41.8	14	84
Bore well	71	30.4	10	61
Supply water	65	27.8	5	60
Keeping pets				
Cat	42	17.9	21	21
Dog	165	70.5	15	150
Dog and Cat	27	11.5	3	24
Type of living room				
Mud house	195	83.3	26	169
Cement house	39	16.7	13	26

We found eating meat 38(97.4%) and fish 39(100.0%), non-pasteurized simple boiled milk 39(100.0%) and vegetables like salad eater 32(82.1%) has highest rate of listeriosis. The results showed most of the listeriosis were occurring in a 3rd trimester 17(43.6%) compared with the 2nd and 1st trimester (Table-2).

Table 2. Association of risk factors for Listeriosis from the pregnant women attending antenatal care clinic in CMS-TH, Bharatpur, Nepal.

Variable	Number	Percentage	*L. monocytogens*	
			Positive	Negative
Eating habit				
Meat				
Yes	222	84.9	38	184
No	12	5.1	1	11
Fish				
Yes	224	95.7	39	185
No	10	4.3	0	10
Boiled milk				
Yes	226	96.6	39	187
No	8	3.4	0	8
Uncooked vegetables (Salad)				
Yes	210	89.7	32	178
No	24	10.3	7	17
Gestational period				
1st trimester	154	65.8	8	146
2nd trimester	52	22.2	14	38
3rd trimester	28	12.0	17	11

Overall, Table-3 and Figure-1 shows the antibiotics resistance pattern was low in *L. monocytogens*. This investigation also reveled less resistance in erythromycin 9(23.1%), ciprofloxacin 8(20.5%), chloramphenicol 16(41.1%). The increased resistance pattern among *Listeria* species were found in drugs like penicillin 31(79.5%) cotrimoxazole 20(51.3%), amoxicillin 21(53.1%) and vancomycin 29(74.4%).

Table 3. Antibiotics susceptibility test of *L. monocytogens* (n=39) isolated from pregnant women, antenatal mother care center of central part of Nepal.

Antibiotics	Sensitive		Resistance	
	Number	Percentage	Number	Percentage
Penicillin	8	20.5	31	79.5
Co-trimoxazole	19	48.7	20	51.3
Amoxycillin	18	46.2	21	53.8
Erythromycin	30	76.9	9	32.1
Vancomycin	10	25.6	29	74.4
Clindamycin	25	64.1	14	35.9
Ciprofloxacin	31	79.5	8	20.5
Chloramphenicol	23	58.9	16	41.1

Hi

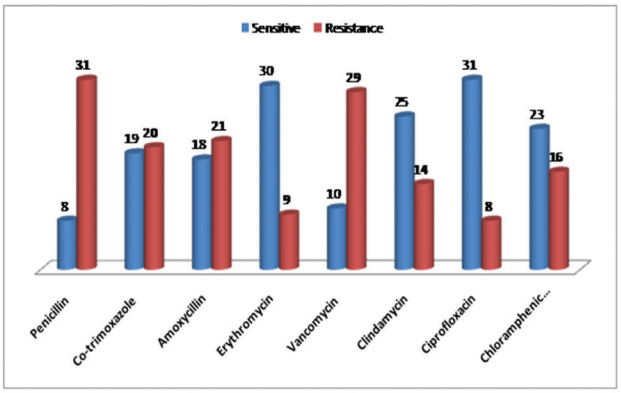

Figure 1. Shows the susceptibility patterns among isolated *L. monocytogens.*

DISCUSSION

In this present research, the prevalence of *L. monocytogens* was found 39/234 (16.7%) clinically suspected listeriosis among pregnant women. This prevalence rate is higher compared to previous studies, where rate of prevalence was 0.1% reported. This is due to selectively taken clinically suspected cases of *Listeria* infection among patients. Listeriosis is a disease that can cause mild asymptomatic infection to devastating fetal problems in pregnant women like, preterm delivery, stillbirth, and fetal death. In few cases, it may complicate to serious neonatal morbidity *viz* septicemia, pneumonia, meningitis and encephalitis.

The percentage of *Listeria monocytogens* in our study was 16.7% compared to other bacterial infections which accounted for 83.3%. Further, pathogenicity study was essential to prove pathogenic role of isolated *Listeria* species. Different study has proven the close association of Listeriosis and immunocompromised condition of patients.[9,10]

In our study, pregnant women with age between 25 and 32 had high rate of *Listeria* infection 21(53.1%) and most of the patients were from urban area 26(66.7%). These results were in accordance with the publisher's report, who also reported rural area with all age group patients has more chances to get *Listeria* infection compared to urban area.[11] As we know hygiene has inverse relation with the bacterial infection, therefore similar pattern of result were obtained in the present scenario, where we found unhygienic patients with more listeriosis.

In the present research, patients with mud house 26(66.7%), farmer 20(51.3%), house wife 12(30.8%) and cat as pet animas 21(53.8%) in their house were using pipe water 14(35.9%) has more incidence of listeriosis compared to other

group of people in the community. Similar type of data were also obtained in another research done by (Linke *et al*, 2018).[12] It is well known that *Listeria* species is a ubiquitous bacteria therefore, it has high chances to cause infection in low immunity host like pregnant women.[13]

Maternal listeriosis is usually mild but it is very sever for neonates. Hence, the current research emphasizes the need to screen hygienic condition and gestational period of infection which also gives us clue for association of bacteria with food habits among patients. We found eating meat 38(97.4%) and fish 39(100.0%), non-pasteurized simple boiled milk 39(100.0%) and vegetables like salad eater 32(82.1%) has highest rate of listeriosis. Mostly similar types of results were also seen in different research, where farmer, domestic animals in their house and eating habits showed high rate of listeriosis.[14]

There are some research which shows time period of pregnancy and its close association of *Listeria* infection[15] the similar pattern of research result was also found in our reaearch regarding gastesnal period. The results showed most of the listeriosis were occurring in a 3rd trimester 17(43.6%) compared with the 2nd and 1st trimester. It is due to sudden change in hormonal status of a pregnant woman. These types of reports were also found from the Ethopia and Uganda.[16] The developing countries has similar pattern of infectivity rate where there is less practice of hygiene.

Overall, the antibiotics resistance pattern was currently low in *L. monocytogens*. This investigation also showed quite comparable less resistance in erythromycin 9(23.1%), ciprofloxacin 8(20.5%), chloramphenicol 16(41.1%). The increased resistance pattern among *Listeria* species were found in penicillin 31(79.5%) cotrimoxazole 20(51.3%), amoxicillin 21(53.8%) and vancomycin 29(74.4%). This type of resistance pattern shows red alarm and also makes us more attentive towards the drug susceptibility pattern among *Listeria* species. This may be due to acquaring resistance or plasmid mediated resistance among the *Listeria* species as reported by (Emmanuelle and Patrice, 1999).[17] It is very much confirmatory that *Listeria* might have acquired drug resistance gene from different sources by different modes.

Limitations

All *Listeria* species isolated should be characterized by molecular technique. Virulence factor and Pathogenicity should be continuous monitored by performing research. Routine antibiotics susceptibility test must be performed to see the resistance among the isolates.

CONCLUSIONS

In the current scenario of mostly ignored gram positive rods *Listeria* infection is increasing day by day. It is very important to consider listeriosis in a clinical practice now a day. Maternal Listeriosis should be considered in all age group of pregnant women due to diligence must be followed in a lab. It is mostly a food borne disease therefore; a government guideline must be made to ensure food safety.

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