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Research Article

Prevalence, Antibiotics Susceptibility Profile of *Listeria Monocytogenes*, and Its Associated Risk Factors Among Pregnant Women Attending Millennium Health Center

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Abstract

Background: *Listeria monocytogenes* is a facultative intracellular pathogen that causes serious invasive infections in pregnant women, resulting in disseminated fetal infections, abortions, and still-births. Although it causes a greater burden on pregnant women and their infants, the case in Ethiopia is overlooked, and there is a scarcity of data on *L. monocytogenes,* particularly in the southern parts of Ethiopia. Therefore, this study aimed to determine the prevalence and, antibiotics susceptibility of *L. monocytogenes* and its associated risk factors among pregnant women attending the Millennium Health Center, Hawassa, Ethiopia. *Method*: A cross-sectional study was conducted on 203 pregnant women between May 30 and July 30, 2022. Socio-demographic data and 5 ml venous blood samples were collected from pregnant women using structured questionnaire. Data were analyzed using SPSS version 25. Bivariate logistic regression was carried out then variables with $p<0.25$ were further analyzed by multivariate logistic regressions, p-values <0.05 were accepted as statistically significant. *Results*: The overall prevalence of *L. monocytogenes* among pregnant women was found to be 11/203 (5.42%; 95% CI=2.88-9.38). *L. monocytogenes* was resistant to benzyl penicillin (90.91%) and meropenem (81.82%), but susceptible to ampicillin (90.91%). Raw meat (AOR=6.99; 95% CI=1.39-35.14), $p=0.018$), uncooked vegetables (AOR=6.62; 95% CI=1.04-42.30), $p=0.046$), unpasteurized milk (AOR=7.56; 95% CI=1.33-42.97), $p=0.023$) and fever (AOR=14.65; 95% CI=3.15-68.15), $p=0.001$) were significantly associated with *L. monocytogenes* infection. *Conclusion*: *L. monocytogenes* was resistant to benzyl penicillin, meropenem, erythromycin, and sulfamethoxazole but susceptible to ampicillin. Raw meat, uncooked vegetables, unpasteurized milk, and fever were significantly associated with *L. monocytogenes*.

Keywords

Antibiotics Susceptibility, *Listeria monocytogenes*, Pregnant Women, Prevalence, Hawassa, Ethiopia

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1. Introduction

L. monocytogenes (LM) is a facultative intracellular gram-positive rod [1] which causes listeriosis in both humans and vertebrates. It is the primary pathogen, while *L. ivanovii*, *L. seeligeri*, *L. grayi*, and *L. innocua* are less medically important [2]. It causes septicemia, meningitis, and abortion in pregnant women [3] and morbidity of 20–30% in immunocompromised patients [4], with a high death rate [5]. The World Health Organization reported that pregnancy-related listeriosis is 43% and is more likely to occur in the third trimester than in the first trimester [6], because successful pregnancy requires timely adaptation by the maternal immune system [7]. The Center for Disease Control estimated 1,600 people get listeriosis annually and 260 will die [8]. Pregnant women experience listeria infection 20-times more frequently than the general population due to pregnancy-related suppression of cell-mediated immunity and placental tropism. This encompasses fetal and neonatal listeriosis, with death rates of 25–35% and 20%, respectively [9]. If the infection remains unrecognized, it may result in interruption of pregnancy or premature abortions [10]. Pregnant women may exhibit flu-like fever, headache, diarrhea, myalgia, or digestive-related symptoms [11].

Meat and dairy products can transmit listeria to human [12, 13]. Approximately 99% of human listeriosis cases are transmitted by the consumption of ready-to-eat foods [14], which are introduced into the food chain from raw materials [15]. It is also transmitted vertically to the fetus [16]. It survives and replicates over a wide range of temperatures (4 - 42 ℃), pH, salt, and oxygen concentrations [17] and forms biofilms, which makes elimination difficult [18]. It can be isolated from maternal/neonatal blood, body fluid, and the placenta [19] by growing on different culture media [20, 21]. A mixture of ampicillin and aminoglycoside, ampicillin and gentamicin is the current therapy of choice [22] despite the emergence of drug-resistant strains [23]. Although LM causes a greater burden on pregnant women and their infants, data are scarce particularly in the southern parts of Ethiopia. Therefore, this study aimed to determine the prevalence, antibiotic susceptibility of LM, and associated risk factors for infection among pregnant women attending the Millennium Health Center, Hawassa, Ethiopia.

2. Methodology

2.1. Study Setting

A health center-based, cross-sectional study was conducted from May 30 to July 30, 2022 at the Millennium Health Center, which is located in Hawassa 275 km from the capital city of Ethiopia, Addis Ababa [24]. The city has a population of approximately 419,655, of which 51.4% are male and 48.6% are female [25]. The Millennium Health Center provides services for 80,074 individuals annually. The antenatal care clinic of the health center serves a mean of 30 pregnant women per day, and eight beds are available for both antenatal and postnatal services within the health center (health center- based data).

2.2. Eligibility Criteria

Pregnant women aged 18-45 years who were willing to provide informed consent were included in the study, whereas pregnant women who were on antibiotic treatment 2 weeks prior to the time of data collection were excluded from the study.

2.3. Sample size Determination and Sampling Technique

A total of 206 pregnant women who fulfilled the eligibility criteria were recruited between May 30 and July 30, 2022. The participants were selected using a systematic sampling technique, and the first participant was selected using the lottery method.

2.3.1. Dependent Variable

Prevalence of *L. monocytogenes.*

2.3.2. Independent Variables

Socio-demographic variables and possible risk factors.

2.3.3. Co-variables

Clinical sign and symptoms.

2.4. Data and Specimen Collection

Clinical and socio-demographic data were collected using structured questionnaires after obtaining written informed consent from study participants. Venous blood (5 ml) was collected aseptically by disinfection with 70% alcohol and 2% tincture of iodine, transferred into sterile 45 ml tryptose soy broth (Oxoid, UK) with 0.6% yeast extract (TSBYE) [26] and transported within 1h to the SNNP Regional Public Health Laboratory.

2.5. Culture and Identification

Blood culture bottles were incubated at 35 ℃ with daily inspection for visible microbial growth for 7 days by observing visually the turbidity of broth. For blood cultures that show turbidity (microbial growth), subcultures were prepared on sheep blood agar (Oxoid, UK) and was incubated at 35 ℃ for 24 h. *L. monocytogenes* typically grows as gray to white beta-hemolytic colonies on 5% sheep blood agar (Oxoid, UK). Preliminary identification of *L. monocytogenes* was performed using Gram staining and hemolytic patterns on 5% sheep blood agar (Oxoid, UK). For blood cultures that did not show turbidity (microbial growth), blind sub culturing was also performed on the 2nd, 5th, and 7th day of inoculation. Blood culture results with no microbial growth after 7 days were recorded as culture-negative. Suspected colonies were further identified using Catalase, and CAMP test to confirm the presence of *L. monocytogenes* [26].

2.6. Antibiotics Susceptibility Testing

The standardized disk diffusion method was performed on Mueller-Hinton agar (Himedia, India) with 5% defibrinated sheep blood using an inoculum of McFarland 0.5, and was incubated at 5% CO_2, 35 ± 1 ℃, for 18 ± 2 h. The result was interpreted by reading the zone edges as the point showing no growth when viewed from the front of the plate with the lid removed and with reflected light. Antibiotics tested [27] included: ampicillin (2 µg), erythromycin (15 µg), benzyl penicillin (1 unit), sulfamethoxazole (5 µg), and meropenem (10 µg). Suspended isolates were inoculated on MHA supplemented with 5% sheep blood and antibiotic discs were placed firmly and incubated at 37 °C for 24 h. *Streptococcus pneumoniae* ATCC 49619 was used as quality control. The zone of inhibition was measured and interpreted according to CLSI 2020 [28] and EUCAST guidelines for *L. monocytogenes* [27].

2.7. Quality Assurance

Standard operational procedures were followed and *Streptococcus pneumoniae* (ATCC 49619) was used as the reference strain. A negative control was performed by randomly taking the prepared culture media and incubating overnight to check for growth [28].

2.8. Data Entry and Analysis

Data entry and analysis were performed using Epi data version 4.6 and the Statistical Package for Social Science (SPSS) version 25 software. Descriptive statistics and binary and multivariate logistic regression analyses were performed. Binary logistic regression was used to show the association of each variable with the dependent variable, and $p< 0.05$, with a 95% confidence interval, was considered statistically significant. Bivariate logistic regression was performed, and variables with $p<0.25$ were further analyzed by multivariate logistic regression. Statistical significance was set at $p<0.05$. The results are presented in the text, tables and charts.

3. Results

3.1. Socio-Demographic Characteristics

A total of 206 pregnant women were included in this study, of whom 203 responded, with a response rate of 98%. The mean age of the women was 26.57 (± 6.31 SD) years. The median age of the pregnant women was 25 years (range: 18-43 years). The findings of this study showed that 91 (44.80%) of women were aged between 18 and 24 years old, 160 (78.80%) were married, 68 (33.50%) had completed secondary school, 71 (35%) were house wives, and the place of residence of the pregnant women was urban for 164 (80.80%) (Table 1).

Table 1. *Socio-demographic characteristics of pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).*

Variables	Category	Frequency	Percent (%)
Age (years)	18 – 24	91	44.8
	25 – 34	72	35.5
	35 – 44	40	19.7
Residence	Urban	164	80.8
	Rural	39	19.2
Marital Status	Single	20	9.9
	Married	160	78.8
	Divorced	14	6.9
	Widowed	9	4.4
Educational status	Read and Write	20	9.9
	Elementary School (1 – 8)	54	26.6
	Secondary School (9 – 12)	68	33.5

Variables	Category	Frequency	Percent (%)
Occupation	College/University	61	30
	Government employee	54	26.6
	Merchant	38	18.7
	Daily Worker	40	19.7
	House Wife	71	35

3.2. Gestational Age and Frequent Food Habits

Of the study participants, 85 (41.90%), 105 (51.70%), 95 (46.80%), 78 (38.40%), and 75 (36.90%), pregnant women were in the 2nd trimester, and had frequent feeding habits of uncooked/raw fish, uncooked vegetables, unpasteurized milk, and uncooked meat, respectively (Table 2).

Table 2. *Gestational age and frequently feeding habit of pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).*

Variables	Category	Frequency	Percent (%)
Gestational age	1st trimester	41	20.2
	2nd trimester	85	41.9
	3rd trimester	77	37.9
Frequent eating/drinking habits			
Uncooked meat	Yes	75	36.9
	No	128	63.1
Uncooked/raw fish	Yes	105	51.7
	No	98	48.3
Uncooked vegetables	Yes	95	46.8
	No	108	53.2
Unpasteurized milk	Yes	78	38.4
	No	125	61.6

3.3. Clinical Characteristics of *L. monocytogenes* Infection

Gastroenteritis was noted in 58 patients (28.6%), nausea/vomiting in 49 (24.1%), and headache in 42 (20.7%) (Table 3).

Table 3. *Clinical characteristics of L. monocytogenes infection among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).*

Variables	Category	Frequency	Percent (%)
Fever	Yes	24	11.8
	No	179	88.2
Headache	Yes	42	20.7
	No	161	79.3
Gastroenteritis	Yes	58	28.6
	No	145	71.4
Nausea/Vomiting	Yes	49	24.1
	No	154	75.9
Backache	Yes	35	17.2
	No	168	82.8
Muscle pains	Yes	33	16.3
	No	170	83.7

3.4. Prevalence of *L. monocytogenes*

The overall prevalence of *L. monocytogenes* among the pregnant women was 11/203 (5.42%; 95% CI=2.88–9.38). The prevalence of *L. monocytogenes* was 8 (11.11%) in the age group 25–34 years, 1 (11.11%) in widowed, 4 (10%) in daily workers, 2 (10%) in those who could read and write, 6 (7.79%) in the third trimester, and 3 (7.69%) in rural locations of residence (Table 4).

Table 4. Bivariate analysis of socio-demographic characteristics among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).

Variables	Category	*L. monocytogenes*		*p*-value	COR (95% CI)
		Positive (n=11) (%)	Negative (n=192) (%)		
Age (years)	18 – 24	2 (2.20)	89 (97.80)	Ref	
	25 – 34	8 (11.11)	64 (88.89)	0.034*	5.56 (1.14-27.07)
	35 – 44	1 (2.50)	39 (97.50)	0.915	1.14 (0.10-12.96)
Residence	Urban	8 (4.88)	156 (95.12)	Ref	
	Rural	3 (7.69)	36 (92.31)	0.489	1.63 (0.41-6.43)
Marital status	Single	2 (10.00)	18 (90.00)	Ref	
	Married	7 (4.38)	153 (95.62)	0.291	0.41 (0.08-2.14)
	Divorced	1 (7.14)	13 (92.86)	0.773	0.69 (0.06-8.47)
	Widowed	1 (11.11)	8 (88.89)	0.928	1.13 (0.09-14.28)
Educational status	Read and Write	2 (10.00)	18 (90.00)	0.252	3.28 (0.43-24.95)
	Elementary School	3 (5.56)	51 (94.44)	0.555	1.74 (0.28-10.80)
	Secondary School	4 (5.88)	64 (94.12)	0.489	1.84 (0.33-10.44)
	College/University	2 (3.28)	59 (96.72)	Ref	
Occupation	Government employee	2 (3.70)	52 (96.30)	Ref	
	Merchant	2 (5.26)	36 (94.74)	0.719	1.44 (0.19-10.73)
	Daily Worker	4 (10.00)	36 (90.00)	0.235*	2.89 (0.50-16.62)
	House Wife	3 (4.23)	68 (95.77)	0.883	1.15 (0.19-7.12)

COR, crude odds ratio; CI, confidence interval; Ref, reference; (*), candidate variables for multivariable analysis at *p*< 0.25.

3.5. Association of Possible Risk Factors for *L. monocytogenes* Infection

Pregnant women who frequent consumed uncooked meat (COR=4.98; 95% CI=1.28-19.38), *p*= 0.021), uncooked vegetable (COR=5.55; 95% CI=1.17-26.35), *p*=0.031), and unpasteurized milk (COR=8.02; 95% CI=1.69-38.19), *p*=0.009) were 4.98-, 5.55-, and 8.02- times more at risk for *L. monocytogenes* infection, respectively, which was statistically significant. In contrast, gestational age (2nd trimester (COR=1.98; 95% CI=0.21-18.26), *p*=0.549), 3rd trimester (COR=3.38; 95% CI=0.39-29.08), *p*=0.267), and consumption of uncooked/raw fish (COR=1.68; 95% CI=0.48-5.92), *p*=0.421) were not statistically associated with *L. monocytogenes* infection (Table 5).

Table 5. Bivariate analysis of possible risk factors of L. monocytogenes among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).

Variables	Category	*L. monocytogenes*		*p*-value	COR (95% CI)
		Positive (n=11) (%)	Negative (n=192) (%)		
Gestational age	1st trimester	1 (2.44)	40 (97.56)	Ref	
	2nd trimester	4 (4.71)	81 (95.29)	0.549	1.98 (0.21-18.26)
	3rd trimester	6 (7.79)	71 (92.21)	0.267	3.38 (0.39-29.08)
Frequently feeding habit					

Variables	Category	*L. monocytogenes*		*p*-value	COR (95% CI)
		Positive (n=11) (%)	Negative (n=192) (%)		
Uncooked meat	Yes	8 (10.67)	67 (89.33)	0.021*	4.98 (1.28-19.38)
	No	3 (2.34)	125 (97.66)	Ref	
Uncooked/raw fish	Yes	7 (6.67)	98 (93.33)	0.421	1.68 (0.48-5.92)
	No	4 (4.08)	94 (95.92)	Ref	
Uncooked vegetables	Yes	9 (9.47)	86 (90.53)	0.031*	5.55 (1.17-26.35)
	No	2 (1.85)	106 (98.15)	Ref	
Unpasteurized milk	Yes	9 (11.54)	69 (88.46)	0.009*	8.02 (1.69-38.19)
	No	2 (1.60)	123 (98.40)	Ref	

COR, crude odds ratio; CI, confidence interval; Ref, reference; (*), candidate variables for multivariable analysis at $p< 0.25$.

3.6. Clinical Signs and Symptoms

Fever (COR=18.02; 95% CI=4.79-67.81), *p*=0.001) and nausea/vomiting (COR=6.25; 95% CI=1.75-22.37), *p*=0.005) were significantly associated with *L. monocytogenes*. In contrast, headache (COR=1.47; 95% CI=0.38-5.81), *p*=0.581), gastroenteritis (COR=1.46; 95% CI=0.41-5.19), *p*=0.558), backache (COR=1.88; 95% CI=0.47-7.45), *p*=0.372), and muscle pains (COR=2.03; 95% CI=0.51-8.07), *p*=0.317) were not statistically associated with *L. monocytogenes* infection (Table 6).

Table 6. *Bivariate analysis of clinical signs and symptoms of L. monocytogenes among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).*

Variables	Category	*L. monocytogenes*		*p*-value	COR (95% CI)
		Positive (n=11) (%)	Negative (n=192) (%)		
Fever	Yes	7 (29.17)	17 (70.83)	0.001*	18.02 (4.79-67.81)
	No	4 (2.23)	175 (97.77)	Ref	
Headache	Yes	3 (7.14)	39 (92.86)	0.581	1.47 (0.38-5.81)
	No	8 (4.97)	153 (95.03)	Ref	
Gastroenteritis	Yes	4 (6.90)	54 (93.10)	0.558	1.46 (0.41-5.19)
	No	7 (4.83)	138 (95.17)	Ref	
Nausea/Vomiting	Yes	7 (14.29)	42 (85.71)	0.005*	6.25 (1.75-22.37)
	No	4 (2.60)	150 (97.40)	Ref	
Backache	Yes	3 (8.57)	32 (91.43)	0.372	1.88 (0.47-7.45)
	No	8 (4.76)	160 (95.24)	Ref	
Muscle pains	Yes	3 (9.09)	30 (90.91)	0.317	2.03 (0.51-8.07)
	No	8 (4.71)	162 (95.29)	Ref	

COR, crude odds ratio; CI, confidence interval; Ref, reference; (*), candidate variables for multivariable analysis at $p< 0.25$.

In bivariate analysis, six variables had a *p*-value <0.25, including the age of participants, uncooked meat, uncooked vegetables, unpasteurized milk, fever and nausea/vomiting, which fulfilled the variable screening criteria (*p*<0.25) and were entered into multivariable logistic regression analysis. In the multivariate analysis, uncooked meat (AOR=6.99; 95% CI=1.39-35.14), *p*=0.018), uncooked vegetable (AOR=6.62; 95% CI=1.04-42.30), *p*=0.046), unpasteurized milk (AOR=7.56; 95% CI=1.33-42.97), *p*=0.023) and fever (AOR=14.65; 95% CI=3.15-68.15), *p*=0.001) were significantly associated with *L. monocytogenes* infection (Table 7).

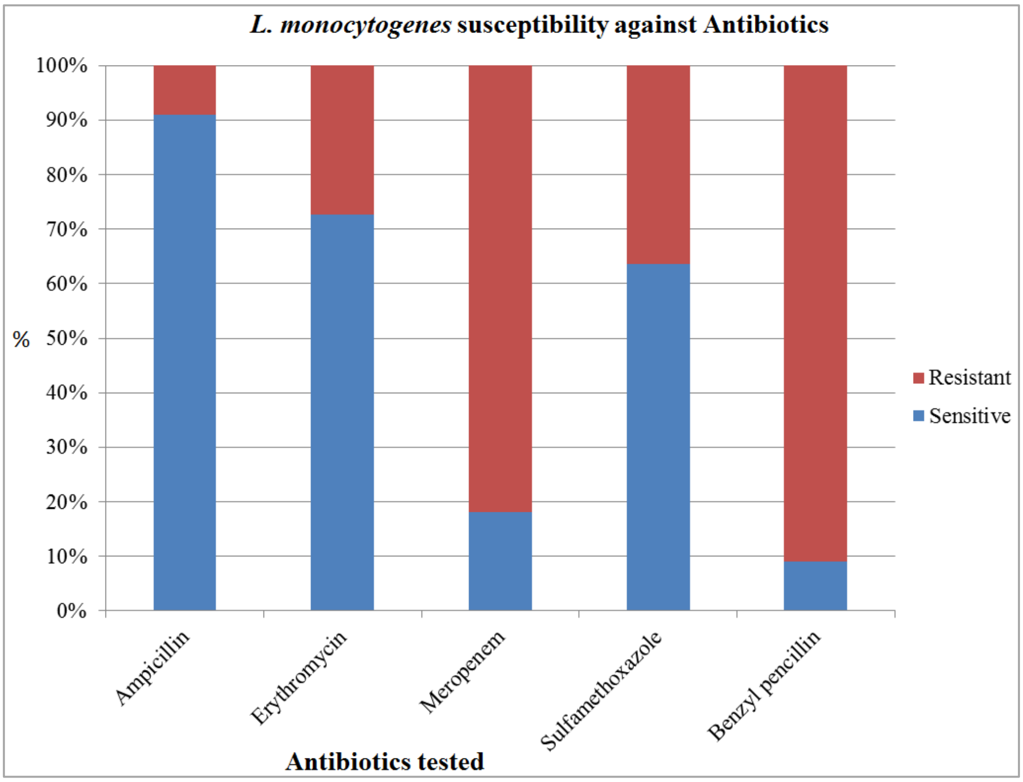

Figure 1. *Antibiotics susceptibility test of L. monocytogenes among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022.*

Table 7. *Multivariate analysis of factors associated with L. monocytogenes among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022 (n=203).*

Variables	Category	*L. monocytogenes* Positive (n=11) (%)	Negative (n=192) (%)	*p*-value	COR (95% CI)	*p*-value	AOR (95% CI)
Age (years)	18 – 24	2 (2.20)	89 (97.80)	Ref		Ref	
	25 – 34	8 (11.11)	64 (88.89)	0.034*	5.56 (1.14-27.07)	0.141	4.40 (0.61-31.74)
	35 – 44	1 (2.50)	39 (97.50)	0.915	1.14 (0.10-12.96)	0.783	1.51 (0.80-28.53)
Uncooked meat	Yes	8 (10.67)	67 (89.33)	0.021*	4.98 (1.28-19.38)	0.018**	6.99 (1.39-35.14)
	No	3 (2.34)	125 (97.66)	Ref		Ref	
Uncooked vegetables	Yes	9 (9.47)	86 (90.53)	0.031*	5.55 (1.17-26.35)	0.046**	6.62 (1.04-42.30)
	No	2 (1.85)	106 (98.15)	Ref		Ref	
Unpasteurized milk	Yes	9 (11.54)	69 (88.46)	0.009*	8.02 (1.69-38.19)	0.023**	7.56 (1.33-42.97)
	No	2 (1.60)	123 (98.40)	Ref		Ref	
Fever	Yes	7 (29.17)	17 (70.83)	0.001*	18.02 (4.79-67.81)	0.001**	14.65 (3.15-68.15)
	No	4 (2.23)	175 (97.77)	Ref		Ref	

Variables	Category	*L. monocytogenes*		*p*-value	COR (95% CI)	*p*-value	AOR (95% CI)
		Positive (n=11) (%)	Negative (n=192) (%)				
Nausea/Vomiting	Yes	7 (14.29)	42 (85.71)	0.005*	6.25 (1.75-22.37)	0.716	1.47 (0.18-11.96)
	No	4 (2.60)	150 (97.40)	Ref		Ref	

AOR, adjusted odds ratio; COR, crude odds ratio; CI, confidence interval; Ref, reference; (*), candidate variables for multivariable analysis at $p< 0.25$; (**), variables statistically significant in multivariate logistic regression at $p<0.05$.

3.7. Antibiotics Susceptibility of *L. monocytogenes*

L. monocytogenes was resistant to benzyl penicillin (10, 90.91%), meropenem (9, 81.82%), sulfamethoxazole (4, 36.36%) and erythromycin (3, 27.27%). The strain was susceptible to ampicillin (10, 90.91%) (Figure 1).

3.8. Antibiogram of *Listeria monocytogenes*

Antibiotic susceptibility tests were performed on 11 *L. monocytogenes* isolates, and 10 isolates were resistant to one of the five antibiotics. One (10%), two (20%), one (10%), and five (50%) isolates were resistant to five, four, three, and two of the antibiotics tested, respectively. One isolate of *L. monocytogenes* was resistant only to benzyl penicillin, and the remaining nine (90%) isolates of *L. monocytogenes* were resistant to one or five antibiotics tested (Table 8).

Table 8. *Antibiograms of 10 L. monocytogenes isolates among pregnant women attending Millennium Health Center, Hawassa, Ethiopia, May 30 to July 30, 2022.*

Resistance pattern	Resistant strain, n (%)
BP	1 (10)
BP, Mem	5 (50)
BP, Mem, SXT	1 (10)
BP, Ery, Mem, SXT	2 (20)
Amp, BP, Ery, Mem, SXT	1 (10)
Total	10 (100)

Amp, ampicillin; BP, benzyl penicillin; Ery, erythromycin; Mem, meropenem; SXT, sulfamethoxazole.

4. Discussion

In the present study, the prevalence of *L. monocytogenes* was found to be 11/203 (5.42%; 95% CI=2.88-9.38). This finding was similar to those of the Tertiary Care Hospital in Tehran, Iran (5.5%) [29], and in Jimma University Medical Center, Southwest Ethiopia (5.56%) [30]. However, it was greater than the study findings in Arash Hospital in Tehran, Iran (4, 4.0%) [31] and a study conducted on fecal carriers in HIV-uninfected pregnant women in Brazil (2.9%) [32]. On the other hand, it was less than the findings of the Gynecology Department, Bharatpur, Nepal (16.7%) [33], Salah Aldeen Province (13.82%) [21], Tigray region, Northern Ethiopia (8.5%) [34], and India (6.3%) [35]. The variation in the study findings might be due to differences in socio-demographic characteristics, study population, access to health facilities, and methodology used.

In this study, the prevalence of *L. monocytogenes* among pregnant women was found to be higher in the age group 25–34 years (8/72; 11.11%) with AOR=4.40 (95% CI=0.61-31.74), *p*=0.141), agreement with findings [21, 33], daily worker (4/40; 10%) with COR=2.89 (95% CI=0.50-16.62), *p*=0.235), and rural (3/39; 7.69%) with COR=1.63 (95% CI=0.41-6.43), *p*=0.489), agreement with findings [34], third trimester (6/77; 7.79%) with COR=3.38 (95% CI=0.39-29.08), *p*=0.267), agreement with findings [33, 6]. The possible reasons for the high prevalence in the present study could be the high-risk group, populated area, pregnancy-related suppressed cell-mediated immunity, placental tropism of *L. monocytogenes* and sudden changes in hormones during pregnancy.

Pregnant women who had a frequent feeding habit of uncooked meat (8; 10.67%) with AOR=6.99 (95% CI=1.39-35.14), *p*=0.018) were highly infected with *L. monocytogenes*, which is in agreement with previous findings [33, 36]. The odds of uncooked meat consumption among pregnant women who had the habit of frequently eating uncooked meat was 6.99-times higher than that among those who did not frequently eating uncooked meat (AOR=6.99; 95% CI=1.39-35.14, *p*=0.018). This might be due to the fact that cattle and sheep are natural hosts for *L. monocytogenes* and it can easily be transmitted by eating contaminated uncooked meat.

Pregnant women who frequently ate uncooked vegetables (9; 9.47%) with AOR=6.62 (95% CI=1.04-42.30), *p*=0.046) were highly infected with *L. monocytogenes*, which is in agreement with the previous findings [37, 33]. This study showed that pregnant women who had the habit of frequently eating uncooked vegetables were 6.62-times at greater risk than those pregnant women who had no habit of frequently eating uncooked vegetables (AOR=6.62; 95% CI=1.04-42.30, *p*=0.046). This may be because vegetables are the primary reservoirs for *L. monocytogenes* and can easily be transmitted by eating uncooked vegetables because they are food-borne diseases and are present in contaminated raw vegetables.

Pregnant women who frequently drank unpasteurized milk (9; 11.54%) with AOR=7.56 (95% CI=1.33-42.97), *p*=0.023) were highly infected with *L. monocytogenes*, which was in agreement with previous findings [33, 38, 34]. Pregnant women who frequently drank unpasteurized milk were 7.56-times more at risk of *L. monocytogenes* infection (AOR=7.56; 95% CI=1.33-42.97, *p*=0.023). This might be due to the fact that *L. monocytogenes* can easily be transmitted by drinking unpasteurized milk because it might be contaminated during milking from cows, during preservation, or from the environment.

Additionally, the prevalence of *L. monocytogenes* was high in pregnant women who showed clinical signs and symptoms such as nausea/vomiting (n=7; 14.29%) with AOR=1.47 (95% CI=0.18-11.96), *p*=0.716) and fever (n=7; 29.17%) with AOR=14.65 (95% CI=3.15-68.15), *p*=0.001) which was in agreement with previous findings [11, 34, 30]. The results of this study indicated that the probability of isolating *L. monocytogenes* was 14.65-times greater in pregnant women who showed clinical signs and symptoms of fever than those who did not show clinical signs and symptoms of fever (AOR=14.65; 95% CI=3.15-68.15, *p*=0.001). This might be due to the fact that *L. monocytogenes* infections usually showed clinical signs and symptoms of fever.

In our study, we observed high antibiotic resistance in *L. monocytogenes* to benzyl penicillin (90.91%) and meropenem (81.82%). This is because, *L. monocytogenes* infections are usually treated clinically without culture isolation, and antibiotic susceptibility tests are performed for *L. monocytogenes*. Similar findings were reported in other studies [33, 34, 30]. In contrast, ampicillin showed better antibiotic activity against *L. monocytogenes*. This is in agreement with the findings of previous studies [39, 29, 34, 30].

5. Conclusions and Recommendations

The prevalence of *L. monocytogenes* among pregnant women attending the Millennium Health Center is high. *L. monocytogenes* was resistant to benzyl penicillin, meropenem, erythromycin, and sulfamethoxazole, and susceptible to ampicillin. Uncooked meat, raw vegetables, unpasteurized milk, and fever were significantly associated with *L. monocytogenes* infection. Pregnant women should avoid eating foods commonly contaminated with *L. monocytogenes*, such as uncooked meat, unpasteurized milk, and raw vegetables. Early detection of *L. monocytogenes* via blood culture and drug sensitivity testing may reduce the rate of antibiotic resistance. Further studies are needed to isolate species and strains using molecular methods and to determine the most feasible combination of antibiotics for the management of *L. monocytogenes* infection during pregnancy.

6. Limitations of the Study

This study could not determine cause-and-effect relationship because it was a cross-sectional study. In addition, the status of HIV, diabetes, history of abortion, and other immunological disorders were not assessed.

Abbreviations

ATCC: American Type Culture Collection
CAMP: Christie, Atkins, Munch-Petersen
CLSI: Clinical Laboratory Standard Institute
EUCAST: European Committee on Antimicrobial Susceptibility Testing
MHA: Muller Hinton Agar
TSB: Tryptic Soy Broth
TSBYE: Tryptose Soy Broth With 0.6% Yeast Extract

Declarations

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Hawassa University College of Medicine and Health Sciences Institutional Review Board (IRB) (reference number IRB/147/14). Permission was obtained from the Millennium Health Center. Data were collected from study participants after obtaining written informed consent. All information obtained from the study participants was confidential and was used only for this study. The results of patients were kept confidential by using codes, and whether positive or negative results were reported to clinicians by the assigned runner.

Consent for Publication

Not applicable.

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Author Contributions

FD was involved in the proposed design, study participant selection, materials provided, laboratory investigation, data analysis, and manuscript preparation. AA contributed in laboratory investigation and manuscript preparation, DD and DY supervised laboratory work and all the investigation processes and contributed in the manuscript preparation and approval to the final version of this paper and AW contributed in data analysis and revision of the manuscript. All the authors read and approved the final manuscript.

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Data Availability Statement

There are no remaining data and materials, and all this information is presented in the main manuscript. The raw datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interests

The authors declare no conflicts of interest.

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